In this issue of *Clinical Cancer Research*, Canel et al. (1) describe a new study investigating the role of focal adhesion kinase (FAK) in head and neck squamous cell carcinoma (HNSCC). Using immunohistochemical analysis of >200 HNSCC clinical specimens, they show FAK overexpression in the majority of samples, including benign hyperplasias and preinvasive dysplasias. Furthermore, they found FAK overexpressed in all lymph node metastases, correlating with expression in the matched primary tumors. These findings provide important clinical data supporting the possible involvement of enhanced FAK signaling in the onset and progression of HNSCC. Cell adhesion to the extracellular matrix is essential for survival, proliferation, and migration of most cell types. Cell/extracellular matrix adhesion occurs through integrin receptors that span the plasma membrane and establish a physical linkage between the extracellular matrix and the actin cytoskeleton. Numerous cellular proteins are recruited to the sites where integrins cluster into large aggregates called focal adhesions or focal contacts. The tyrosine kinase FAK localizes prominently to focal adhesions and is activated upon integrin-mediated adhesion. Indeed, the discovery of FAK in the early 1990s was a key to understanding that integrins are not simply adhesion proteins but also potent signaling receptors. Early on, it was widely speculated that enhanced or deregulated FAK signaling could be a factor in tumor onset and progression. Over the years, this idea has taken root, in part from numerous studies showing elevated FAK expression in a variety of human tumors, including those of epithelial origin (reviewed in refs. 2, 3). In most cases, high expression was observed in invasive and metastatic tumors, implicating FAK in malignant progression.

Basic science studies have provided insight into how FAK overexpression could contribute to cancer. Here, we present a brief overview of this topic with emphasis on recent advances. Interested readers may consult several extensive reviews (4–7) for more information, including references to other primary literature.

**FAK Signaling: a Partnership with Src**

FAK structural features include an NH2-terminal FERM domain, a central kinase domain, two proline-rich motifs, and a COOH-terminal focal adhesion targeting domain (Fig. 1). A critical event in integrin-mediated FAK signaling is phosphorylation of Tyr397 (reviewed in refs. 2–7). Tyr397 lies in the linker region between the FERM and kinase domains, and phosphorylation of this site may be achieved through trans-autophosphorylation. Notably, Tyr397 phosphorylation creates a high-affinity binding site for SH2 domains of Src family kinases, and this interaction promotes Src kinase activity through a conformational change. Thus, a major function of FAK is to recruit and activate Src at cell-extracellular matrix adhesion sites. Src bound to the FAK Tyr397 site can then phosphorylate other FAK tyrosines, including Tyr576/Tyr577 in the kinase domain, leading to further stimulation of FAK activity that could trigger a positive feedback loop for signal amplification (8). Tyr397 is not strictly an autophosphorylation site, and signal amplification could also result from phosphorylation of this site by Src. FAK residues Tyr407, Tyr561, and Tyr225 can also be phosphorylated by Src. Of these, Tyr925 site is recognized as a binding site for the growth factor receptor binding protein 2 adaptor protein (Grb2), and this is one of several mechanisms by which adhesion can promote activation of the Ras/mitogen-activated protein kinase pathway (reviewed in ref. 6). Given its location in the focal adhesion targeting domain, Tyr225 phosphorylation may also negatively affect focal adhesion targeting.

Recent studies showed the FERM domain as a key regulatory element (9–11). The FERM domain seems to directly interact with the central region of FAK to inhibit kinase activity. As adhesions form, the FERM domain may make new higher-affinity interactions with the integrin cytoplasmic tails or other integrin-associated proteins to release the autoinhibition and allow FAK Tyr397 phosphorylation and Src recruitment (Fig. 1). A crystal structure of the FAK NH2-terminal region suggests that Src binding may also contribute to FAK activation by promoting release of the FERM domain from its binding sites on FAK (12).

In addition to Src family kinases, the phosphorylated Tyr397 site can mediate interactions with SH2 domains of several other signaling proteins, including PLC-γ1, and adaptor proteins, such as Src homology and collagen protein (SHC), Grb7, Nck-2, and the p85 subunit of phosphatidylinositol 3-kinase (reviewed in refs. 2, 4–7). These observations hint at additional complexity in FAK signaling that remains largely unexplored.

