Tensins: bridging AMPK with integrin activation

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Keywords

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Abstract

Integrin activation is essential for cell adhesion and for connecting the extracellular matrix to the actin cytoskeleton. Thus, inappropriate integrin activation has been linked to several diseases, including cancer. Recent insights demonstrate that the main fibrillar adhesion component tensin maintains β1-integrin active in these mature adhesions. Depletion or silencing of AMPK, the energy sensor involved in maintaining the energy balance of the cell, enhances integrin activity by increasing the expression of tensin and thereby promoting cell adhesion, matrix formation and mechanotransduction. Here, we discuss the role of tensin and AMPK in the regulation of integrin activity and integrin-dependent processes and their implication in diseases such as cancer and tissue fibrosis.

Trends box

The energy sensor AMPK negatively regulates gene expression of the scaffold proteins tensin1 and tensin3.

Loss of AMPK enhances the formation of fibrillar adhesions, rich in tensin and active α5β1-integrin.

Direct binding of tensins to the β1-integrin tail is required for maintaining β1-integrin activity; however, tensins support β1-integrin activity only in the presence of talin.

Tensins promote cell spreading, fibronectin fibrillogenesis and traction force in the absence of AMPK.

AMPK negatively regulates several proteins involved in cell adhesion and migration, either directly or indirectly.

Integrin activation, fibrillar adhesions and tensins

Integrins are heterodimeric cell-surface receptors, composed of an α and a β subunit, that mediate the interactions between cells and the extracellular matrix (ECM; see Glossary) [1]. Integrins become activated upon binding of talin and kindlin to the cytoplasmic tail of β-integrin (inside-out activation), facilitating binding to extracellular ligands (outside-in signaling) and triggering the recruitment of a large network of cytoskeletal and signaling proteins, collectively called the adhesome [1, 2]. Integrin activation controls essential cellular functions such as adhesion, migration and survival, and defective integrin activation underlies several human diseases. Hence, substantial effort has been put into identifying the molecules involved in the regulation of integrin activity, including large unbiased screens aimed at identifying novel integrin activity regulators [3, 4].

Depending on the size, stability, location and molecular composition there are different types of integrin-dependent adhesions (reviewed in [5]). Fibroblasts bound to the ECM ligand fibronectin have three types of adhesions: the initially-formed nascent adhesions, which mature into larger focal adhesions and the long-lived, highly elongated fibrillar adhesions [5]. Fibrillar adhesions are formed from the medial ends of focal adhesions that translocate centripetally in an actomyosin-dependent manner along fibronectin fibrils. They are rich in α5β1-integrin and tensin and their main function is to promote fibronectin fibrillogenesis and re-organization of the ECM [6, 7].

Tensins are a family of four ubiquitously expressed proteins (tensins 1-4). Tensins 1-3 contain PTP (protein tyrosine phosphatase), SH2 (Src homology 2) and integrin-binding PTB (phosphotyrosine binding) domains, whereas tensin4 (also known as C-terminal tensin like protein; CTEN) is shorter and lacks the PTP domain [8]. Apart from integrins, tensins have been shown to interact with other proteins, such as activated receptor tyrosine kinases (RTKs) and the Rho GAP DLC1, suggesting that, apart from scaffolding functions, tensins also possess signaling properties [8-11]. Of the four tensins, tensin1 and tensin3 mediate the formation of mature fibrillar adhesions involved in matrix remodeling; however whether these isoforms perform different or redundant functions is unclear. In contrast, tensin2 localizes to dynamic focal adhesions at the cell periphery [11] and tensin4 expression is restricted within

normal tissue, in line with the reported oncogenic functions of this isoform in many cancers [9].

Recent data demonstrate that the major metabolic sensor AMPK, identified in the previously published RNAi screens as an integrin inhibitor [3, 4], negatively regulates β1-integrin activity and integrin-dependent processes by decreasing the expression of tensin1 in human telomerase-immortalized fibroblasts or tensin3 in mouse embryonic fibroblasts. [12]. Gain and loss-of function studies indicate that both tensin1 and tensin3 can positively regulate β1-integrin activity. Therefore, loss of AMPK leading to increased integrin activity, cell adhesion, mechanotransduction and fibrillogenesis may be a consequence of either tensin1 or tensin3 upregulation; however, the specific tensin isoform involved may be dependent on the cell-type or the relative expression of the tensin molecules. Given that the original link between AMPK and integrin activity was observed in cancer cells [3, 4], it is highly likely that this AMPK-dependent mechanism, established in fibroblasts, is also relevant in other cell types.

