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# Vinculin, cell mechanics, and tumor cell invasion



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*Review*

**Vinculin, cell mechanics, and tumor cell invasion**

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**Abstract**

The focal adhesion protein vinculin is important for transmitting mechanical forces and orchestrating mechanical signaling events. De-regulation of vinculin results in altered cell adhesion, contractility, motility, and growth, all of which are important processes in cancer metastasis. This review summarizes recent reports on the role of vinculin in cellular force generation and signaling, and discusses implications of vinculin's function for promoting cancer cell migration in 3D environments.

*Keywords:* Vinculin; focal adhesions; adherence junctions; cancer; cell mechanics; ECM; 2D -and 3D environment

*Abbreviations:* ECM = extracellular matrix; FA = focal adhesions; AJ = adherence junctions; 2D = two dimensional; 3D = three dimensional; Vt = vinculin-tail; Vh = vinculin-head

## Introduction

The mechanical integration of cells in tissues through contacts with the extracellular matrix (ECM) and neighboring cells is essential for tissue development. Cell adhesion is the result of complex and highly coordinated interactions of many proteins. Among them, the transmembrane cell adhesion receptors of the integrin family are the best studied. Integrins cluster in focal adhesions, where they recruit cytoplasmic focal adhesion proteins that connect the cytoplasmic tails of integrins to F-actin. These connections enable the bidirectional transmission of mechanical forces between the cytoskeleton and the ECM (Alonso et al. , 2002, Hynes, 2002). In addition, focal adhesion proteins modulate intracellular signaling pathways upon integrin ligation to the ECM, which controls diverse cellular processes such as proliferation, differentiation, apoptosis, or motility (Critchley, 2000). The majority of these processes are de-regulated in tumor cells, and it is therefore reasonable to ask to which degree adhesion proteins are implicated in the course of the disease.

Vinculin is an abundant, prominent, and well-characterized F-actin binding protein localized in focal adhesions as well as in cell-adherence junctions (AJ). Vinculin provides a mechanical link (Ezzell et al., 1997, Grashoff et al. , 2010, Hu et al. , 2007, Li et al. , 2012), controls cell signaling processes (Chen et al. , 2002, Peng et al. , 2011, Subauste et al. , 2004a, Subauste et al. , 2004b), affects contractility (Mierke et al. , 2008b), and adhesion protein turnover (Humphries et al. , 2007, Möhl et al. , 2009). Vinculin has been suggested to function as a tumor suppressor by supporting anchorage-dependent cell growth (Rodriguez Fernandez et al. , 1993, Rodriguez Fernandez et al. , 1992b) and by suppressing tumor metastasis through reducing cell motility (Liu et al. , 2007, Rodriguez Fernandez et al. , 1992a, Rodriguez Fernandez et al., 1992b). Recent reports, however, have demonstrated that the role of vinculin in regulating cell migration is more complex and fundamentally differs between 2D and 3D environments (Mierke et al. , 2010).

## Vinculin's interaction with proteins and lipids

Vinculin simultaneously binds F-actin and the focal adhesion (FA) protein talin and $\alpha$-actinin, which in turn connect to ECM-bound integrins (Giannone et al. , 2003, Goldmann, 2002, 2012, Jiang et al. , 2003, Margadant et al. , 2011). Structurally, vinculin is located in a layer between actin and talin within focal adhesions (Kanchanawong et al. , 2010). The connection between vinculin and partner molecules is mechanically strong and thus important for force transmission from the ECM to the actin cytoskeleton and vice versa (Ezzell et al. , 1997, Grashoff et al., 2010). Vinculin contains 1066 amino acids (MW 117 kDa), which can be cleaved with protease V8 into a 95 kDa (residues 1–838) head and a 30 kDa (residues 894–1066) tail fragment (Johnson and Craig, 1994). It binds to various other FA and actin regulatory proteins including paxillin, tensin, zyxin, ezrin, p130Cas, Arp2/3, VASP, and also binds to itself through an intramolecular head-tail-interaction (Brabek et al. , 2005, Brindle et al. , 1996, Burridge and Mangeat, 1984, Crawford et al. , 1992, DeMali et al. , 2002, Drenckhahn and Franz, 1986, Geiger and Ginsberg, 1991, Geiger et al. , 1980, Goldmann et al. , 1996, Johnson et al. , 1998, Lo et al. , 1994, Reinhard et al. , 1992, Svoboda et al. , 1999, Turner, 1998, Turner and Burridge, 1991, Volberg et al. , 1995). The role of

