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Focal Adhesion Kinase as a Regulator of Cell Tension in the Progression of Cancer

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Abstract

Growing evidence indicates that critical steps in cancer progression such as cell adhesion, migration, and cell cycle progression are regulated by the composition and organization of the microenvironment. The adhesion of cancer cells to components of the microenvironment and the forces transmitted to the cells via the actinomyosin network and the signaling complexes organized within focal adhesions allow cancer cells to sense the local topography of the extracellular matrix and respond efficiently to proximal growth and migration promoting cues. Focal adhesion kinase (FAK) is a nonreceptor tyrosine kinase that is over expressed in a variety of cancers and plays an important role in cell adhesion, migration, and anchorage-dependent growth. In this review, we summarize evidence which implicate FAK in the ability of cells to sense and respond to local forces from the microenvironment through the regulation of adhesion dynamics and actinomyosin contractility, and we discuss the potential roles of FAK as a mechanosensor in the progression of cancer.

Keywords

Focal adhesion kinase; tension; mechanotransduction; cell migration

1. Introduction

The loss of cell growth regulation leads to a diverse array of pathological states, including defects in embryonic development and aberrant wound healing, and is the key event in the initiation and progression of the cascade of events leading to cancer. Cell growth has long been known to be regulated by signaling pathways triggered by extracellular cues, (e.g., growth factors, hormones) and terminating in changes in gene expression. However, there is increasing evidence that the physical architecture of the cell (i.e., the cell's shape), which is governed by cellular mechanical forces and the cell's interaction with the extracellular matrix (ECM) and other cells, also plays a critical role in regulating cell proliferation. For example, early studies showed a correlation between cell shape and proliferation [1]. Normal adherent cells fail to divide when they are confined to a spherical shape, either by placing them in suspension, or by allowing them to adhere but not to spread. However, when placed on a rigid substrate on which they can exert strong forces, cells will flatten and proliferate. Many cancer cells appear to overcome this geometrical barrier and are able to proliferate even when placed in growth conditions that limit ECM and cell-cell interactions (so-called anchorage independent growth),

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suggesting that the cells have established a compensatory mechanism(s) [2]. However, *in vivo*, many tumors will modify their microenvironments to create a more rigid scaffold to stimulate proliferation [3,4]. These studies point to a key role for the forces generated by interactions of cells with the ECM and other cells, as well as more conventional growth promoting signals in cancer initiation and progression.

In spite of the studies that implicate the role of structural forces in cell proliferation and tumor progression, the signaling pathways that are involved in detecting and responding to forces (i.e., mechanotransduction) are still unclear. Although there are several general ways in which cells can detect changes in force, such as the opening of ion channels and the transmitting of forces through cell-cell contacts [5], focal adhesions have received considerable attention because they serve as a nexus between the contractile actin cytoskeleton and the ECM. In particular, the protein tyrosine kinase, focal adhesion kinase (FAK), has been shown to be an important mediator in mechanotransduction pathways by both responding to substrate rigidity on the outside of the cell and regulating tension via the actin cytoskeleton within the cell. In many cancers there is a correlation between increased FAK expression and progression to a more malignant state, consistent with the importance of signaling via mechanotransduction pathways and the progression of cancer. The purpose of this review is to summarize the observations that implicate FAK in the regulation of mechanotransduction pathways through the organization and regulation of the cytoskeleton and cell adhesion dynamics, and to establish a role for FAK in the generation and maintenance of cellular tension during the growth and invasion of tumor cells.

2. Adhesion and the development of tension

The generation of tension occurs in any physical object on which there is a pulling force at one end and a resisting or opposite-pulling force at the other end. Likewise, the cellular tension that determines the cell's shape is derived from two components, forces generated by the contraction of the cytoskeleton within the cell (the network of actin and myosin), and an "anchor" that resists the contraction (adhesions to the ECM or another cell) (Figure 1).

