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Focal adhesion kinase: switching between GAPs and GEFs in the regulation of cell motility

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Abstract

Summary—Focal adhesion (FA) kinase (FAK) is a cytoplasmic protein-tyrosine kinase involved in cytoskeleton remodeling, formation and disassembly of cell adhesion structures, and in the regulation of Rho-family GTPases. Therefore, FAK is widely accepted as an important promoter of directional cell movement. Recent studies have elucidated new molecular connections of FAK in these processes. Specifically, FAK facilitates the localized and cyclic activation of guanine nucleotide exchange factors (GEFs) and GTPases-activating proteins (GAPs). In general, GEFs activate, while GAPs inactivate RhoGTPases. Therefore, FAK is in a unique signaling position to modulate RhoGTPase activity in space and time, thereby affecting various steps (integrin activation, leading edge formation, FA turnover, and trailing edge retraction) needed for efficient directional cell migration.

Keywords

FAK; GEF; GAP; focal adhesion; cell motility

Introduction

Directional cell migration is important in physiological and pathological processes such as embryonic development, angiogenesis, wound repair, tumor invasion and metastasis. Dynamic polarization of cells in response to growth factors and extracellular matrix (ECM) interactions, formation of cell protrusions and focal adhesions (FAs) at the leading edge, reorientation of the golgi and microtubule organizing center, and coordination of FA disassembly at trailing cell regions are all required for directional cell motility (reviewed in [1]). RhoGTPases, including Cdc42, Rac1 and RhoA are critical effectors of cell migration and function as molecular switches cycling between an active GTP-bound state and inactive GDP-bound state (reviewed in [2]). RhoGTPases are held in balance by the opposing action of guanine nucleotide exchange factors (GEFs), that activate and GTPases activating proteins (GAPs), that inactivate GTPases (reviewed in [3]). How cell surface receptors connect to RhoGTPase is a focus of much research effort.

Focal adhesion kinase (FAK) is an intracellular protein-tyrosine kinase (PTK) recruited to and activated at FA sites. FAK is a key signaling PTK that acts downstream of various growth

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factors and ECM components. Activated FAK recruits c-Src (referred to as Src herein) at FA sites to form a FAK-Src signaling complex. This signaling complex phosphorylates other FA signaling proteins such as paxillin and p130Cas, thereby activating diverse signaling pathways important in regulation of cell migration (previously reviewed in [4,5]). In this review, we will discuss recent advances in the role of FAK in directional cell migration that is achieved in part by regulating: (i) FA dynamics at the leading edge; (ii) both GEFs and GAPs thereby facilitating the cyclic activation of RhoGTPases; and (iii) FA disassembly at the trailing edge.

FAK: A Leading Edge Organizer

Polarized motility is governed by organization of a leading edge in the direction of cell movement. The leading edge is stabilized by the formation of new FAs or cell-ECM contact sites [1]. Although soluble chemokines are believed to initiate and drive the directional motility response, ECM-generated signals and the location of nascent FAs can also influence the positioning of leading edge [6].

Integrin clustering results in FAK activation at nascent FA sites [7]. However, a recent study demonstrated that FAK can also activate integrins, resulting in increased integrin-ECM interaction and adhesion strengthening [8]. Therefore, during the initial steps of cell spreading, there is a cycle where integrin activation (outside-in signaling) causes FAK activation and FAK can further enhance the pool of activated integrins (inside-out signaling). Interestingly, adhesion strengthening involves force-dependent conformational transition of integrins. The interaction of $\alpha 5 \beta 1$ integrins to fibronectin (FN), an important ECM component occurs in two stages. First, under low cell contractility (less tension) $\alpha 5 \beta 1$ binds to the RGD (arginineglycine-aspartate) motif of FN and second, under high cell contractility (high tension), $\alpha 5 \beta 1$ integrins interact with a synergy site on FN, causing increased adhesion strengthening which was found to be important for full FAK activation [9]. Thus, there is a complex interplay and potentially sequential series of events initiated by integrins leading to FAK activation and resulting in leading edge cytoskeletal organization.