these interactions is poorly understood, but is likely to have a critical impact on cell signaling (Carisey and Ballestrem, 2011).

The binding and activation of vinculin at adhesion sites is rather complex and currently still not fully understood. Unbound, cytoplasmatic vinculin shows a high affinity between the vinculin-head (Vh) and -tail (Vt) domain, which renders the molecule in an auto-inhibited, closed conformation, such that numerous of its binding sites are masked (Bakolitsa et al. , 2004, Borgon et al. , 2004, Cohen et al. , 2005, Johnson and Craig, 1995, Ziegler et al. , 2006). Releasing this high affinity Vh-Vt interaction to open the molecule is thought to require the binding of vinculin to focal adhesion proteins (Bakolitsa et al. , 2004, Bois et al. , 2006, Ziegler et al. , 2006). In particular, talin and F-actin are required for vinculin activation, as was shown in a FRET assay (Chen et al. , 2006) (Fig. 1). This view is at the core of the so-called combinatorial model for vinculin activation.

Vinculin also associates with membrane lipids (Diez et al. , 2009, Diez et al. , 2008, Johnson et al. , 1998, Tempel et al. , 1995). Phospholipid-binding of vinculin is discussed as a potential mechanism for vinculin activation (Johnson et al. , 1998, Ziegler et al. , 2002). In the presence of acidic phospholipids, tyrosine phosphorylation of vinculin is increased (Ito et al. , 1982, Ito et al. , 1983, Niggli et al. , 1990), which in turn is believed to promote the opening and hence activation of the molecule (Moese et al. , 2007, Zhang et al. , 2004). However, the view that phospholipid-binding leads to vinculin activation has recently been challenged by a study that demonstrated that $PIP_2$-binding enhances the dissociation of vinculin from focal adhesions (Chandrasekar et al. , 2005) (Fig. 1).

Several studies have demonstrated that the phosphorylation of vinculin on residues Y100 and Y1065 by Src family kinases (SFKs) might be important for its activation (Moese et al. , 2007, Zhang et al. , 2004). Downregulation of Src-kinase or mutations of these residues that prevent phosphorylation caused marked cell mechanical alterations. Specifically, reduced phosphorylation at residue Y1065 was associated with increased exchange dynamics in nascent focal adhesions and reduced insertion of the vinculin C-terminal residues into lipid membranes, yielding a decrease in cell traction and force generation (Diez et al. , 2009, Möhl et al. , 2009). Hence, preventing vinculin phosphorylation had a similar effect as complete vinculin knockout (Fig. 1).

It was subsequently suggested that vinculin might first be recruited to the lipid membrane to become activated. Upon binding to phosphatidylinositol(4,5)-biphosphate ($PIP_2$) at the cell membrane, vinculin unfolds, exposing its talin binding sites that are critical for vinculin's focal adhesion localization in cells. Phosphorylation at residues Y100/Y1065 may therefore increase the affinity of vinculin for other binding partners, but phosphorylation alone is not sufficient to cause a complete activation and opening of the molecule. It is therefore believed that, in addition to phosphorylation, intracellular forces that are coupled to vinculin through its connection with talin, actin, and $\alpha$-actinin, may mechanically open and thereby fully activate the vinculin molecule (Golji et al. , 2012 , Shams et al. , 2012). In support of this view is the observation that phosphorylation of vinculin is required for a reinforcement of the talin-actin -and $\alpha$-actinin-actin linkage in response to mechanical forces (Huang et al. , 2011). This would mean that vinculin can only be fully activated under a condition in which it is already bound, at least weakly, to talin, actin, or $\alpha$-actinin (Bershadsky et al. , 2006, Chen et al. ,2006). Accordingly, vinculin