Although FAK signaling is best understood in integrin-mediated responses, FAK can also be activated upon stimulation by various growth factors, chemokines, and G-protein-linked receptors. In many of these cases, FAK activation seems to be a secondary event resulting from other pathways affecting integrin function. At any rate, FAK activation is a point of convergence for different signaling pathways to effect changes in cell behavior.
FAK Signaling in Cell Motility

Numerous studies have implicated FAK signaling as contributing to efficient cell motility (reviewed in refs. 4–6). FAK-null mouse embryo cells, for example, have motility defects (13) that can be rescued by reconstitution with wild-type FAK but not mutants lacking Tyr397 (14, 15). Cell motility is a complex process involving protrusion of the plasma membrane and formation of adhesions that stabilize the leading edge and generate contractile forces, adhesion turnover, and release of the cell rear. These events must be tightly coordinated and are largely controlled by Rho family GTPases that regulate actin cytoskeleton dynamics (reviewed in ref. 16). Like other members of the Ras superfamily, Rho family GTPases cycle between an active GTP-bound state and an inactive GDP-bound state, driven, respectively, by the activities of guanine-nucleotide exchange factors (GEF) and GTPase-activating proteins (GAP). Among the Rho family GTPases, FAK/Src signaling has in particular been implicated in regulating activities of Rac1 and RhoA. A major function of activated Rac1 is to stimulate actin polymerization via the Arp 2/3 complex, leading to plasma membrane protrusion and extension of lamellipodia. RhoA activity promotes the formation of contractile actin-myosin filaments important for focal adhesion assembly and maintenance, cell contractility, and retraction of the cell rear.

Insight into how FAK/Src complex signaling can regulate Rac1 activity has come from studies on two major downstream substrates: CAS (p130Cas, Crk-associated substrate) and paxillin. CAS and paxillin are both nonenzymatic docking/scaffold proteins that bind directly to FAK, are enriched in focal adhesions, and undergo adhesion-dependent tyrosine phosphorylation. CAS has an NH$_2$-terminal SH3 domain that binds to FAK proline-rich motifs, but surprisingly, CAS bound to FAK is not phosphorylated by FAK. Rather, FAK acts as a classic signaling scaffold in recruiting Src to phosphorylate CAS (reviewed in ref. 5). The FAK/Src complex contributes substantially, but not entirely, to adhesion-mediated CAS tyrosine phosphorylation. Major sites of CAS tyrosine phosphorylation are Tyr-x-x-Pro (YxxP) motifs that lie in a central substrate domain. Antibodies specific to phosphorylated CAS YxxP sites prominently stain cell/extracellular matrix adhesions, including those newly formed at the leading edge of migrating cells (17). The consequence of CAS substrate domain phosphorylation is recruitment of additional SH2-containing effectors into the signaling complex, most notably adaptor proteins of the Crk family. Crk proteins also interact with DOCK180/ELMO that has GEF activity toward Rac1 (reviewed in ref. 18). Thus, recruitment of the Crk/DOCK180/ELMO complex to CAS in adhesions is a likely important event regulating plasma membrane protrusion downstream of Rac1 (Fig. 2). In addition to Crk, phosphorylated CAS YxxP sites may also signal to effect motility-related changes through interactions with other SH2-containing proteins, including Nck family adaptors and SH2-containing inositol 5-phosphatase 2 (reviewed in ref. 5).

Paxillin (reviewed in ref. 19) interacts with the FAK focal adhesion targeting domain and plays a major role in the focal adhesion targeting of FAK, thus acting as an upstream regulator in addition to a downstream effector in FAK signaling. Unlike CAS, FAK seems to directly phosphorylate paxillin, but the relative roles of FAK versus Src are uncertain. Tyr118 and Tyr138 are primary sites of paxillin phosphorylation, and these can also promote Crk binding and thus some of the same downstream pathways achieved by CAS. Alternatively, phosphorylated...
Tyr$^{11}$/Tyr$^{118}$ sites can bind to p120RasGAP, an interaction that releases the inhibitory interaction of p120RasGAP with p190RhoGAP and thereby acts to suppress activity of RhoA (20). As RhoA is inhibitory to Rac1, this pathway could also be important for efficient integrin-stimulated activation of Rac1 (Fig. 2). FAK, Src, and paxillin deficiencies have been linked to inefficient focal adhesion turnover (21), which could involve local suppression of RhoA activity (22).

Other recent studies point to further complexity in FAK regulation of Rho family GTPases. For example, FAK/Src signaling has also been implicated in down-regulation of Rac1 activity. RNA interference–mediated knockdown of FAK or paxillin in HeLa cells results in increased peripheral Rac1 activity associated with a disruption of cell-cell contacts (23), and phosphorylation of the ArfGAP paxillin kinase linker by FAK and/or Src family kinases was implicated in suppression of excessive Rac-induced lamellipodia formation (24). The recent finding that FAK associates with p190RhoGEF and promotes RhoA activity in neuronal cells (25) indicates that FAK can also have both positive and negative effects on RhoA.

Taken together, these studies support diverse mechanisms by which FAK/Src signaling may act to coordinate Rac1 and RhoA activities in the precise spatiotemporal manner critical for maintaining proper cell polarity and directional movement. A failure to properly regulate Rho family GTPases is consistent with observed motility defects associated with FAK deficiency in fibroblast-like cells, such as aberrant lamellipodial extensions (26) and loss of directional persistence (27).