The actin cytoskeleton is the contractile network of the cell. Actin filaments are crosslinked by myosin II complexes which exert forces on adjacent filaments, resulting in the bundling and contraction of stress fibers. The activation of myosin downstream of signaling pathways is mediated mainly by the phosphorylation of the myosin light chain subunit by myosin light chain kinase, a calmodulin-dependent serine/threonine kinase. Myosin light chain phosphorylation augments the affinity of myosin for actin filaments, leading to increased contractility [6-9]. In addition, activation of the Rho-GTPase pathway leads to the activation of Rho-activated kinase (ROCK), which phosphorylates and inhibits myosin phosphatase, resulting in sustained light chain phosphorylation [10]. Because of the high correlation between myosin light chain phosphorylation and contractility, myosin light chain phosphorylation is commonly used as a biochemical marker for the state of tension in the cell.

Focal adhesions (FAs) bridge the cytoskeleton on the inside of the cell with components of the ECM on the outside of the cells [11-13]. FAs are comprised of integrins, heterodimeric transmembrane receptors that bind directly to the ECM on the outside of the cell and link to the actin cytoskeleton through a scaffold of several actin-binding proteins. In addition, FAs also contain signaling proteins that regulate cell growth and cytoskeletal dynamics, including changes in actinomyosin contractility. The spatial organization of FAs at the junction of the cytoskeleton and the ECM underscores their role in anchoring the contractile actinomyosin network of the cell with the ECM.

FAs play an important role in sensing the forces generated by the cell via its cytoskeletal network. These forces are relayed through the FAs to the microenvironment, leading to

aggregation/remodeling of ECM fibers and the establishment of a denser, highly cross-linked matrix [14]. In turn, cells are more likely to exert stronger forces on a more rigid ECM; thus, a state of equilibrium exists between the actinomyosin and the ECM networks, with the status of the ECM being an extension of that of the cytoskeleton, and vice-versa (Figure 1).

The organization and rigidity of the ECM appears to play an important role in the formation and maintenance of FAs. For example, cells plated on rigid substrates have more robust adhesions than those on more flexible substrates [15-17], presumably because rigid substrates are more capable of resisting the contractile forces from the cell. Likewise, cells treated with agents that increase actinomyosin contractility such as Lysophosphatidic acid (LPA), thrombin, or bombesin also lead to a further increase in FA size in cells plated on rigid substrates [18,19]. This is mediated by activation of the Rho pathway, which stimulates actinomyosin contractility, leading to FA growth [20]. In addition, the growth of FAs in response to Rho activation appears to be due to an increase of force on the adhesion, because FAs will also grow when physical force is applied to the cell by stretching it [21,22]. Likewise, if force on an adhesion is decreased, the adhesion will disassemble [23,24]. This basic mechanism of reinforcement in response to an increase in force, and release in response to a decrease in force, is a fundamental property of FAs that plays an important role in several physiological processes of the cell such as migration and proliferation.

3. The role of tension in cell migration and proliferation

Cell migration consists of several temporarily and spatially coordinated events, including protrusion of the leading edge, adhesion of the leading edge to the substrate, translocation of cell body, and release of the trailing edge [25]. It is not surprising that cell migration requires the tight regulation of tension at specific times and in specific areas of the cell [26]. Measurements of traction forces (i.e, the measurable external forces exerted by the cell on the substrate) in migrating cells show that the highest amount of forces exerted by the cell are at its front, immediately behind the leading edge [27]. This is supported by the findings that the highest traction forces are associated with newly forming adhesions at the periphery of the cell [28], and increased membrane protrusion occurs at areas of higher tension [29]. Thus, a concentration of tension at the front contributes to the establishment of cell polarity, where the accumulation of forces would not only serve to pull the cell forward, but would also stimulate additional protrusions at the leading edge. This coordination of force generation and protrusion leads to persistent, unidirectional movement, as long as traction forces are maintained at the front of the cell.

