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Review

Focal adhesion kinase: Protein interactions and cellular functions

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Summary. Integrin-mediated cell adhesion to extracellular matrix (ECM) plays important roles in a variety of biological processes. Recent studies suggested that integrins mediate signal transduction across the plasma membrane via activating several intracellular signaling pathways. Focal adhesion kinase (FAK) is a non-receptor tyrosine kinase that has been shown to be a major mediator of integrin signal transduction pathways. Upon activation by integrins, FAK undergoes autophosphorylation as well as associations with several other intracellular signaling molecules. These interactions in the signaling pathways have been shown to regulation a variety of cellular functions such as cell spreading, migration, cell proliferation, apoptosis and cell survival. Recent progress in the understanding of FAK interactions with other proteins in the regulation of these cellular functions will be discussed in this review.

Key words: Extracellular matrix (ECM), Integrins, Focal adhesion kinase (FAK), Signal transduction

Introduction

Extracellular matrix (ECM) proteins such as collagen, laminin, vitronectin, fibrin and fibronectin (FN) mediate cell attachment which plays an important role in various biological processes including embryonic development, wound healing, homeostasis, angiogenesis and tumor metastasis (Dustin and Springer, 1991; Rosen et al., 1992; Adams and Watt, 1993; George et al., 1993; Yang et al., 1993; Brooks et al., 1994; Qian et al., 1994; Sheppard et al., 1994; Yang et al., 1995). The interaction of the cell with the ECM is mediated to a large extent by the integrin family of cell surface receptors. Structurally, integrins are αß heterodimers of transmembrane glycoproteins. The integrin family consists of more than 20 members assembled from 16α subunits and 9ß subunits. Recent studies have shown that integrins can function not only as physical links connecting the ECM to the actin cytoskeleton but are also capable of transducing biochemical signals across the plasma membrane. The ECM-integrin interaction is followed by enhanced tyrosine phosphorylation of various signaling proteins within the cell, prominent among which is a non-receptor tyrosine kinase called focal adhesion kinase (FAK) (Guan et al., 1991; Guan and Shalloway, 1992; Hanks et al., 1992; Schaller et al., 1992). In this review, we will summarize our current understanding of FAK interactions with other signaling molecules in the regulation of various cellular functions.

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FAK-interacting proteins

FAK interacts with various other signaling molecules which play functional roles in various cellular activities. FAK has several phosphorylation sites that serve as binding sites for the SH2 containing proteins. The major phosphorylation site of FAK, Tyr397, binds the SH2-domian of Src, p85 subunit of PI3K, Grb7, Shc, Nck-2 and PLC-γ. FAK also has proline-rich domains that bind SH3-contaning proteins. Several other proteins serve as substrates for FAK.

Src

The major autophosphorylation site of FAK Tyr397 serves as the binding site for Src SH2 domain (Schaller et al., 1994; Eide et al., 1995). However, a proline-rich region upstream of the Tyr397 in FAK also forms a binding site for Src SH3 domain (Thomas et al., 1998). This is supported by the evidence that the binding of FAK with SH2-Src alone is weaker than its binding with SH3-SH2-Src (Xing et al., 1994). Various studies have also demonstrated the association of FAK and Src both *in vitro* and *in vivo*.

Src binds FAK and phosphorylates various residues within FAK such as Tyr 407, 576, 577, 861 and 925. The Tyr567 and 577 phosphorylations are within the activation loop of FAK kinase domain and function to increase FAK activity to the maximal level (Calalb et al., 1995). In turn, FAK binds to the SH2 domain of Src thus

preventing the closed conformation of Src caused by intramolecular interaction with Tyr527 on Src. The role of Tyr407 and Tyr861 phosphorylations are not clear but the neighboring residues suggest that these sites may function to bind SH2 domains of other proteins. The phosphorylation of Tyr925 by Src creates a binding site for SH2 domain of Grb2 adaptor protein which mediates activation of the Erk family of MAPK (Schlaepfer et al., 1994; Chen et al., 1998).