The talin-tensin switch

In the recent study linking AMPK signaling to regulation of integrin activity, AMPK inhibition in fibroblasts was demonstrated to augment the amount of active-α5β1-integrin- and tensin1-containing adhesions, and conversely to reduce talin1-positive adhesions [12]. Notably, there were more tensin1- and active-β1-integrin-positive adhesions both at the cell periphery (focal adhesions) and in the center of the cell (fibrillar adhesions) in cells lacking AMPK [12]. Loss- and gain-of-function studies indicated that both tensin1 and tensin3 positively regulate β1-integrin activity, however their ability to enhance integrin activity was dependent on talin expression [12]. Interestingly, tensin2 which is absent from fibrillar adhesions and localizes to focal adhesions, was unable to activate integrins [12]. These findings support a previously-proposed model of a “talin-tensin switch” in fibrillar adhesions [13]. While the spatio-temporal occurrence of this switch in cells remains unknown, we hypothesize that talin activates β1-integrin in focal adhesions and that an increase in the tensin to talin stoichiometry in maturing adhesions favors replacement of talin with tensin on the β1-integrin tail. Subsequently, tensin would promote the centripetal movement of active α5β1-integrin towards the cell center leading to the maturation of

these adhesions into fibrillar adhesions. However, further investigation is required to prove this hypothesis.

The unexpected role of tensin as an integrin activator

The role of tensin1 and tensin3 as integrin activators is somewhat unexpected. Tensin1 and tensin3 mediate β1-integrin activation by directly binding to the integrin cytoplasmic tail through their PTB domain. Indeed tensin1 and tensin3 mutants with reduced binding to β1-integrin were compromised in their capacity as integrin activators [12]. PTB domains of integrin activity regulators bind to two well-defined motifs at the β-tails, the membrane proximal (MP) NPxY motif and the membrane distal (MD) NxxY motif. The MP NPxY motif serves as a binding site for talin, Dok1 and α-actinin, whereas the MD NxxY motif is the binding site for kindlin and ICAP1 [1]. Direct binding of talin to integrin β-tails is the key final step in integrin activation, thus the binding of other proteins to the talin-binding site is expected to be competitive and inhibitory [1]. Nevertheless, given that tensin binds the β-tail with a 100-fold lower affinity than talin [14], it is unlikely that it will directly compete with talin for integrin binding. Therefore, talin and tensin binding seem to be two consecutive events occurring at spatially distinct subcellular compartments. But what leads to the replacement of talin by tensin? Talin binds integrin tails when the tyrosine of the MP NPxY motif is unphosphorylated [15], whereas tensin binding is unaffected by phosphorylation [14]. Thus, one plausible hypothesis is that upon tyrosine phosphorylation tensin replaces talin (Figure 1A). Dok1 interacts preferentially with a phosphorylated MP NPxY motif. However, in contrast to tensin, Dok1 acts as an integrin inactivator [16] (Figure 1A). A major difference between tensin and Dok1 is that tensin interacts with the actin cytoskeleton providing mechanical integrin coupling, whereas Dok1 binding is expected to prevent the integrin-actin linkage, correlating with cell rounding (Figure 1A). Nevertheless, further studies are required to confirm that the tensin-actin interaction is essential for integrin activation. Further questions that arise are whether Dok1 and tensin are in competition, what is the role of β-tail phosphorylation in regulating tensin binding, and which conditions would favor binding of tensin over talin or Dok1 (Figure 1A).

Another example of an integrin-binding protein acting as an integrin inactivator is the large actin cross-linking protein filamin. Filamin binds partly to both the talin- and the

kindlin-binding NPxY motifs, thus competing with both integrin activators [17]. Most likely the difference between tensin and filamin lies in the localization of tensin. Tensin is supposed to bind integrin in mature focal adhesions and fibrillar adhesions, where talin is mostly absent. Moreover, filamin keeps integrin inactive by additional mechanisms, including through binding to the integrin α-tail to clamp the receptor at a resting state [18, 19].

While many of the identified mechanisms regulating integrin activity are applicable to several different integrins, important distinctions are emerging between β1- and β3-integrin heterodimers. The actin-binding protein α-actinin regulates integrin activity, cell adhesion and mechanotransduction [20]. Interestingly, α-actinin has been proposed to compete with talin for binding to the β3-integrin tail, but to be neutral or even co-operate with talin in β1-integrin activation [20]. The role of tensin as an integrin activator may also be distinct among integrin subfamilies. Even though the effect of tensin on β3-integrin activation has not been studied, it is likely that tensin would not be involved in its activation as β3-integrin is localized in focal adhesions. Congruently, AMPK silencing in human fibroblasts (which increased tensin expression) promoted cell spreading on fibronectin, but did not affect spreading on the αvβ3-integrin ligand vitronectin [12].