These findings are summarized in a simplistic model whereby FAK facilitates the formation of a stable leading cell edge (Fig. 1A). The cytoskeletal protein talin binds to FAK and integrin cytoplasmic tails both initiating and also enhancing integrin activation [10]. This integrin-talin complex promotes localized increase in cell tension resulting in unfolding of talin rod domain, binding of vinculin and actin filaments to talin, and promoting the assembly of nascent FAs [10]. Paxillin is also an important cytoskeletal and scaffolding protein recruited early to FAs (reviewed in [11]). FAK is recruited to nascent FAs by its FAT (focal adhesion targeting) domain that binds to both talin and paxillin. Structural and mutagenesis studies have shown that FAK exists in an auto-inhibited conformation, where the N-terminal FERM (protein 4.1, ezrin, radixin and moesin homology) domain of FAK interacts with the FAK kinase domain [12]. In this auto-inhibited conformation, it is possible that the FAK FERM domain may also bind to the actin nucleating protein Arp3 and promote the recruitment of the Arp2/3 complex to nascent adhesions in a kinase-independent manner [13] (Fig.1A). Thus, specific FAK FAT- and FAK FERM-mediated protein interactions can link integrins with the actin polymerizing cell machinery; thereby facilitating leading edge protrusion.

Alternative mechanisms regulating FAK activation have also been elucidated recently. In addition to C-terminal domain mediated clustering of FAK within integrins, the FAK FERM auto-inhibited conformation can be disrupted by FERM domain binding to phospholipids such as PIP_2 (phosphoinositol 4,5 biphosphate) through a basic amino acid enriched patch (KAKTLR) within the FERM F2 lobe [14]. Additionally, tension induced conformation changes in FAK, similar to what has been reported for FA proteins like, p130Cas [15], talin [10] and integrins [9], can also disrupt FAK auto-inhibited conformation, resulting in FAK

activation with rapid phosphorylation of FAK at tyrosine (Y) 397. Interestingly, phosphorylation at Y397 destabilizes the FAK-Arp2/3 complex, resulting in the release of Arp2/3 from sites of FAs, and allowing for Arp2/3 recruitment to the extended lamellipodia [13]. Y397 FAK phosphorylation promotes FAK-Src complex formation, resulting in complete FAK activation (Fig. 1B).

Importantly, by insertion of fluorescent proteins flanking the FAK FERM and FAK kinase domains, fluorescence resonance energy transfer (FRET)-based FAK probes have been created to visualize conformational changes of FAK in living cells. Independent studies by two groups using similar FRET-based FAK biosensors confirmed that the FERM and kinase domains of FAK undergo conformational changes following cellular adhesion to ECM [14,16]. Interestingly, one of the two biosensors created was inverted and showed a decrease in FRET signal upon FAK activation [14], thus supporting the model of FAK FERM domain separation from the kinase region upon FAK activation [12]. However, the other FAK biosensor exhibited increased FRET signal upon cell spreading on ECM [16]. Notably, introduction of a kinase-inactivating point mutation within the positive FAK biosensor did not alter the increased FRET signal detected upon cell binding to ECM [16]. Thus, it was concluded that these biosensors measure conformational changes in FAK or the FAK FERM regions and that this is independent of FAK catalytic domain activation.

The FAK FERM domain is also a point of contact with transmembrane growth factor receptors such as hepatocyte growth factor and epidermal growth factor (EGF) receptors [17]. Growth factor stimulated trans-phosphorylation of FAK at Y397 promotes the formation of a FAK-Src complex where direct FAK-mediated phosphorylation of Src at Y416 facilitates Src activation [18]. It remains unclear whether EGF may directly promote FAK activation, as EGF also promotes protein tyrosine phosphatase D1 binding to and activation of Src leading to FAK-Src interactions at FAs [19]. One of the major downstream phosphorylation targets of the FAK-Src complex is the scaffolding protein, p130Cas [20]. Binding of adaptor proteins to p130Cas can facilitate the activation of small GTPases such as Rap1 and Rac [21]. Regulation of Rac activity by FAK constitutes an important step in leading edge organization and FAK serves as a key regulator of phosphorylated paxillin and p130Cas localization to FAs [22]. However, FAK does not just promote Rac activation as primary bone marrow macrophages isolated from conditionally-deleted FAK mice exhibited altered leading edge cell projections and surprisingly high de-regulated Rac activity [23]. Altered leading edge formation was also observed in fibroblasts with siRNA knockdown of FAK or fibroblasts isolated after conditional deletion of FAK [24]. The notion that FAK could function as both an activator (through the p130Cas pathway) and inhibitor of Rac (unknown pathway elucidated from FAK knockout cells) likely stems from the fact that FAK can interact with both GEFs and GAPs at FAs that will be discussed below.