The FAK Tyr397 is crucial for many established roles of FAK like promotion of cell spreading, cell migration, cell cycle progression and cell survival (Cary et al., 1996; Frisch et al., 1996; Zhao et al., 1998). It is difficult to specifically delineate the role of Src in these processes since Tyr397 serves as a binding site for a large number of proteins. However, there is evidence that Src binding to Tyr397 of FAK is required for stimulation of cell migration through phosphorylation of p130cas (Cary et al., 1998). Also, it is suggested that Src-mediated phosphorylation of paxillin can rescue FRNK-mediated inhibition of cell spreading (see below). Characterization of the role of Src in FAK mediated cellular functions is further complicated by activation of Src by various other stimuli like growth factors.

Paxillin

Paxillin was discovered in much the same way as FAK; a highly phosphorylated protein in v-Src transformed fibroblasts (Glenney and Zokas, 1989). Its role in integrin-mediated signaling was demonstrated by it localization to focal adhesion sites, binding to vinculin, another prominent focal adhesion assembly protein, and its concomitant phosphorylation with FAK upon integrin activation (Turner et al., 1990; Burridge et al., 1992).

Paxillin is a multi-domain adaptor protein. Various *in vitro* and *in vivo* binding assays show that paxillin can directly bind to integrin ß subunit (Schaller et al., 1995; Tanaka et al., 1996), FAK (Turner and Miller, 1994; Hildebrand et al., 1995; Tachibana et al., 1995), vinculin (Turner et al., 1990; Turner and Miller, 1994), Src-family-members (Weng et al., 1993; Schaller et al., 1995), Csk (Schaller and Parsons, 1995), Crk (Birge et al., 1993; Schaller and Parsons, 1995) and tyrosine phosphatase PTP-PEST (Shen et al., 1998). Because of its lack of enzyme activity paxillin is generally believed to act as a scaffolding protein in focal adhesions that provides multiple docking sites for interaction with other signaling and/or cytoskeleton proteins.

The FAK/Src complex phosphorylates paxillin at Tyr31 and Tyr118 which creates a binding site for Crk (Birge et al., 1993; Schaller and Parsons, 1995). It has been demonstrated that mutation of Tyr31 and Tyr118 to Phe inhibited paxillin's binding to Crk and impaired cell motility (Petit et al., 2000). Thus, FAK mediated phosphorylation of paxillin seems to play an important role in cell migration events. The phosphorylation of N-terminal region of paxillin by the FAK/Src complex recruits Crk which can associate with p130cas which plays a pivotal role in cell motility and activating the MAP kinase cascade. Paxillin can also be involved in the negative regulation of this pathway. It binds to Csk, a inhibitor of Src, as well as to PTP-PEST which dephosphorylates p130cas. This implies that FAK association with paxillin can also recruit negative regulators of focal adhesion proteins.

Paxillin also binds PKL (paxillin-kinase linker) (Turner et al., 1999), that interacts with a complex of PIX (a cdc42/Rac guanine exchange factor), PAK (Hashimoto et al., 2001) and a SH3-SH2 adaptor, Nck (Hashimoto et al., 2001). This protein complex plays a role in integrin-mediated cell spreading and locomotion and in regulation of Rac activity during these events.

The mechanism of paxillin localization to focal contacts is not clear although it seems to be upstream of FAK or vinculin binding. Paxillin constructs containing both deletion and point mutations that abrogate binding to FAK and vinculin still targeted to focal contacts (Brown et al., 1996). Additionally, expression of the N-terminus 313 amino acids of paxillin that contains intact vinculin- and FAK-binding domains, failed to target to focal adhesions. It was found that the LIM3 domain in the C-terminal region of paxillin was the principle determinant of paxillin localization to focal contacts (Brown et al., 1996). The protein(s) that interacts with this LIM domain has not been identified yet. Furthermore, phosphorylation of various Ser/Thr residues within LIM2 and LIM3 effect paxillin localization to focal contacts (Brown et al., 1998) but the kinase(s) responsible for these phosphorylations are not yet known. Expression of paxillin mutants targeting the Ser/Thr phosphorylation sites in the LIM domain have been shown to effect cell adhesion to FN.