phosphorylation is only needed for the initial, weak binding of the molecule to its binding partners. It is possible that these bonds are catch-bonds that strengthen under mechanical load, and hence that the reinforcement process is in fact not an actively regulated but a passive mechanical process, but catch-bond behavior of vinculin has been recently challenged by a molecular dynamics study (Hytönen and Vogel, 2008).

**Force-dependent vinculin activation and force transmission**

Vinculin may also be mechanically activated by tensile forces acting through F-actin and talin. Indeed, force-dependent recruitment of vinculin to focal adhesions has been reported in several studies (Galbraith et al. , 2002, Grashoff et al. , 2010). Mechanical stress acting across the vinculin molecule could separate the Vh from the Vt domain, which then exposes binding sites for other FA proteins (Chen et al. ,2006, Küpper et al. , 2010, Möhl et al. , 2009). Such a mechanical unfolding has been suggested to trigger integrin-dependent mechano-sensitive signal transduction pathways (Hoffman et al. , 2011), although this has never been shown directly.

Integrin-dependent mechano-sensitive signal transduction gives adherent cells the ability to reinforce their integrin-FA-actin connection when (i) external forces are exerted (Choquet et al. , 1997), or when (ii) increased internal forces are applied (Deng et al. , 2004). This reinforcement process leads to locally increased concentrations of integrin (clustering), an increased accumulation of focal adhesion proteins (recruitment) as well as actin polymerization (Coussen et al. , 2002, Huveneers et al. , 2012, Nishizaka et al. , 2000). Reinforcement allows the cell to generate higher traction forces and to withstand greater external forces (Balaban et al. , 2001, Grashoff et al. , 2010, Hoffman et al. , 2011).

Evidence that vinculin is particularly important in tissues exposed to high mechanical load comes from several in vivo models. For instance, vinculin is required for the normal development of the body wall musculature in *Caenorhabditis elegans* embryos (Barstead and Waterston, 1989). Vinculin-deficient mouse embryos show heart edemas as well as defects in neural tube closure and nerve growth, and die at mid-gestation (Xu et al. , 1998a). Cardiomyocyte-specific vinculin gene disruption in mice is lethal and associated with the disintegration of intercalated discs, cardiac arhythmias, and dilated cardiomyopathy (Zemljic-Harpf et al. , 2007). Even heterozygous inactivation of the vinculin gene predisposed mice for cardiomyopathy (Zemljic-Harpf et al. , 2004), and vinculin-deficient smooth muscle tissue showed diminished force generation (Saez et al. , 2004).

Consistent with these *in vivo* data, cell culture studies of vinculin-deficient murine embryonic fibroblasts (MEF), F9 cells, and PC12 neuronal cells showed that vinculin is required for cell spreading, firm adhesion to various extracellular matrix proteins, and the stabilization of focal adhesions and lamellipodia (Goldmann et al. , 1995, Saunders et al. , 2006, Varnum-Finney and Reichardt, 1994, Xu et al. , 1998b). These observations can be explained by the mechano-coupling and stabilizing function of vinculin through direct interaction with talin and F-actin (Ezzell et al. , 1997, Goldmann and Ezzell, 1996, Goldmann et al. , 1998, Humphries et al. , 2007). Indeed, vinculin transmits forces of ~2pN per molecule (Grashoff et al., 2010). In line with this, vinculin-deficient MEFs show diminished traction force

generation and reduced cytoskeletal stiffness on two-dimensional cell culture substrates (Mierke et al. , 2010).