FAK: A Key Regulator of Localized GAP and GEF Activity

Rho-family GTPases are critical molecular switches that regulate directional cell movement [3]. Until recently, cell migration models widely assumed that Rac promotes membrane protrusion at the leading edge and Rho regulates contractility in the cell body. However, recent studies with FRET-based probes for Rho-family GTPases revealed high levels of RhoA activity at both the leading and trailing edges of cells [25] whereas high Rac activity was observed only at leading edge projections [26]. As increased Rac activity can be antagonistic to Rho activation [27], we postulate that the occurrence of high Rac and Rho activity at leading edge is likely cyclical. At the leading edge, Rac activation can provide the necessary “push” (decrease in cell contractility) needed for lamellipodial growth and Rho activation then facilitates the “pull” (increase in cell contractility) to stabilize growing lamellipodia.

It is the coordination of GEFs and GAPs that is critical for cyclic RhoGTPase regulation. Recent findings show that FAK may facilitate cycles of RhoA inactivation followed by RhoA activation through the selective association with p190ARhoGAP [28] and p190RhoGEF (termed Rgnef) [29], respectively during cell spreading on fibronectin (FN); conditions that mimic a growing lamellipodia (Fig. 1B and 2). We do not believe that these associations with FAK occur simultaneously. Instead, evidence supports temporal constraints with FAK-p190RhoGAP interactions occurring prior to FAK-Rgnef associations during cell spreading on FN. Notably, FAK expression and activity were required for leading edge FA localization and tyrosine phosphorylation of p190RhoGAP, a GAP specific for RhoA [28]. This FAK-p190RhoGAP interaction was not direct and was dependent upon the binding of p120RasGAP to both FAK and p190RhoGAP. Previous studies showed that p190RhoGAP activity was associated with increased p190RhoGAP tyrosine phosphorylation and was important in mediating RhoA inhibition at early stages (15-45 min) of FN-stimulated cell spreading [30]. We find that the FAK-p190RhoGAP interaction was important in promoting cell polarity [28] and we hypothesize that this is related to the transient decrease in RhoA activity observed early upon cell binding to FN [31].

At later stages of FN-stimulated cell spreading (+60 min), FAK forms a complex with Rgnef and paxillin that was also localized to FAs [29]. Knockdown of Rgnef decreased RhoA activation and FA formation in fibroblasts, but did not inhibit cell spreading. As Rgnef localization to FAs was dependent upon FAK binding and mutation of the FAK binding site on Rgnef disrupted tyrosine phosphorylation of Rgnef [29], FAK activity is thus linked to early RhoA inactivation (FAK-p190RhoGAP) followed by RhoA activation (FAK-Rgnef) during the time course of FN-stimulated cell spreading. Collectively, a simplistic model can be proposed from these findings. FAK can generate “push” at earlier stages of cell spreading by activating p190RhoGAP and inhibiting RhoA, and subsequent “pull”, by activating Rgnef and RhoA at later stages of cell spreading (Fig. 2). Additionally, the antagonism between RhoA and Rac activity can also result in indirect Rac regulation via cyclic regulation of Rho by FAK.

FAK has also been implicated in the tyrosine phosphorylation and FA localization of other RhoA-specific GEFs such as PDZ-RhoGEF and LARG [32,33]. The localization of PDZ-RhoGEF to FAs is associated with contractility-dependent FA movement [34]. Similar to Rgnef, LARG is important for full RhoA activation during FN-stimulated cell spreading of fibroblasts [35] and FAK-LARG connections facilitate RhoA activation downstream of the neogenin receptor associated with neuronal growth cone collapse [33]. As the number of FAK-associated GEFs and GAPs grows [36], it is likely that FAK may facilitate the spatial and temporal management of multiple GEFs and GAPs. These connections may be both cell type and/or stimulus specific and for FAK-p190RhoGAP interactions, this complex is important for fibroblast, endothelial, and carcinoma cell two-dimensional polarity needed for efficient directional cell movement [28].