Paxillin seems to lie both upstream and downstream of FAK. Substantial evidence demonstrates that paxillin can recruit FAK to focal contacts, thus in this model paxillin is upstream of FAK. At the least paxillin can localize to focal contacts independent of FAK. On the other hand paxillin is a FAK substrate. Mutation of the two FAK binding sites within the LD motifs in paxillin dramatically impairs phosphorylation of paxillin in response to cell adhesion and Src-transformation in fibroblasts (Schaller et al., 1999). These results suggest that paxillin must bind to FAK for its maximal phosphorylation in response to cell adhesion and that FAK may function to directly phosphorylate paxillin. Indeed, paxillin has been shown to play a role in FAK downstream signaling events mediating cell spreading and migration (Salgia et al., 1999; Turner et al., 1999; Ito et al., 2000; Ivankovic-Dikic et al., 2000; Nakamura et al., 2000; Petit et al., 2000; Yano et al., 2000; Zhao and Guan, 2000).

p130cas

P130cas binds FAK directly through its SH3 domain (Polte and Hanks, 1995; Burnham et al., 1996; Harte et

al., 1996). There are two proline-rich regions in FAK that participate in binding p130cas and both sites must be mutated in order to completely abolish the ability of p130cas to bind FAK. P130cas is also the substrate for several phosphatases including PTP-PEST (Garton et al., 1996), Yersinia YopH protein (Black and Bliska, 1997) and PTP1B (Liu et al., 1996, 1998).

P130cas is localized to focal adhesion in rat fibroblasts (Petch et al., 1995; Harte et al., 1996) and in COS cells plated on FN (Nakamoto et al., 1997). FAK overexpression in either rat fibroblasts (Vuori et al., 1996) or CHO cells (Cary et al., 1998) increases p130cas phosphorylation levels. Although FAK can phosphorylate p130cas directly, this event allows Src to bind to p130cas which further phosphorylates it at sites in the substrate domain (Tachibana et al., 1997). Thus, both FAK and Src function to phosphorylate p130cas which then binds Crk in an cell adhesion dependent manner (Vuori et al., 1996). This interaction plays a role in p130cas and Crk-mediated increases in cell migration which is dependent on their respective binding sites (Klemke et al., 1998). Crk has been shown to associate with GEF (GTPase exchange factor); C3G and SOS (Vuori et al., 1996), which can activate the Erk family of MAPK (Schlaepfer and Hunter, 1997). However, no study directly supports the activation of Ras/Erk pathway downstream of p130cas activation. On the other hand, a Crk mutant unable to bind p130cas blocked p130cas stimulation of JNK. Crk interaction with C3G has been shown to activate JNK (Dolfi et al., 1998).

P130cas binding to FAK functions to promote cell motility as has been demonstrated in CHO cells (Cary et al., 1998) and Cos-7 cells (Klemke et al., 1998). P130cas deficient fibroblasts showed significant defects in cell movement towards FN which was rescued by re-expression of p130cas (Honda et al., 1999). Also, expression of p130cas with FAK increased migration towards FN in CHO cells and inhibition of the FAK/p130cas complex failed to promote cell motility (Cary et al., 1998).