Tension also plays an important role in cell proliferation. When cultured in sheets of various shapes, endothelial cells show the highest degree of proliferation in the areas that were under the highest amount of tensional stress [30]. Likewise, fibroblasts cultured in attached (mechanically stressed) matrices undergo proliferation, while cells in floating (relaxed) matrices show low levels of DNA synthesis and were less responsive to growth factors [31]. Culturing fibroblasts in stressed matrices and then releasing the stress on the matrix inhibits DNA synthesis. Similarly, while epithelial cells in stressed collagen gels proliferate in response to growth factors, cells in floating collagen gels stop proliferating and form tube-like acini [32]. Restoration of tension in acini leads to cell proliferation and a collapse of these structures into a more disorganized state [4]. These observations have led to the proposition of a “threshold model” whereby cells will not be able to progress through the cell cycle unless there is sufficient tension to do so [33].

4. The involvement of FAK in contractility

FAK is a ubiquitously expressed nonreceptor tyrosine protein kinase that is over expressed in many tumors [34-36]. FAK is recruited to FAs and activated upon cellular adhesion to the

ECM [37,38]. The molecular structure of FAK suggests that it acts as a scaffold in addition to its role as a kinase. The central tyrosine kinase domain is flanked on the N-terminus by a FERM domain, which may serve as a site of interaction with ezrin and membrane receptors [35], and on its C-terminus by a proline-rich region and the focal adhesion targeting (FAT) domain (Figure 2). The FAT domain is necessary for the localization of FAK to FAs, and it contains sequences which bind to paxillin, another FA protein, although it appears that these sequences are not necessary for FAK localization to FAs [39]. The proline-rich region provides binding sites for several SH3-domain containing proteins including p130Cas, GRAF and ASAP1 [40-42].

FAK is maintained in an inactive and autoinhibited state by the binding of the FERM domain to the kinase domain [43]. Engagement of integrins with the ECM leads to the activation of FAK and phosphorylation at tyrosine 397 (Y397), apparently by trans-phosphorylation of adjacent FAK molecules [44]. Phosphorylated Y397 provides a binding site for c-Src, and the binding of Src to FAK sustains Src and FAK in their activated states, thereby creating a functional bipartite kinase complex [45,46]. Activated c-Src phosphorylates other sites on FAK and other FA proteins such as paxillin and p130Cas [47,48]. The phosphorylation of these proteins has been implicated in the activation of the small GTPase Rac and other signaling pathways [49-51].

While the molecular biology of FAK has been well characterized, its physiological role is still unclear. FAK knockout mice exhibited an embryonic lethal phenotype with severe defects in mesoderm development [52]. Surprisingly, the fibroblasts derived from these mice form robust adhesions in the absence of FAK, suggesting that while FAK is dispensable for FA assembly, it is required for FA disassembly ("turnover"). Further studies demonstrated that FAK $-/-$ cells migrated more slowly and appeared more contractile than wild-type cells, with both of these phenotypes attributed to a lack of adhesion disassembly. FAK $-/-$ cells were found to be unable to downregulate Rho activity upon adhesion, and treatment of FAK $-/-$ cells with a Rho kinase inhibitor enabled the cell to spread similar to a wild-type cell [53,54].

These initial studies hinted at the role of FAK in regulating cellular tension, but perhaps the most intriguing studies dealing with FAK relate to its potential as a mechanotransducer. When migrating on flexible substrates, normal fibroblasts preferentially migrate towards a stiffer substrate, while FAK $-/-$ cells show no such preference [55]. This suggests that FAK plays an important role in sensing the rigidity of the substrate. The mechanism behind this phenomenon is unclear. One possibility is that FAK participates in the strengthening of adhesions with regard to the state of the substrate; when the leading edge of the cell comes into contact with a more rigid substrate, the adhesions will respond by FAK-dependent strengthening with subsequent pulling of the cell in the direction of the higher rigidity. This is supported by the observation that wild-type cells increase their traction forces as the substrate rigidity increases, while FAK $-/-$ cells maintain lower traction forces [55]. In addition, there have been numerous studies which correlate FAK activity with the strengthening of adhesions. Both FAK and paxillin phosphorylation are required for the contraction of smooth muscle cells [56,57]. FAK is also activated in endothelial cells under conditions in which the vessels are stressed by distension, such as reperfusion following ischemia [58]. In addition, FAK becomes phosphorylated on Y397 under conditions where adhesions are growing in response to an increase in force, such as treatment of cells with LPA or bombesin [59,60]. The mechanism behind FAK activation in response to an increase in tension is unclear. It may be due to an increase in FAK recruitment or clustering in FAs, or FAK itself may become unfolded and subsequently activated due to physical stretching of the molecule, as was demonstrated to be the case for p130Cas [61]. Nevertheless, this correlation between FAK activity and adhesion growth, coupled with the observation that focal adhesion strength increases in response to an increase in force, suggests that FAK may be involved in a positive feedback loop involving focal adhesion growth, FAK