FAK: In Focal Adhesion (FA) Turnover

Directional cell migration requires continuous spatio-temporal formation and turnover or maturation of FAs at the leading edge. Whereas, FA disassembly is best visualized at the trailing edge of cells [37], nascent FAs that assemble under the lamellipodium exert tractional forces on the substrate that lead to lamellipodium growth and stability. The nascent FAs either undergo rapid turnover or mature in response to contractile forces [38]. Mature FAs facilitate increased cell contractility to pull the cell forward and are subsequently disassembled, or modified to form fibrillar adhesions that play critical role in remodeling of ECM (reviewed in [39]).

Evidence is accumulating that FAK is a key component promoting FA turnover. FAK-null fibroblasts show increased number of peripheral FAs [29,40] and FA turnover at the leading

edge and trailing edge is decreased in FAK-null cells [37]. It is important to note that FAK is a cytoplasmic kinase that can be recruited to the nucleus or nascent FAs in a highly regulated manner [41,42]. It is the recruitment of FAK to FAs that is linked to the processes of FA turnover through incompletely understood mechanisms. Therefore, signals that promote increased FAK FA localization have been connected to increased turnover. These signals include local calcium [43], phosphorylation of caveolin-1 [44], and phosphorylation of paxillin [45]. All of these stimuli were associated with increased FAK Y397 phosphorylation and fluorescence recovery after photobleaching (FRAP) experiments performed in astrocytoma cells revealed that Y397 phosphorylation increased the time-residency of FAK at FAs [46].

A simplistic model is that increased residency of activated FAK at FAs also enables recruitment of effectors that lead to FA disassembly and turnover. For nascent FAs, we hypothesize that the cyclic regulation of Rac/Rho activity may be an important determinant of FA turnover. This is due to the fact that nascent FA turnover or maturation is dependent upon the balance of contractile forces at the leading edge. Nascent FAs mature under high contractility and turnover upon loss of contractility [47]. In a rapidly growing lamellipodia, FAK activity inhibits Rho via spatial regulation of p190RhoGAP [28], and concurrently, FAK can enhance Rac activation through phosphorylation of adaptor proteins such as p130Cas or PIX [20,48,49]. FAK-null fibroblasts or cells expressing inactive FAK mutants exhibit high Rho activity and severe FA turnover defects [29,37]. Therefore, FAK mediated decrease in RhoA and increase in Rac activity at leading edge FAs may prevent FA maturation and indirectly facilitate nascent FA turnover.

In an opposite manner, high Rho activity and increased actomyosin contractility is an important 'trigger' for promoting trailing edge FA disassembly [47]. In this context, FAK association with various RhoGEFs (Rgnef, PDZ-RhoGEF, or LARG) could result in increased RhoA to ROCK activation, increased contractility, thereby facilitating trailing edge FA disassembly [34]. This is supported by the fact that Rgnef knockdown results in fibroblasts that exhibit reduced motility and time-lapse imaging showed that these cells possess tail retraction defects [29]. Alternatively, FAK has also been proposed to facilitate the recruitment of intracellular proteases such as calpains that cleave FA-associated proteins leading to the destabilization of FA structures [50]. Recent studies have added caspase-8 to this group of FAK-associated proteases as caspase-8 forms a complex with FAK and calpain 2 and is also needed for efficient cell motility [51]. Therefore, FAK serves as a unique regulator of FA turnover and disassembly, processes that are fundamental for efficient directional cell movement.

Conclusions and Perspectives

In this review, we have tried to simplify the multiple roles of FAK in the regulation of directional cell migration. We have highlighted key steps for FAK in promoting leading edge organization, FA turnover, GEF/GAP mediated RhoGTPase regulation, and trailing edge retraction (Fig. 3). We have emphasized that the activation of Rac and Rho at the leading edge is cyclic and is modulated by FAK activity. This unique signaling position of FAK reinforces the role of FAK as a "master regulator" of directional cell migration.