**Vinculin and cell motility**

Impaired traction force generation, spreading, and ECM-adhesion of cells lacking vinculin is generally associated with increased cell motility in 2D (Coll et al. , 1995, Mierke et al. , 2008a, Rodriguez Fernandez et al. , 1993, Rodriguez Fernandez et al. , 1992b, Saunders et al. , 2006, Xu et al. , 1998b). In contrast, vinculin overexpression was shown to reduce cell motility in 2D (Rodriguez Fernandez et al. , 1992b). These data suggest that a lack of vinculin, in addition to promoting cell growth and inhibiting anoikis, could also contribute to the malignancy of cancer cells by promoting their invasive behavior (Rodriguez Fernandez et al. , 1992b). Consistent with this, the re-expression of vinculin in malignant fibroblasts and epithelial cells with low levels of endogenous vinculin led to reduced primary tumor formation after subcutaneous injection into mice and strongly reduced metastatic spreading into lungs (Rodriguez Fernandez et al. ,1993). But it remains unclear whether the reduced metastatic capacity of these cells after vinculin restoration was primarily the result of reduced cell invasion, reduced proliferative capacity, or both. These possibilities are discussed in more detail below.

**Vinculin-dependent cell growth, apoptosis, and tumorigeneity**

Vinculin depletion was shown to promote anchorage-independent growth of BALBc/3T3 cells on soft agar colonies (Rodriguez Fernandez et al. , 1993). Moreover, restoration of vinculin expression in transformed fibroblasts and pancreatic adenocarcinoma cells with low endogenous vinculin levels suppressed both anchorage-independent growth in soft agar, and the tumorigenic ability of these cells upon injection into nude mice (Rodriguez Fernandez et al. , 1992b). Reduced apoptotic behavior of vinculin-deficient cells is a consequence of changes in the activity of focal adhesion kinase (FAK), paxillin, and extracellular signal-regulated kinase ($ERK^{1/2}$) (Subauste et al. , 2004b). Vinculin has also been proposed to directly influence other key signaling proteins such as p130Cas and CrkII (Janoštiak et al. *unpublished observation*) (Xu et al. , 1998b). In addition, vinculin-deficient F9 embryonic carcinoma cells lack the tumor suppressor PTEN (phosphatase and tensin homologue deleted on chromosome ten) (Subauste et al. , 2004a). These data suggest that vinculin deficiency induces alterations in cell signaling in a direction that may increase the tumorigeneity of the cells (Table 1).

**Vinculin's role in tumor cell invasion**

Cancer metastasis requires cells to invade connective tissue, which is inherently a mechanical event that involves adhesion, shape changes, movement, and force generation of cells (Friedl and Brocker, 2000, Rolli et al., 2003, Wolf et al., 2003, Paszek et al., 2005, Zaman et al., 2006, Brabek et al., 2010, Friedl and Wolf, 2010, Bradbury et al., 2012). Since the vinculin molecule connects the ECM through integrins and

talin to the actomyosin cytoskeleton and is therefore critical for the transmission of contractile forces, and since vinculin regulates cell motility on 2D substrates, it is conceivable that vinculin may also affect cell invasion. However, whether reduced cell adhesion and force generation in the absence of vinculin lead to an increased cell motility in 3D tissue environments similar to 2D substrates, or whether loss of vinculin inhibits 3D cell migration, has only recently been addressed (Mierke et al. , 2010).