activation, and the development of tension (Figure 3). In support of this model, mice in which FAK was specifically knocked out of Purkinje cells showed an abnormally high number of branched axons and synapses in the cerebellum, and this was attributed to an inability of neurons to retract due to a defect in the activation of the Rho pathway [62].

Because much evidence points to a role of FAK in adhesion strengthening, its mechanistic role in adhesion turnover remains unclear. In FAK $-/-$ cells, adhesions assemble at rates similar to those in wild-type cells; however, the rate of adhesion disassembly is markedly decreased [63]. FAK $-/-$ fibroblasts, and keratinocytes derived from mice with an epidermal-specific knockout of FAK, appear more contractile with large adhesions and an abundance of stress fibers [52,64]. However, FAK knockdowns in fibroblasts, epithelial, or endothelial cells, and fibroblasts or neurons derived from mice with a conditional knockout of FAK, exhibit an elongated or spindle-shaped morphology consistent with the inability of the cells to contract [65-67,62]. These new methods for inhibiting FAK expression along with recently-developed small molecules that inhibit FAK kinase activity [68,69], combined with methods to monitor the ability of the cells to contract [70,71], will shed new light on the role of FAK in regulating cell contraction and adhesion turnover.

5. Signaling pathways downstream of FAK involved in contractility

Rho-family GTPase pathways are important targets for regulation by FAK and their activation is directly linked to regulation of cytoskeletal contractility. Activation of G-coupled protein receptors (GPCR), such as the LPA and thrombin receptors, trigger guanine nucleotide exchange factors (GEFs) which in turn will activate members of the Rho family, including RhoA. RhoA stimulates actomyosin contractility by sustaining myosin light chain phosphorylation through activation of ROCK and by stimulating actin polymerization by the activation of the formin, mDia, resulting in an accumulation of contractile stress fibers in the cell [72]. RhoA can be activated by multiple GEFs, several of which have been shown to be downstream of FAK signaling. In neurons, p190RhoGEF has been shown to interact with the FAT domain of FAK, resulting in p190RhoGEF phosphorylation and activation of RhoA following adhesion to laminin [73]. Expression of an endogenous inhibitor of FAK, FRNK, inhibited RhoA activation. Expression of a myristylated form of FAK (myr-FAK), which sequesters FAK to the plasma membrane and renders it constitutively active [74], induces RhoA activation in human embryonic kidney 293T cells [75]. This appears to be mediated by PDZ-RhoGEF, which is phosphorylated in cells expressing myr-FAK, and this is inhibited by expression of FRNK, although FAK does not appear to be necessary for thrombin-induced RhoA activation. These observations suggest that FAK can augment GPCR-dependent RhoA activity by stimulating RhoGEF activation at sites of adhesion.

FAK has also been shown to be important for the activation of another member of the Rho GTPase family, Rac1, which is involved in lamellipodium formation via the assembly of the cortical actin meshwork [76]. Phosphorylation of p130Cas by the FAK/Src complex provides a binding site for the SH2 domain of CrkII, which in turn serves as a docking site for DOCK180/ELMO, a GEF for Rac1 [77]. Another connection from FAK to Rac is through the activation of PI 3-kinase by the binding of the p85 subunit of PI 3-K to phosphorylated Y397 of FAK [78,79]. The generation of phosphoinositides by PI 3-K provides binding sites for Rac GEFs such as P-REX1 or Sos-1 [80]. The FAK-dependent activation of the Rac pathway, while it may not stimulate contractility in itself, may provide signals promoting protrusiveness in areas of the cell that are under tension, in agreement with previous findings that lamellipodia formation occurs at areas of high tension [29]. This coupling of tension with protrusiveness is critical for the maintenance of polarity during cell migration.