Grb7

Grb7 (Growth factor receptor bound protein 7) is a member of an emerging family of SH2-domain containing adaptor molecules including Grb2, Grb10 and Grb14. Recently, Grb7 has been found to play a role in signal transduction of integrin-mediated cell migration via FAK (Han and Guan, 1999). The SH2 domain of Grb7 was found to directly interact with FAK both *in vitro* and *in vivo*. Grb7 bound to the major autophosphorylation site of FAK, Tyr397, in a cell adhesion dependent manner. It was demonstrated that inducible expression of Grb7 in NIH3T3 cells could enhance cell migration towards FN as well as transient overexpression of Grb7 in CHO cells stimulated cell motility. It was also demonstrated that Grb7 is a direct substrate of FAK. The focal adhesion targeting of Grb7 was found to be essential for its role in stimulation of cell motility. However, the focal adhesion targeting of Grb7 in FAK-/- cells was not sufficient for promotion of cell migration. These results demonstrate that both the focal adhesion targeting of Grb7 as well as its phosphorylation by FAK is important in the regulation of cell migration by Grb7.

The Grb7 overexpression and FAK/Grb7 complex formation has also been linked to increased invasion and metastasis of esophageal carcinoma cell (Tanaka et al., 2000). Ectopic expression of the Grb7-SH2 fragment inhibited the FN dependent Grb7/FAK complex formation, phosphorylation of endogenous Grb7, and reduced migration of these carcinoma cells on FN. Furthermore, inhibition of endogenous Grb7 protein expression level using an antisense Grb7 RNA suppressed the *in vitro* invasive phenotype of these cells (Tanaka et al., 1998). These studies suggest a potential role of Grb7 interaction with FAK in tumor metastasis.

Shc

The Shc family of adaptor proteins contains multiple protein-protein interaction domains (Pelicci et al., 1992). Shc exists in three isoforms of 46, 52, and 66 Kda and was found to associate with and subsequently get phosphorylated by the EGF receptor. (Ricketts et al., 1996). These phosphorylations create binding site for the SH2-domain of Grb2 (Pelicci et al., 1992; Rozakis-Adcock et al., 1992) ultimately resulting in activation of Ras/MAPK pathway which plays a role in cell mitogenesis (Bonfini et al., 1996). Recent reports also implicate Shc in integrin signaling and integrin-mediated activation of Shc has been shown to promote cell cycle progression (McGlade et al., 1992; Mainiero et al., 1995; Wary et al., 1996; Schlaepfer et al., 1998).

Shc phosphorylation is dependent on its binding to the Tyr397 of FAK (Schaller, 1997). Evidence that FAK could directly phosphorylate Shc came from the study where inhibition of Src PTK activity by herbimycin A or PP1, did not effect FN-stimulated FAK activity and still resulted in Shc binding to and phosphorylation by FAK (Schlaepfer et al., 1998). *In vitro*, FAK can directly phosphorylate Tyr317 of Shc to promote Grb2 binding. Grb2 binding to Shc was also observed *in vivo* in herbimycin A treated fibroblasts upon FN stimulation. Thus, FAK autophosphorylation can also result in MAPK activation via Shc phosphorylation. The activation of Shc downstream of FAK has been implicated in promotion of cell proliferation and cell migration (Wary et al., 1996; Collins et al., 1999).

Shc has also been shown to directly bind to the ß subunit of integrins to promote cell cycle progression. Shc was found to be both necessary and sufficient for activation of MAPK pathways in response to integrin clustering. This study demonstrated that specific integrin/Shc interactions could regulate cell survival as well as cell cycle progression (Wary et al., 1996).

PI3K

PI3K is an important lipid kinase which has been

shown to play a role in integrin signal transduction. PI3K is a heterodimer of a 85Kda regulatory subunit (p85) and a 110Kda catatlytic subunit (p110). These lipid products of PI3K act as second messengers in a variety of signaling processes including cell survival, spreading and cell migration (Kapeller and Cantley, 1994).

