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Opinion

Endosomes: Emerging Platforms for Integrin-Mediated FAK Signalling

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Integrins are vital cell adhesion receptors with the ability to transmit extracellular matrix (ECM) cues to intracellular signalling pathways. ECM–integrin signalling regulates various cellular functions such as cell survival and movement. Integrin signalling has been considered to occur exclusively from adhesion sites at the plasma membrane (PM). However, recent data demonstrates integrin signalling also from endosomes. Integrin-mediated focal adhesion kinase (FAK) signalling is strongly dependent on integrin endocytosis, and endosomal FAK signalling facilitates cancer metastasis by supporting anchorage-independent growth and anoikis resistance. Here we discuss the possible mechanisms and functions of endosomal FAK signalling compared with its previously known roles in other cellular locations and discuss the potential of endosomal FAK as novel target for future cancer therapies.

Trends

Active integrins signal from endosomes to activate focal adhesion kinase (FAK).

Integrin endosomal signalling contributes to FAK-dependent anoikis suppression.

Integrin trafficking is thoroughly linked to cellular signalling.

FAK targeting to endosomes is distinct from focal adhesion recruitment.

Signalling from integrin-containing 'endoadhesomes' supports cancer metastasis.

Integrins Signal from Endosomes

The PM of mammalian cells is a highly dynamic barrier with its multiple transmembrane receptors undergoing active traffic between distinct subcellular membrane compartments in response to external stimuli and the requirements of the cell. Endocytosis was initially considered a means to terminate receptor signalling starting from the PM by directing receptors for lysosomal degradation. Since then several different receptor tyrosine kinases (RTKs) have been shown to signal also from endosomes and now signalling endosomes are seen as active signalling platforms that allow spatiotemporally regulated signalling for complex cellular functions such as cell migration and polarity. Examples from RTKs show that receptor endocytosis can function to generate specific signalling unique to endosomes or sustain and amplify existing PM signalling (reviewed in [1]).

Integrins are a family of essential cell surface adhesion receptors with the unique ability to sense the content and stiffness of the surrounding ECM and translate it to cytoplasmic signalling pathways [2,3]. Integrins are heterodimers comprising α and β subunits and can be bidirectionally activated by the binding of specific activators to integrin cytoplasmic tails (inside-out signalling) or by binding of ECM components such as collagen or fibronectin at the PM (outside-in signalling) [2]. This binding triggers integrin clustering on the PM and the formation of multiprotein signalling complexes called focal adhesions (FAs), where one of the first downstream components to become activated by integrins is FAK [4]. FAK becomes autophosphorylated at Y397, which leads to activation of Src family kinases and activation of the PI3K–Akt and Raf–MEK–Erk signalling pathways [2]. Therefore, integrin outside-in signalling regulates almost every aspect of the behaviour of adherent cell types from cell proliferation and survival to migration and invasion [5]. Integrins also undergo constant endo- and exocytic trafficking to enable receptor turnover and targeted recycling to facilitate motility and invasion [6]. Integrin

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endocytosis is regulated by numerous proteins including the small GTPase Rab21, which binds directly to integrin ∝ tails and guides the receptors to early endosome antigen 1 (EEA1)-containing early endosomes [7–9]. Following endocytosis, active and inactive integrins traffic with different kinetics and several distinct mechanisms regulate context- and cell type-specific integrin recycling [9].

Integrins are conceptually thought to signal exclusively from PM adhesions, but recent data show that integrins also signal from endosomes. Inhibition of integrin endocytosis by dynamin inhibitors, or more specifically with Rab21 silencing or by expressing endocytosis-defective integrin mutants, leads to reduced integrin-mediated pFAK-Y397, pAkt-S473, and pErk1/2 signalling [10]. In line with this, overexpression of Rab21 or active Rab5 (Q79L) leads to increased FAK signalling in breast cancer cells [10] and overexpression of wild-type Rab5 increases pFAK-Y397 and pAkt-S473 levels in hepatocellular carcinoma cell lines [11]. Interestingly, the activation of Src is not significantly dependent on integrin endocytosis, suggesting a degree of specificity for integrin endosomal signalling.Integrin traffic has been shown to influence cellular signalling, although evidence for actual endosomal signalling was missing. Several studies have shown that active integrins colocalise with their ligands in endosomes [12,13]. In addition, FAK localises and becomes activated on LAMP-1-positive *Salmonella*-containing vacuoles in mouse macrophages, but whether these vacuoles contain active integrin is unclear [14]. Furthermore, integrin co-trafficking with RTKs stimulates RTK-induced PM signalling [15,16] and the endocytosis of active and ECM-bound integrin induces TIAM-mediated activation of Rac1 on endosomes [17]. Moreover, FAK phosphorylation promotes FA disassembly [18] and integrin endocytosis couples with the activation of mTOR on late endosomes [19]. Thus, integrin traffic is coupled to signalling on multiple levels and new findings of the ability of integrins to signal from endosomes expand this concept further [10].

Recruitment and Activation of Endosomal FAK

FAK is a ubiquitously expressed non-RTK regulating a wide range of cellular functions from embryonic development to wound healing, cell migration, and cancer (Figure 1, Key Figure). FAK comprises an N-terminal FERM (band 4.1, ezrin, radixin, moesin) domain, a central kinase domain, and a C-terminal FA-targeting domain (FAT), which are separated by long linker regions (reviewed in [20]). The FAT domain links FAK to integrin-containing FAs by binding to other FA components such as paxillin and talin. In addition, integrin binding to the ECM and the following receptor clustering increases locally the production of PI(4,5)P2 at the PM [21]. This mediates FAK recruitment and clustering to FAs [22,23]. FAK exists in an autoinhibited conformation in the cytoplasm, but the binding of PI(4,5)P2 to a basic patch in the FAK FERM domain relieves the autoinhibition thus triggering autophosphorylation of Y397 *in trans* [23]. This first phosphorylation site constitutes a binding site for Src family kinases, which leads to further Src-dependent phosphorylation and activation of FAK (including Y576/Y577, Y861, and Y925) [24]. Active FAK–Src complex has several downstream targets including p130Cas, GRB7, the p85 subunit of PI3K, and p120RasGAP [24]. Interestingly, p120RasGAP interacts with integrins in early endosomes and drives integrin recycling by replacing Rab21 [8]. In addition to PM enriched PI(4,5)P2, FAK is able to bind PI(3,4,5)P3 [23], which was reported to exist as a small fraction in early endosomes [25], suggesting a possible mechanism for FAK recruitment to endosomes. The FERM domain mediates the PI(4,5)P2 binding of FAK and the FERM domain alone is also sufficient to localise to the integrin-containing endosomal fraction [10]. Whether FAK FERM is able to bind other PIPs remain to be discovered, but FAK was found in a mass spectrometry-based study from a PI3P interactome, the main phosphoinositide in early endosomes [26]. This finding, and the fact that FAK can directly bind purified endosomes in a reconstituted setup [10], suggest that one possible mechanism of FAK targeting could involve endosomal PIPs.