Tensins in mechanotransduction

The molecular clutch refers to a series of protein-protein interactions that mediate the mechanical linkage between ECM-bound integrins and the actomyosin cytoskeleton. Talin and vinculin are two important players within this clutch, not only in connecting integrin-mediated adhesions to the actomyosin machinery but also in generating traction force to the substrate [21]. In peripheral focal adhesions talin is under high tension reinforcing the integrin–cytoskeletal bonds and transducing mechanical information [22]. In central focal adhesions talin is under low tension, even though tension is critical for adhesion maturation and stability, suggesting that other molecules are involved in the transmission of force leading to adhesion maturation and fibrillar adhesion formation [22]. A molecular clutch protein proposed to transmit forces between β3-integrins and the actin cytoskeleton in mature adhesions is α-actinin [20]. In addition, tensin1 is also exposed to forces in a similar manner to talin, acting as a mechanosensor [23]. AMPK-silenced fibroblasts, with

157 increased tensin expression, exert elevated traction forces on the ECM, whereas
158 tensin silencing reduces force generation, indicative of an important role for tensins
159 in mechanotransduction to the ECM [12]. As tensin is the major fibrillar adhesion
160 component and may link integrin to the actin cytoskeleton in these structures, it is
161 highly likely that it would also be responsible in mediating the mechanosensitive
162 events required for fibrillar adhesion formation and stability, but this hypothesis
163 requires further investigation.

164 Recent studies have identified the kank family of proteins as novel talin-binding
165 components of focal adhesions [24, 25]. Furthermore, kank2 was shown to activate
166 talin leading to integrin activation [25]. Similar to tensins, kanks promote integrin
167 activation, fibrillar adhesion formation and fibronectin fibrillogenesis, however in
168 contrast to tensins [12], kanks reduce traction force [25] (Figure 1B). A possible
169 explanation is that kanks diminish the connection between integrins and the actin
170 cytoskeleton, as they displace actin from talin, whereas tensins are expected to
171 maintain the integrin-actin linkage through their actin-binding sites. Thus, a
172 fundamental difference is that tensins promote integrin signaling whereas kanks
173 suppress it. Whether kanks and tensins co-operate or compete during the process of
174 adhesion maturation and what their respective contributions to fibrillar adhesions and
175 matrix assembly regulation are remain to be investigated.

176 The link between AMPK and integrins

177 AMPK inhibits integrin activity, fibrillar adhesion formation, ECM assembly and
178 mechanotransduction by transcriptionally reducing tensin levels, without affecting the
179 expression of other known integrin activity regulators [12]. The regulation of tensin
180 expression by AMPK is expected to be indirect, most likely occurring through a
181 transcription factor or a miRNA [26, 27]. AMPK may also regulate integrin-dependent
182 processes via mechanisms unrelated to the modulation of tensin transcription.
183 Indeed, according to mass-spectrometry-based studies, AMPK is a component of the
184 fibronectin-induced integrin adhesome [2]. This pool of AMPK, present within
185 adhesions, could potentially impact on integrin activity and signaling by either direct
186 phosphorylation of adhesome components, to influence protein activity and/or
187 localization, or by some other indirect and tensin-independent mechanism.

Some studies have associated AMPK with the transcriptional regulation of integrins [28]. Adiponectin, a potent activator of AMPK *in vivo* [29], was found to increase α5- and β1-integrin gene expression and surface levels in prostate cancer cells [30] and α2β1-integrin expression in human chondrosarcoma cells [31] in an AMPK-dependent manner. Moreover, activation of AMPK by a nutraceutical called berberine led to reduced β1-integrin protein levels in colon cancer cells [32]. However, mechanisms of AMPK-controlled integrin expression remain to be explored.

Integrin endocytic and exocytic trafficking regulates integrin signaling and adhesion dynamics [33]. Interestingly, AMPK may also control integrin traffic. In retinal pigment epithelial cells, short-term AMPK activation triggers α5- and α11-integrin depletion from the cell surface, as measured by mass spectrometry, and reduces β1-integrin surface levels, based on a biotinylation assay [34]. Furthermore, a chemical genetic screen coupled to a peptide capture identified several proteins involved in endosomal traffic, cell adhesion and migration as direct AMPK targets [35]. SNX17, a regulator of endosomal recycling implicated in β1- and β5-integrin recycling back to the plasma membrane [36, 37], was found to be phosphorylated and degraded by AMPK [35], suggesting that in the absence of AMPK SNX17 would prevent integrin lysosomal degradation by promoting receptor recycling (Figure 2 A and B).