Association of FAK with PI3K in response to integrin activation has been demonstrated in both platelets (Guinebault et al., 1995) and fibroblasts (Chen and Guan, 1994). FAK/PI3K association is also stimulated by treatment of cells with PDGF (Chen and Guan, 1994) suggesting a cross talk between integrin and growth factor signaling pathways. PI3K binds to Tyr397 of FAK via its SH2 domain while the SH3 domain of PI3K also contributes to its association with FAK (Chen et al., 1996).

A number of studies imply a role for PI3K in cell migration. A FAK mutation proximal to Tyr397 which selectively disrupts FAK binding to p85 but not Src, abolishes FAK promoted cell migration (Reiske et al., 1999). It has also been shown that PI3K inhibitors, Wortmanin and Ly294002, which specifically inhibits the lipid kinase activity of PI3K, inhibited CHO cell migration.

FAK/PI3K association is required for the ability of FAK to resist apoptosis induced by UV radiation in MDCK cells (Chan et al., 1999). PI3K activates the Akt pathway which has been shown to regulate cell survival pathways (Franke et al., 1997). FAK mediated matrix survival signals are also mediated by Akt activation downstream of FAK/PI3K association (Tamura et al., 1999b). Interestingly, PI3K association with FAK is not needed for FAK regulated cell cycle progression or FAK promoted Erk activation (Reiske et al., 2000).

Graf, Nck 1, Nck-2, PLCγ

The proline-rich regions of FAK also interact with a protein called Graf (GTPase regulator associated with FAK) via Graf's SH3 domain (Hildebrand et al., 1996). Graf can stimulate the GTPase activity of Rho and cdc42 suggesting that it may link FAK to the regulation of the small-G-protein family.

FAK has also been found to associate with the adaptor protein Nck-1 in response to thrombin. Nck-1 can further bind to SOS and activate the Ras/MAPK pathway presenting yet another link between FAK and mitogenesis. An analog of Nck, Nck-2, also associates with FAK (Goicoechea et al., 2002). The SH2 domain of Nck-2 associates with Tyr397 of FAK in an adhesion dependent manner while the SH3 domain of Nck-2 binds FAK in an adhesion independent manner. Nck-2 mutants lacking the SH3 domain alter FAK promoted migration (Goicoechea et al., 2002) suggesting the role of Nck-2 in cell migration.

PLC-γ1 is a lipase that binds FAK at Tyr397 in a cell adhesion dependent manner. This binding results in increased phosphorylation of PLCγ and its subsequent activation (Zhang et al., 1999a). The formation of FAK/PLCγ complex results in activation of PKC and Ca^{+} release which leads to changes in cytoskeleton rendering the possibility that FAK/PLCγ interaction may regulate cellular functions like cell spreading and migration.

Cellular functions of FAK

Integrin signaling is known to play a role in a wide variety of biological functions and FAK has been identified as a key player in many of these including cell spreading, cell migration, cell proliferation, cell survival and apoptosis. Many FAK associated proteins have been implicated to play a role in one or more of the cellular functions involving FAK.

Cell spreading

Since FAK was initially discovered as a protein tyrosine kinase that gets phosphorylated and localizes to focal contacts upon integrin simulation, it was postulated that FAK may play a role in cell attachment to ECM proteins. However, to date there is no direct evidence to demonstrate a role of FAK in regulating integrin-mediated cell adhesion. Cultured cells from FAK-/- cells are able to attach to FN just as well as FAK+/+ cells (Ilic et al., 1995). However, the FAK-/- fibroblasts exhibit rounded morphology and are poorly spread when plated on FN compared to their FAK+/+ counterparts (Ilic et al., 1995). Furthermore, re-expression of FAK in these FAK-/- fibroblasts resulted in cell morphology identical to that of FAK+/+ cells (Owen et al., 1999; Sieg et al., 1999). These results support the conclusion that FAK functions to promote cell shape changes and spreading in fibroblasts.