Cells on 2D surfaces experience only negligible frictional (drag) forces from the liquid environment but no steric hindrance, whilst cells in a 3D environment have to overcome the forces that arise from the steric hindrance of the matrix network (Zaman et al. , 2006, Zhong et al. , 2012). Cells have several options: they either deform themselves until they fit through the pores/gaps, or they change the network until the pores/gaps are large enough to pass through. For the latter, cells can either use pushing and pulling forces, or they secrete cellular enzymes such as metalloproteinases (MMPs) (Sanz-Moreno et al. , 2008, Friedl and Wolf, 2003). Switching between cell body deforming versus matrix deforming migration strategies can be deduced from cell morphology changes between rounded versus elongated cell shapes. A cell body-deforming migration strategy is referred to as amoeboid migration, whereas a matrix-deforming migration strategy is referred to as mesenchymal migration. A review of these different migration strategies is given in (Friedl and Gilmour, 2009, Friedl and Wolf, 2003).

To squeeze through small pores/gaps, the cell needs to generate sufficient forces to overcome the elastic and frictional resistance of the cytoskeleton and the nucleus. Here, cells have the option to decrease the cytoskeletal elasticity (stiffness) and friction by depolymerizing the cytoskeletal filaments; this reduces the forces that are necessary to deform the cell, but at the same time this strategy also reduces the force-generating capacity of the actomyosin contractile apparatus (Petrie et al. , 2012). The cellular changes after a loss of vinculin, i.e. reduced adhesion, increased focal adhesion turnover, reduced cell stiffness and contractile forces are all associated with an amoeboid migration strategy, but whether vinculin-deficient cells do indeed exhibit an amoeboid phenotype in 3D needs to be investigated. As reported above, the cellular changes induced by a loss of vinculin lead to an increased migration speed in 2D. It is, however, not obvious how these changes affect migration through a dense 3D environment with a high degree of steric hindrance. If the pores of the 3D matrix fall below a cell-specific minimum size through which the cell can conveniently squeeze, amoeboid-like migration becomes less effective and a mesenchymal migration strategy may need to be employed, including cell elongation, strong adhesion, and large contractile force generation, all of which require vinculin. Indeed, wildtype MEFs invade deeper and with higher motility into dense and relatively stiff 3-D collagen gels compared to vinculin-deficient cells (Mierke et al. , 2010), suggesting that vinculin may be an important promoter of tumor cell invasiveness in dense environments with a high degree of steric hindrance. We speculate that vinculin, beyond increasing adhesiveness and force generation, also promotes cell polarization and directionality of traction force generation. These mesenchymal attributes have recently been shown to be a prerequisite for the migration of tumor cells through dense 3-D matrices (Koch et al. , 2012).

This raises the question whether vinculin also promotes the 3-D migration of tumor cells *in vivo*. The reduced metastatic capacity of vinculin-expressing cells as reported in several studies (Lifschitz-Mercer et al. , 1997, Rodriguez Fernandez et al. , 1992a, 1993, Rodriguez Fernandez et al. , 1992b) seems to

contradict data from *in vitro* 3-D migration assays but may be explained by a reduced cell proliferation. Studies that specify between vinculin functions in cell migration and regulation of cell growth at the tissue level or *in vivo* will likely yield new insight into the mechanism underlying vinculin's function as tumor suppressor.

**Future directions**

A recent study reported the presence of mechanical tension across vinculin in cells (Grashoff et al. , 2010). These authors suggested a regulatory mechanism by which FA stabilization requires both the recruitment and force transmission of vinculin. However, major questions still remain unanswered: (i) is vinculin only a mechano-coupler or also a mechano-sensor, and (ii) to what degree is vinculin, beyond its mechanical function, involved in signaling processes that enable the cell to react to its physical environment?

Moreover, it is still an open question how vinculin is activated in cells, whether (i) by phosphorylation through $PIP_2$ or Src-kinase on residue Y1065/Y100, (ii) through binding to talin/alpha-actinin to vinculin`s head and its binding to actin, (iii) through the binding of vinculin`s tail to the cell membrane, (iv) through internal/external forces, or (v) by the combination of many parameters. Vinculin activation, in turn, triggers a cascade of downstream events via proteins such as paxillin, FAK, ERK, MLCK, but the precise pathway and the dynamics of these events are still debated.