The functional coupling of integrin traffic and metabolism can be extended to Rab25-expressing cancer cells. Here, tensin-dependent translocation of ligand-bound α5β1-integrin to fibrillar adhesions has been proposed to prime a subpopulation of the receptor for internalization into late endosomes/lysosomes and to be required for the recruitment and activation of the nutrient sensor mTORC1 in these compartments [38]. Importantly, this process is triggered under low mTORC1 activity providing a link between energy sensing and ECM internalization in cancer [38]. Given that AMPK is a well-established inhibitor of mTORC1 [39], these findings suggest that AMPK would promote integrin endocytosis to late endosomes/lysosomes and thus inhibit integrin-dependent signaling (Figure 2A and B).

AMPK may also affect integrin-dependent processes by regulating the microtubule and actin cytoskeletons (Figure 2A). Indeed, inhibition of AMPK has been shown to reduce the phosphorylation of the microtubule plus-end-tracking protein CLIP-170

leading to impaired microtubule polymerization, formation of enlarged (paxillin-rich) focal adhesions and reduced directional migration [40]. Moreover, active AMPK directly phosphorylates the actin-associating protein Pdlim5, which localizes in actin stress fibers and focal adhesions [41]. Pdlim5 phosphorylation by AMPK inhibits lamellipodia formation and cell migration by suppressing Rac1 activity, and displacing the Arp2/3 complex, at the cell periphery [41]. Finally, AMPK-mediated phosphorylation of the actin anti-capping protein VASP suppresses vectorial actin filament assembly at focal adhesions causing increased contractility and stabilization of ventral stress fibers, whereas inhibition of AMPK promotes the formation of long dorsal stress fibers [42]. Dorsal stress fiber assembly is required for fibrillar adhesion formation [43], therefore these long stress fibers, generated in the absence of AMPK, might lead to the formation of tensin-rich fibrillar adhesions.

The role of AMPK and tensin in cancer and fibrosis

AMPK, an important energy sensor [44], has been extensively studied as a central regulator of cellular metabolism [27], but in recent years it has become apparent that AMPK directly phosphorylates non-metabolic proteins and regulates other cellular functions, such as transcription, cell polarity, mitosis, migration and adhesion [27, 35, 40, 45]. Loss or silencing of AMPK in fibroblasts increases adhesion to fibronectin [12], in agreement with previous findings showing that AMPK activation by AICAR decreases endothelial progenitor cell adhesion to fibronectin [46]. Upon matrix detachment AMPK is rapidly activated to promote cell survival during anoikis [47]. Integrin-mediated adhesion to the ECM is essential to suppress anoikis and thus maintaining low AMPK levels would be required to prevent matrix detachment and anoikis in cancer cells.

AMPK-depleted fibroblasts promote fibronectin fiber assembly, in a tensin–dependent manner [12], and in this way may resemble serum-depleted fibroblasts (an in vitro model of quiescent cells), which show enhanced secretion of specific ECM molecules [48]. The ability of AMPK to regulate matrix turnover could be important for tumor-stroma crosstalk (Figure 2B). Matrix secreted by serum-depleted fibroblasts is utilized by starved epithelial cells as a nutrient source [49]. Nutrient-deprived cells are expected to exhibit high AMPK and low mTORC1 activity promoting integrin and ECM internalization [38, 50] and degradation. These

processes are potentially also supported by AMPK-driven SNX17 phosphorylation [35]. High AMPK activity is, additionally, expected to correlate with lower tensin1 and/or tensin3 expression levels. This would be concordant with the tensin switch - downregulation of tensin3 and upregulation of the oncogenic tensin4 - often observed in cancer cells compared to normal cells [9, 51-53]. The energy and nutrient requirements of cancer cells are very high and most likely exceed those of stromal resident cells. Thus, one likely scenario within the tumor microenvironment is that while cancer-associated fibroblasts (CAFs) maintain low AMPK levels and therefore synthesize and deposit excessive amounts of ECM [54], the constantly energy craving ("starved") malignant cells display high AMPK levels and take up the deposited ECM to generate nutrients essential for their survival and unbridled proliferation (Figure 2B). In line with this hypothetical model, AMPK activation, with the widely used anti-diabetic drug metformin, was found to reduce the desmoplastic reaction (ECM deposition giving rise to fibrous tissue) and ultimately prevent tumor growth in a pancreatic ductal adenocarcinoma (PDAC) mouse model [55]. However, whether AMPK activation occurs specifically in the stromal cells, and in this way impacts on tensin levels, integrin endocytosis and matrix deposition in the tumor stroma remains to be investigated. Interestingly, high tensin1 expression has been associated with poor prognosis in PDAC [38], although it remains to be determined whether this high tensin expression is associated specifically with the stromal cells or with the cancer cells.