Overexpression of FRNK, which is a negative regulator of FAK, in CEF cells resulted in delayed cell spreading (Richardson and Parsons, 1996). Furthermore, this reduced cell spreading correlated with reduced tyrosine phosphorylation of FAK and could be overcome by co-expression of wild-type FAK. In addition, cell spreading in this system correlated with paxillin phosphorylation but not with phosphorylation of other FAK targets like tensin (Richardson et al., 1997). Overexpression of Shp2 and PTEN which dephosphorylate FAK also demonstrated impaired cell spreading (Tamura et al., 1998; Manes et al., 1999).

Cell migration

Cell migration plays a role in physiological processes such as embryogenesis, inflammation and wound healing. Cell motility also plays a role in cancer invasion and metastasis. Various evidence suggest the role of FAK in integrin-mediated cell motility events. This was first suggested by the observation that FAK kinase activity correlated with endothelial cell migration (Romer et al., 1994). More direct evidence for FAK's role in cell motility came from several different studies.

Overexpression of FRNK inhibited endogenous FAK tyrosine phosphorylation (Gilmore and Romer, 1996) and endothelial cell migration in cell wound-healing assays (Richardson et al., 1997). Another study demonstrated that stable overexpression of FAK in CHO cells increased cell motility on FN, which depended both on Tyr397 (Cary et al., 1996) and p130cas binding site (Cary et al., 1998). The strongest evidence for FAK's role in cell migration comes from the analysis of FAK deficient fibroblasts. The FAK-/- fibroblasts derived from FAK null mice showed reduced rates of cell motility on FN (Ilic et al., 1995). Upon re-expression of FAK, the FAK-/- fibroblast resume their cell morphology, spreading and migratory capabilities (Owen et al., 1999; Sieg et al., 1998-2000). Studies have also shown that dephosphorylation of FAK by PTEN resulted in decreased cell migration of glioblastoma cells lines (Tamura et al., 1998). In contrast, Shp-/- cell lines and PTP-PEST deficient fibroblasts which contain elevated tyrosine phosphorylation of FAK, paxillin and p130cas exhibit decreased rates of cell migration *in vitro* (Yu et al., 1998). This can be explained if the role of FAK, paxillin and p130cas phosphorylations are viewed as a part of a dynamic process. It seems that increased tyrosine phosphorylation per se may not be the critical factor for promotion of cell migration but instead repeated cycles of phosphorylation and dephosphorylation may be essential for coordinated cell movement. This is consistent with the model of cell migration where stable attachments with the ECM have to be formed at the leading edge of a migrating cell, accompanied by disassembly of focal contacts at the rear edge of the cell, in order to allow the cell to move forward.

There are several pathways implicated downstream of FAK in stimulation of cell migration. The FAK/Src complex tyrosine phosphorylates p130cas which is believed to regulate migration events (Matsuda et al., 1994). Grb2 association with FAK has also been shown to promote cell migration in some systems (Lai et al., 2000). However, in another system FAK/Grb2 association was not required (Cary et al., 1998). However, the FAK construct used in the latter study hinders FAK interaction with Grb2 which might have masked the contribution of Grb2 in these cellular events.

PI3K association with FAK has also been shown to be required for cell migration events (Reiske et al., 2000) though the exact downstream components are unknown. FAK/Nck-2 complex is localized to lamillopodia and fillopodia (Goicoechea, 2001) and FAK interaction with Grb7 also plays a role in promoting cell migration (Han and Guan, 1999). Thus, there are various pathways downstream of FAK that are involved in FAK's role in cell motility events.