An intriguing question is whether vinculin's effect on traction force generation (Mierke et al. , 2008a, Mierke et al. , 2010) is primarily a result of physically linking the actin cytoskeleton and ECM, or whether vinculin also actively controls actomyosin-based force generation in the cell. Interestingly, it was recently demonstrated that vinculin is required for myosin light chain recruitment to cell-adherence junctions (AJ) under increased mechanical load (le Duc et al. , 2010, Leckband et al. , 2011, Twiss et al. , 2012). Whether similar vinculin-dependent signaling processes contribute to the generation of high ECM-traction forces (Mierke et al. , 2010) remains to be determined.

There is supporting evidence that vinculin fundamentally influences many important cell function, in particular mechanical properties such as contractility, adhesion strength, and stiffness. These mechanical properties affect the ability of cells to migrate, but this depends on the dimensionality, adhesiveness, or steric hindrance of the environment. Therefore, vinculin can be expected to have a crucial effect on the ability of tumor cells to invade tissue and hence to metastasize. We envision that vinculin, similar to numerous other focal adhesion and adherence junction (AJ) molecules that have been implicated in cancer development and metastasis, such as integrins, talin, p130Cas, or cadherins, will keep scientists busy for years to come, addressing not only single cell behavior but also cell populations in complex 3-D environments.

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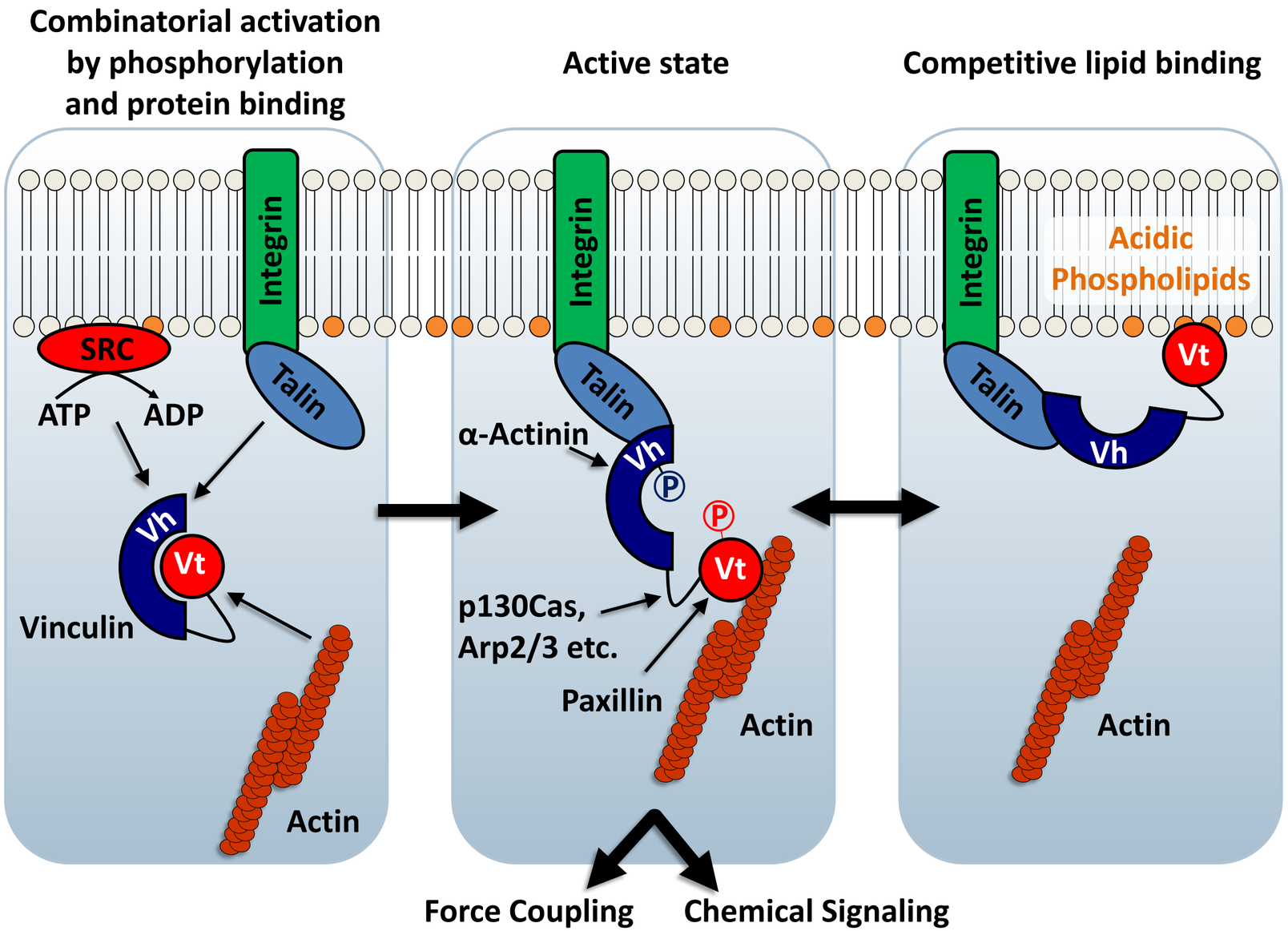
**Table 1: Influence of vinculin on tumorigeneity**