The increase in fibrillogenesis observed upon AMPK loss [12] may also provide mechanistic insight into the link between AMPK and tissue fibrosis, caused by abnormal ECM composition and remodeling. An increasing amount of studies demonstrate that AMPK activation, for example with metformin, inhibits TGFβ signaling, the major regulator of fibrosis, in several mouse models of fibrotic disease, including liver fibrosis [56], cardiac fibrosis [57], obesity [58], lung fibrosis [59] and skin fibrosis [60]. However, a complete molecular mechanism is still lacking. A link between AMPK and integrins in tissue fibrosis has not been suggested thus far, even though some integrins activate TGFβ and thus have a profound role in fibrotic disease [61]. A recent study demonstrated that tensin1 promotes the assembly of fibronectin and collagen matrix in TGFβ-stimulated human lung fibroblasts [62], suggesting a role for tensin1 in pulmonary fibrosis. Taken together, a potential

mechanism of action for metformin in myofibroblasts may involve negative modulation of integrin activity through AMPK-dependent reduction in tensin levels; however this hypothesis has to be explored.

Concluding Remarks

There is increasing evidence that AMPK regulates integrin-dependent processes, such as cell adhesion, migration and matrix formation via several mechanisms. A recent study has shown that loss or silencing of AMPK increases the expression of the scaffold proteins tensin1 and tensin3 in fibroblasts and elevates β1-integrin activity [12]. Tensin1 and tensin3, the major components of the centrally-located fibrillar adhesions, promote integrin activity by directly binding to the β-tail on a site overlapping with the talin-binding sequence. Thus, talin-mediated activation of integrins to generate new adhesions and tensin-induced maintenance of integrin activity to facilitate fibrillar adhesion maturation appear to be consecutive and spatially-restricted events in fibroblasts. Tyrosine phosphorylation of integrins is most likely implicated in the co-ordination of the switch between talin- and tensin-bound integrins. However, the relevant kinases (apart from Src) and especially phosphatases governing this balance remain unknown and an interesting topic for future studies (see Outstanding Questions). Even though AMPK is a serine-threonine kinase and unlikely to affect this balance directly, it could have indirect control over integrin phosphorylation via one of its multiple targets in cells. This possibility, in conjunction with the reported presence of AMPK in the integrin adhesome, advocates several scenarios for AMPK-mediated regulation of adhesions in response to metabolic alterations in health and disease. Integrins coupled to talin and kank localize around focal adhesions and in fibrillar adhesion in cells. The latter are also rich in tensin-bound active integrins. The relationship between tensin and kank, and their relative contribution to the regulation of force transduction and matrix assembly by fibroblasts will be an exciting avenue of investigation. The role of ECM as a source of nutrients for proliferating cancer cells and the emerging link between AMPK-dependent metabolic sensing and integrin-mediated matrix production in fibroblasts is intriguing and may turn out to be an important regulatory loop in cancer progression. Thus, AMPK and tensin can be potential targets for the treatment of tissue fibrosis or a means to disrupt tumor-stroma interactions in cancer.

Outstanding Questions

The effect of AMPK on tensin expression is not direct. What regulates tensin expression downstream of AMPK? Is this effect occurring in other cells, apart from fibroblasts?

How does tensin replace talin for integrin binding? Is the talin-tensin switch dependent on tyrosine phosphorylation at the β1-integrin cytoplasmic membrane proximal NPXY motif? Is AMPK also involved in regulating this tyrosine phosphorylation?

Do AMPK and tensins affect the activity of other integrins?

Is tensin-actin binding essential for β1-integrin activation and signaling?

How is the AMPK-tensin-integrin link involved in tissue fibrosis and the tumor-stroma interactions in cancer?

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Conflicts of interest

The authors declare no competing interests.

Glossary

AMP-activated protein kinase (AMPK): A serine-threonine kinase activated when intracellular ATP levels decrease. It exists as a heterotrimer, containing a catalytic subunit (α) and two regulatory subunits (β and γ).

Cancer associated fibroblasts (CAFs): activated fibroblasts associated with cancer, playing an essential role in regulating the tumor stroma.

Extracellular matrix (ECM): A large network of proteoglycans and fibrous proteins present outside and between cells, providing both a physical scaffold and the biochemical and biomechanical cues necessary for regulating cell behavior. Main fibrous ECM proteins are collagens, fibronectins and laminins, which serve as ligands for the cell-surface receptors integrins.

Fibrillar adhesions: mature, elongated multi-adhesion structures mediating the secretion and remodeling of the ECM.

Fibrillogenesis: the formation and development of thin fibrils usually consisting of collagen or fibronectin.