The complexity of FAK signaling is further reflected by its interaction with Rho GAPs, which stimulate the intrinsic GTPase activity in Rho GTPases and keep them in an inactive state. The Rho GAP, GRAF (GTPase regulator associated with FAK), localizes to stress fibers and FAs and binds to the proline-rich domain at the C-terminus of FAK [41,81], thereby directly linking FAK to a mechanism for the downregulation of the Rho pathway. Another Rho GAP, p190RhoGAP, is phosphorylated (and activated) by Src at focal adhesions [82], and, in endothelial cells, FAK associates with and phosphorylates p190RhoGAP upon treatment with thrombin [83,84]. p190RhoGAP failed to localize to focal adhesions in skin keratinocytes in which FAK expression was conditionally ablated, resulting in elevated Rho activity and enhanced stress fibers [64].

Yet another potential tension-regulator and substrate of FAK in FAs is the actin crosslinker α -actinin, which is found exclusively in large Rho-dependent FAs. α -actinin was found to be a direct substrate of FAK in cells that overexpress both proteins [85]. Upon α -actinin phosphorylation, its affinity for actin decreases, along with its ability to crosslink and stabilize actin filaments at FAs [85]. This destabilization of actin bundles at sites of adhesion could effectively reduce the strength of the adhesion itself, ultimately leading to adhesion release.

Taken together, the observations of activation of multiple signaling pathways downstream of FAK do little to clarify the mechanisms by which FAK regulates cell tension. One possible explanation is that the generation of signaling intermediates downstream of FAK activation may depend upon the spatial setting of FAK activation. For example, in areas under high tension where Rho effectors are abundant, FAK would participate in the Rho pathway and stimulate FA growth, while in areas of lower tension (such as an adhesion undergoing disassembly), FAK would serve to inhibit the Rho pathway and promote FA release and protrusiveness.

6. FAK, tension, and tumor progression

There is strong evidence that FAK expression is important for tumor progression. FAK is over expressed in a variety of tumors, and its expression correlates with increased tumor malignancy [86]. Tumor progression requires several key events that are regulated by cell tension signaling pathways, cell proliferation in the primary tumor, cell migration from the primary tumor through the surrounding tissue, tumor cell survival in anchorage-free conditions, and the establishment of metastases at distant sites by adhesion of the disseminated tumor cells in foreign microenvironments. A role for FAK in several of these steps of cancer progression may prove critical (Figure 4). FAK is important for tumor cell induced contraction/remodeling of the tumor matrix, thereby producing a rigid microenvironment and encouraging cell proliferation. FAK may also be involved in the invasion of the tissue surrounding the tumor by mediating cell migration. Once in the bloodstream or lymphatics, the tumor cell is in anchorage-free conditions, and increased FAK expression may be important for cell survival from anoikis by providing an adhesion signal in the absence of such tension. Similarly, increased FAK expression may support the formation of metastases at sites distant to the original tumor by providing an anchorage signal under circumstances where the cells would not normally survive and thereby enabling the cells to be less selective for their substrates.

7. FAK as a therapeutic target in cancer

A possible role for FAK in the progression of cancer has provided the incentive to develop small molecule inhibitors for use as potential cancer therapies. Two groups have recently generated compounds (PF-573,228; PF-562,271; and NVP-TAE226) that are ATP analogs and effectively inhibit the kinase activity of FAK [69,87]. Treatment of cells with these inhibitors results in a decrease in phosphorylation on Y397 along with inhibition of cell migration. Additionally, PF-573,228 inhibited the FAK-dependent phosphorylation of paxillin and reduced the rate of adhesion turnover in migrating fibroblasts. NVP-TAE226 inhibited the