There are several implications for FAK's role in integrin-mediated cell motility. FAK is required for cell migration events in proper embryonic development at approximately day 8 in mouse embryogenesis. In addition, FAK has been found to be overexpressed in a number of different human tumors (Weiner et al., 1993; Owens et al., 1995; Kornberg, 1998). A direct correlation has been made between increased FAK expression and elevated cell motility in six different cell lines derived from primary human malignant melanomas (Akasaka et al., 1995). This observation indicates that FAK expression may be a common pathway for various tumor types to gain invasive potential as well as that FAK expression levels could be a marker for early events leading to tumor metastasis. FAK's role has also been implicated in wound healing. FAK expression is elevated in epidermal-dermal function of repairing burned wounds as detected by immuno-histochemical staining (Gates et al., 1994). FAK isolated from wounded monolayers also exhibited elevated kinase activity compared to FAK in intact monolayers (Romer et al., 1994). The regions of elevated FAK expression corresponded to only those keratinocytes that were actively migrating and rapidly proliferating in repairing burned wounds.

Cell proliferation

Numerous studies demonstrate the role of FAK in regulation of cell growth. This was first suggested based on studies with FRNK-related FAK construct which when microinjected into cells inhibited DNA synthesis presumably by competitively inhibiting endogenous FAK (Gilmore and Romer, 1996). The inhibition of FAK using a monoclonal antibody against the C-terminal region of FAK also resulted in cells undergoing apoptosis and cell cycle arrest (Hungerford et al., 1996). Recently, inducible expression of FAK was demonstrated to positively regulate cell cycle progression (Zhao et al., 1998). While wild-type FAK was shown to increase DNA synthesis and accelerate G1/S transition, a dominant-negative FAK construct inhibited these steps. It was further demonstrated that this effect of FAK on DNA synthesis was accompanied by enhanced expression of cyclin D1 and repression of p21, whereas the dominant-negative FAK had opposite effects. It was also shown that ectopic expression of cyclin D1 could rescue the cell cycle inhibition by dominant-negative FAK, suggesting that cyclin D1 was the primary functional target of FAK in regulation of cell cycle (Zhao, 2001). The FAK mediated promotion of DNA synthesis depended on the phosphorylation of Tyr397 strongly suggesting the role of FAK/Src complex in this pathway.

The pathways downstream of FAK that regulate the cell cycle are only just being uncovered. A correlation between FAK regulation of DNA synthesis and the activation of MAPK family members Erk and JNK has been established (Zhao et al., 1998; Oktay et al., 1999). Recently, it was shown that FAK regulates cyclin D1 expression at the transcriptional level. This regulation depended on integrin-mediated adhesion and activation of Erk signaling pathway (Zhao, 2001).

Anoikis and cell survival

Normal epithelial and endothelial cells when detached from the ECM begin to undergo apoptosis via a process termed anoikis (Frisch et al., 1996). This process makes sure that cells fulfill the requirement of adhesion to ECM to proliferate. Anoikis, or detachment induced apoptosis, is regulated by integrins and FAK. The observation that expression of an activated, membrane-targeted FAK construct called CD2/FAK (Chan et al., 1994) in epithelial cells prevented anoikis, clearly demonstrates the role of FAK in cell survival. Conversely, inhibition of FAK either by treatment of tumor cell lines with FAK antisense oligonucleotides (Xu et al., 1996), or by microinjection of CEF cells with an anti-FAK monoclonal antibody (Hungerford et al., 1996) induced apoptosis. This demonstrates that FAK is required for mediating cell survival signals. In addition, various cell lines undergoing apoptosis demonstrate concomitant proteolysis of FAK at specific sites (Crouch et al., 1996; Wen et al., 1997; Levkau et al., 1998).

The mechanism of cell survival by FAK is not clear as yet. Both FAK/PI3K and FAK/p130cas associations are implicated in the FAK-mediated cell survival pathway (Chan et al., 1999). In addition, integrin and FAK mediated survival signaling has been shown to suppress the p53 regulated apoptotic pathway (Ilic et al., 1998). Cells can still survive without FAK in the absence of p53 or in the presence of a dominant-negative p53 construct (Ilic et al., 1998). A model emerging from these results is that p53 monitors cell survival signals from integrin/FAK in anchorage dependent cells and that mutations in the p53 tumor suppressor gene during cell transformation, may allow for anchorage independent cell survival.

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