Fibrosis: the pathological accumulation of ECM proteins in the surrounding tissue.

Integrins: heterodimeric cell-surface adhesion receptors composed of an α- and a β – subunit.

Integrin activation: a mechanism involving a conformational shift in the integrin heterodimer through which cells increase the affinity of their cell-surface localized integrin receptors for ECM ligands.

Mechanotransduction: the processes by which cells convert mechanical stimuli into biochemical signals and ultimately cellular responses.

Metformin: an anti-diabetic drug that activates AMPK indirectly by reducing cellular energy status through mild and specific inhibition of the mitochondrial respiratory-chain complex 1 in cells.

Myofibroblasts: activated fibroblasts depositing fibrous proteins and promoting ECM remodeling.

Talin: a cytoplasmic mechanosensitive actin and integrin binding protein which activates integrins by directly binding to the integrin β-subunit cytoplasmic domain.

Figure Legends

Figure 1. Model depicting the control of adhesion formation by phosphorylation and different integrin activity regulators. A. Integrin heterodimers become inside-out activated (primed for ligand binding) upon binding of

talin to the β-integrin cytoplasmic tail and their subsequent binding to the ECM manifests full activation and signalling. Talin links activated integrins to actin, via direct binding or through vinculin, promoting focal adhesion formation and traction force. Tyrosine phosphorylation of the β-integrin tail leads to talin displacement. Dok1 binds to the phosphorylated β-tail and leads to integrin inactivation, disruption of adhesions and cell rounding. Alternatively, in the presence of high tensin levels, tensin may replace talin as the integrin-binder (especially if the integrin is phosphorylated), maintaining integrin activity, promoting adhesion maturation (fibrillar adhesion formation) and force transmission. B. Kank can also promote integrin activity and fibrillar adhesion formation and acts by binding to talin rod to activate talin and integrins. However, kank displaces actin from talin, leading to diminished traction force. PM: plasma membrane; P: phosphorylation.

Figure 2. AMPK regulates proteins involved in cell adhesion, cell migration and matrix formation. A. Apart from a profound role in cell metabolism, AMPK also regulates other cellular processes. AMPK directly phosphorylates the actin-associating proteins Pdlim5 and VASP, and the microtubule plus-end-tracking protein CLIP-170 affecting cell adhesion formation and cell migration. AMPK phosphorylates also SNX17 and the mTORC1 component Raptor, thereby regulating integrin trafficking (recycling and endocytosis, respectively). Finally, AMPK, via an unknown mechanism, inhibits tensin expression leading to enhanced cell adhesion. B. Hypothetical model depicting the potential role of AMPK in ECM turnover and tumor-stroma crosstalk: on the left is an illustration of a CAF with low AMPK levels and on the right a cancer cell with high AMPK (the high energy demand of cancer cells for biosynthetic processes exhausts ATP levels and activates AMPK). Under low AMPK activity/levels in CAFs, SNX17 and mTORC1 are active and tensin expression is upregulated. Active SNX17 binds integrins in the early endosomes promoting their recycling back to the plasma membrane, thereby inhibiting integrin lysosomal degradation. High levels of tensin promote fibrillar adhesion formation and ECM secretion, and mTORC1 inhibits matrix internalization. All these will lead to excessive matrix deposition by the CAF. On the right, high AMPK levels in an energy craving cancer cell leads to the phosphorylation and inhibition of mTORC1 (mTORC1-P) and phosphorylation of SNX17 (potentially downregulating SNX17 function and thus integrin recycling), and suppression of tensin expression. This cell

will internalize the ECM secreted by the CAF and will degrade integrins and ECM in the lysosomes to generate nutrients and support cell survival. Thick black arrows highlight activated pathways and thin grey arrows indicate downregulated processes.