Further efforts will be needed to figure out questions like how does FAK activate integrins? Is localized tension at FA sites sufficient to cause FAK activation? How does FAK coordinate the spatio-temporal cyclic activity of multiple GEFs and GAPs? Are there spatial differences in FAK signaling to RhoA in nascent versus trailing FAs? With the continued development of high resolution real-time imaging techniques and fluorescent fusion proteins of FAK that maintain proper function, coupled with the availability of FRET-based activity probes to study a variety of targets, it should be possible to study the complex interplay of FAK signaling

complexes in space and time within a migrating cell. A complete understanding of FAK-mediated directional cell migration, albeit difficult, is certainly not just “wishful thinking”.

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Abbreviations

ECM, extracellular matrix
 EGF, epidermal growth factor
 FA, focal adhesion
 FAK, focal adhesion kinase
 FAT, focal adhesion targeting
 FERM, band 4.1, ezrin, radixin, moesin
 FN, fibronectin
 FRAP, fluorescence recovery after photobleaching
 FRET, fluorescence resonance energy transfer
 GAP, GTPase-activating protein
 GEF, guanine nucleotide exchange factor
 KAKTLR, Lysine-Alanine-Lysine-Threonine-Leucine-Arginine
 PIP₂, phosphoinositol 4,5 bisphosphate
 PTK, protein tyrosine kinase
 RGD, Arginine-Glycine-Asparagine
 Y, Tyrosine

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Fig. 1. FAK: A Leading Edge Organizer

A) Nascent FAs are formed at cell periphery by integrin and extracellular matrix (ECM) interactions. Inside cells, talin binding to integrin cytoplasmic tails establishes a linkage with the actin cytoskeleton and vinculin. Paxillin is also recruited to nascent FAs and FAK targeting to these sites is via interactions with talin and paxillin. FAK is regulated in part through an inhibitory intra-molecular interaction between the FAK FERM and the FAK kinase domains. In this inactive conformation, FAK FERM can bind the actin nucleating protein Arp3 and facilitate the recruitment of the Arp2/3 complex to nascent FAs. This linkage connects FAK to leading edge protrusions.

B) Integrin clustering, actin cytoskeletal tension, or binding of proteins or phospholipids to the FAK FERM domain can lead to the release of auto-inhibitory constraints and FAK catalytic activation resulting in FAK auto-phosphorylation at tyrosine (Y) 397. FAK activation leads to the formation of a complex with Src-family kinases and the increased phosphorylation of FAK and other substrate proteins within FAs such as p190RhoGAP. FAK-mediated phosphorylation of p190RhoGAP is important in the establishment of cell polarity and in the regulation of RhoA GTPase activity. As Arp2/3 does not interact strongly with activated FAK, its release upon FAK activation allows for Arp2/3 recruitment to the extended lamellipodia and branched actin filament assembly.

Fig. 2. FAK: A Key Regulator of Localized GAP and GEF activity

At early stages of cell spreading (15 to 45 min.), FAK forms a complex with p120RasGAP and p190RhoGAP resulting in localization of p190RhoGAP at nascent FAs. FAK-mediated phosphorylation of p190RhoGAP is associated with the inhibition of RhoA at leading edge FAs. As Rho and Rac activity can be antagonistic, Rho inhibition can also indirectly facilitate Rac activation. Low Rho and high Rac activity at the leading edge results in increased cell spreading, fuelled by f-actin polymerization and decreased cell contractility (PUSH). At later stages of cell spreading (60+ min.), FAK can balance the PUSH signal by recruiting and phosphorylating Rgntef (p190RhoGEF) at FA sites. Rgntef facilitates increased RhoA activation to promote cell contractility (PULL), resulting in increased tension at FA sites and promoting leading edge stability.

Fig. 3. FAK: A master regulator of directional cell migration

FAK can facilitate actin polymerization at nascent FAs by interacting with Arp2/3 complex and modulating Rho/Rac activation cycles, resulting in the stabilization of leading edge formation. FAK also promotes FA turnover at initial stages of cell spreading by RhoA inhibition, resulting in reduced cell contractility at nascent FAs. The organization of a stable leading edge facilitates cell polarity, resulting in Golgi orientation in the direction of cell migration. At later stages of cell migration, FAK promotes FA maturation via RhoA activation and increased cell contractility. At trailing edge FAs, FAK-mediated increased cell contractility via RhoA activation is associated with FA disassembly or turnover. Therefore, FAK acts to regulate multiple and differential steps required for efficient directional cell movement.