adhesion and invasion of glioma cells and inhibited the phosphorylation of the downstream targets Akt and ERK. Interestingly, there were differing effects on anchorage-dependent cell proliferation between these two compounds - namely, NVP-TAE226 inhibits the proliferation of glioma cells [68,87] while PF-573,228 does not affect the proliferation of fibroblasts or prostate cancer cells in short-term growth assays at concentrations which inhibit FAK activity [69]. One explanation for this discrepancy could be differences in specificity between the two compounds, as NVP-TAE226 has been shown to inhibit FAK and the insulin-like growth factor receptor (IGF-IR) at similar concentrations [68], and while PF-573,228 demonstrated significant selectivity against a panel of kinases [69], the effects of NVP-TAE226 on other kinases besides FAK and IGF-IR have not been reported. Preclinical studies on another compound, PF-562,271, have demonstrated tumor stasis or regression in mouse models of prostate, breast, colon, lung, glioblastoma, and pancreatic cancers, and explants from the tumors show a marked increase in apoptotic cells [88]. PF-562,271 has since moved on to Phase I clinical trials, where it has thus far shown minimal toxicity along with some tumor regression [89]. Thus, FAK inhibitors show significant promise for cancer therapy.

8. Summary and prospective

It is becoming more apparent that forces play an important role in the progression of cancer. In vitro studies have demonstrated the importance of tension in cell proliferation and migration. Focal adhesions, which act as anchors for the actin cytoskeleton, are regulated by, and participate in, the establishment of cellular tension. As a tyrosine protein kinase in focal adhesions, FAK serves as a nexus between the ECM/integrins and the actin cytoskeleton. In addition, FAK acts as a mechanosensor, in that it is most active when force is exerted on the adhesions (either by the actin cytoskeleton or external pulling), and there is increasing evidence that FAK regulates tension at sites of adhesion. Exactly how FAK regulates adhesion (modulation of contraction or adhesion strength) is not clear; however, new ways to inhibit FAK will shed light on this subject. Because of the importance of forces and tension in multiple steps of carcinogenesis, FAK has potential to be a target for cancer therapy.

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Figure 1.

Force and the development of tension. A. The exertion of force on an anchored object such as a rope tied at one end results in the development of tension across its length. B. Similarly, the exertion of a force (myosin-mediated contractility) on the cytoskeleton which is anchored by a focal adhesion results in the development of tension across the length of the cytoskeleton. A more rigid substrate provides more resistance which results in the generation of larger contractile forces and more robust adhesions, leading to a higher level of tension across the cytoskeleton.

Figure 2.

The structure of FAK. The binding partners and substrates involved in the regulation of cell tension are shown. Autophosphorylation at Y397 creates a binding site for c-Src and the p85 regulatory subunit for PI 3-K. The Src substrates p130Cas and paxillin bind to the proline-rich and FAT domain of FAK, respectively. Growth factor receptors, integrins, and ezrin interact with the FERM domain of FAK, linking it to the cell membrane. The phosphorylation of α -actinin would inhibit its actin crosslinking activity and destabilize the adhesion. Association with GRAF with the proline-rich (PR) domain of FAK or the phosphorylation of p190RhoGAP would result in an inhibition of Rho, while association with p190RhoGEF or phosphorylation of PDZ-RhoGEF would stimulate the Rho pathway.

Figure 3.

The role of FAK in the establishment of cell tension. FAK is recruited to developing adhesions, resulting in autophosphorylation and activation of c-Src. Downstream signals of FAK and/or Src include mediators of the Rho pathway which would stimulate cytoskeletal contraction, leading to adhesion growth, the development of tension, and further activation of FAK. In addition, exogenous force (such as stretching or resistance by a rigid substrate) would result in increased tension and additional FAK activation. This positive feedback loop between FAK and the contractile cytoskeletal network would serve to maintain tension at critical sites of the cell.

Figure 4.

The role of FAK over expression in cancer progression. FAK could play a role in: 1) the generation of tension within the primary tumor to reorganize the extracellular matrix and stimulate proliferation, 2) the regulation of tension at specific areas of the cell to stimulate adhesion dynamics and cell migration, 3) the generation of a tension signal in anchorage-free conditions, and 4) the provision of a required tension signal in microenvironments at distant metastases where the cells would not normally grow.