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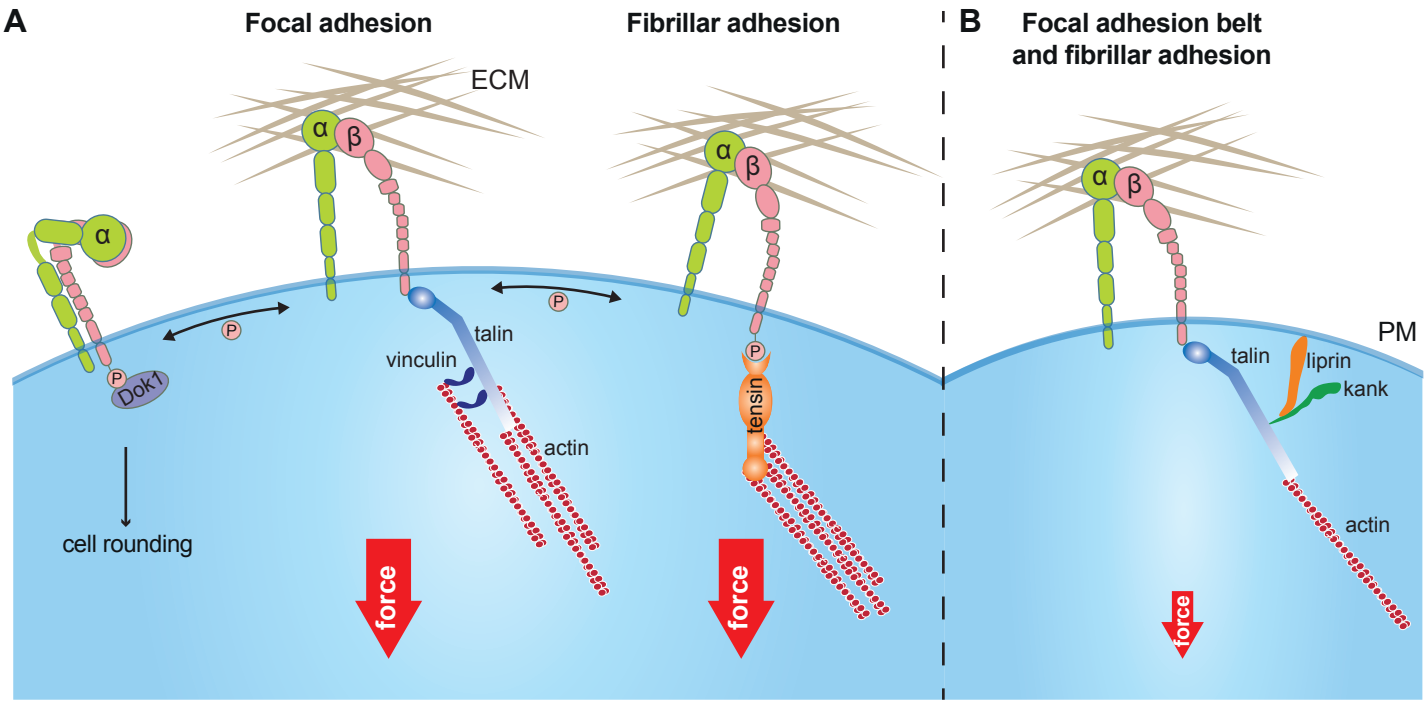
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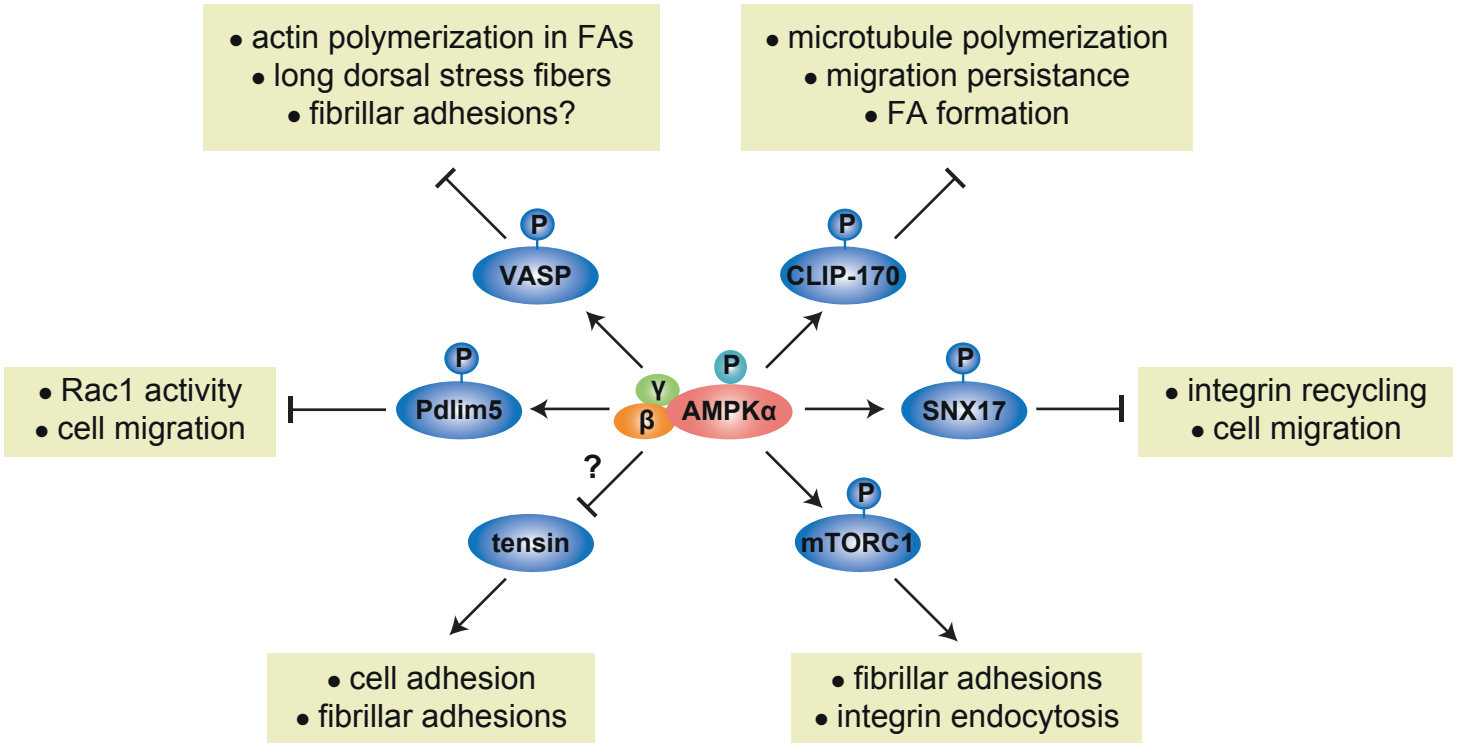