| Effects of vinculin | Expected effects on tumorigeneity | References |
|---|---|---|
| **Conferring anchorage-dependent cell growth** | decreased | (Liu et al., 2007, Rodriguez Fernandez et al., 1992a, 1993, Rodriguez Fernandez et al., 1992b, Volberg et al., 1995) |
| **Increased apoptosis, anoikis** | increased | (Critchley, 2004, Ziegler et al., 2006) |
| **Reduced 2D migration** | decreased | (Coll et al., 1995, Mierke et al., 2010, Rodriguez Fernandez et al., 1992a, Xu et al., 1998b) |
| **PTEN upregulation** | decreased | (Subauste et al., 2004a) |
| **Higher cell stiffness** | decreased | (Mierke et al., 2008a, 2010) |
| **Higher 3-D motility** | increased | (Mierke et al., 2010) |
| **Higher contractility** | increased | (Kraning-Rush et al. , 2012, Mierke et al., 2008a, 2010) |
| **ERK1/2/MAPK activation** | decreased | (Goldmann, 2002, Ziegler et al., 2006) |

**Fig. 1 Possible ways of vinculin activation:** Vinculin interaction by phosphorylation and protein binding (left), or by lipid binding (right), leads to a primed or active state (middle) that may be further activated or stabilized by forces acting across vinculin. The vinculin molecule can either be primed by Src phosphorylation on position Y100 and/or Y1065 before binding to talin and F-actin, or talin/alpha-actinin bind to vinculin to trigger the unmasking of the molecule, which then allows for F-actin binding and phosphorylation. Alternatively, binding of talin to the vinculin-head together with phospholipid membrane binding to the vinculin–tail facilitates F-actin association. The coupling of vinculin to F-actin then enables the transmission of intracellular or extracellular forces and integrin-mediated mechano-chemical signaling. Detailed information can be found in (Cohen, et al., 2005, Cohen et al. , 2006, Dey et al., 2011, Diez et al., 2009, Kanchanawong et al., 2010, Moese et al., 2007, Subauste et al., 2004b, Zhang et al., 2004, Ziegler et al., 2006).


Combinatorial activation by phosphorylation and protein binding
Active state
Competitive lipid binding
Integrin
Talin
SRC
ATP
ADP
Vh
Vt
Vinculin
Actin
α-Actinin
P
p130Cas,
Arp2/3 etc.
Paxillin
Acidic
Phospholipids
Force Coupling
Chemical Signaling