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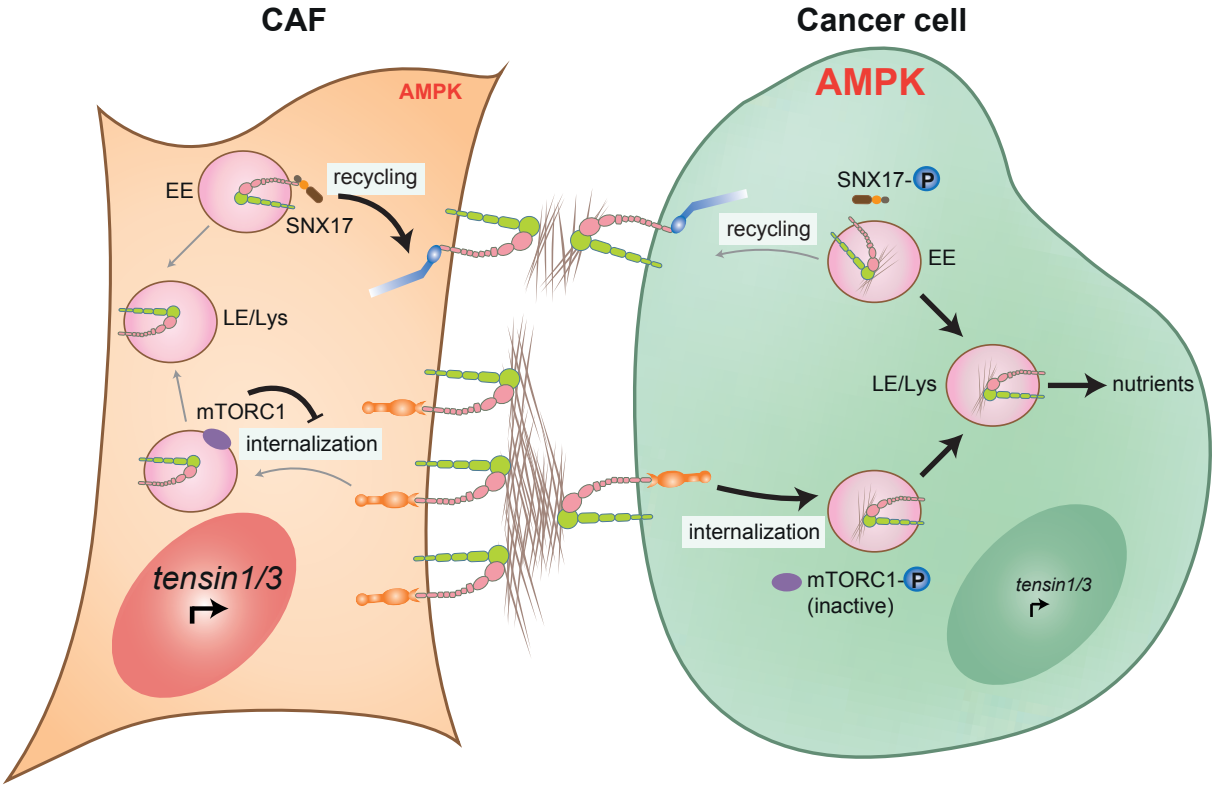
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