**FAK Signaling in Cell Invasion**

Beyond its effects on motility, recent studies have revealed a role for FAK signaling in cell invasion involving proteolytic degradation of the extracellular matrix by matrix-degrading metalloproteinases (MMP). MMPs are strategically concentrated and activated at actin-rich cell/extracellular matrix contacts termed podosomes or invadopodia that are structurally distinct from focal adhesions (reviewed in ref. 28). Podosomes were first observed in Src-transformed fibroblasts, and these cells remain a model for understanding basic mechanisms underlying invasive behavior. FAK signaling was shown to promote matrix-degrading invasive behavior of Src-transformed fibroblasts through a pathway involving c-Jun NH$_2$-terminal kinase–mediated transcriptional activation of MMP-2 and MMP-9 downstream of the CAS/Crk > Rac1 pathway (29). In other studies, CAS expression and signaling enhanced invasive behavior of Src-transformed cells associated with formation of larger podosome structures and increased cleavage of MMP-2 to its active form (30, 31). Thus, FAK/Src signaling through CAS is implicated in multiple pathways leading to the expression and activation of MMPs (Fig. 3). Mechanistically, MMP-2 activation could result from enhanced cell surface expression of MT1-MMP, the enzyme largely responsible for cleavage of pro-MMP-2. FAK/Src signaling was recently shown to promote surface expression of MT1-MMP through phosphorylation of endophilin A2 and inhibition of endocytosis (32).

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**Fig. 2.** Proposed signaling pathways downstream of FAK/Src promoting plasma membrane protrusion. CAS and paxillin are major substrates of the FAK/Src complex. Within nascent integrin adhesion sites at the cell’s leading edge, CAS and paxillin tyrosine phosphorylation cooperate to promote spatial activation of Rac1, a Rho family GTPase that stimulates actin polymerization driving membrane protrusion.

**Fig. 3.** Proposed signaling pathways downstream of FAK/Src promoting ECM degradation. Activation of c-Jun NH$_2$-terminal kinase (JNK) kinase downstream of the CAS >> Rac1 pathway elevates transcription of genes encoding MMP-2 and MMP-9. Pro-MMP-2 cleavage to its active form by MT1-MMP is regulated by FAK/Src-mediated phosphorylation of endophilin A2, resulting in decreased endocytosis of MT1-MMP. CAS also regulates MMP-2 activation, perhaps by increasing the cell surface expression of MT1-MMP through a similar mechanism.
FAK Signaling in Cell Survival

Resistance to anoikis, apoptosis resulting from loss of cell/extracellular matrix adhesion (reviewed in ref. 33), is a common feature of cancer cells and facilitates tumor growth and metastasis. A role for FAK signaling in anoikis resistance was shown with the finding that expression of CD2-FAK, a fusion protein that retains a high level of FAK activity when cells are held in suspension, confers resistance to anoikis requiring both FAK kinase activity and the Tyr397 site (34). A role for FAK in anoikis resistance is supported by other recent studies involving knockdown of FAK expression in oral squamous cell carcinoma (35) and pancreatic adenocarcinoma (36) cells. Moreover, targeted knockout of FAK in mouse endothelial cells causes vascular defects linked to increased apoptosis as well as to aberrant migration (37).

Several pathways downstream of FAK could contribute to cell survival. As mentioned above, phosphorylated FAK Tyr397 can also bind to the p85 subunit of phosphatidylinositol 3-kinase. Phospholipid production by phosphatidylinositol 3-kinase can lead to activation of Akt kinase that inhibits apoptosis by regulating various components of the cell death machinery. A role for FAK upstream of phosphatidylinositol 3-kinase/Akt has been reported to protect fibroblasts from apoptosis triggered during collagen matrix contraction (38), and a FAK > phosphatidylinositol 3-kinase > Akt pathway leading to nuclear factor-κB–mediated expression of caspase inhibitors of the inhibitor of apoptosis family has been implicated in protecting human leukemic HL-60 cells from oxidative stress-induced apoptosis (39). In other cell culture systems, FAK has been shown to suppress apoptosis through other mechanisms, such as activating c-Jun NH2-terminal kinase downstream of CAS (40) and inhibiting the interaction of the protein kinase RIP with the death receptor complex (41).

Prospects

The findings of Canel et al. (1) are consistent with those from an earlier study showing FAK expression significantly increased in a smaller sample of preinvasive and invasive oral cancers (42). Correlative studies of this nature lay a foundation for future experiments testing if and how FAK contributes to various cancers. In an important step in this direction, McLean et al. (43) found that conditional FAK deletion in skin cells results in reduced papilloma formation and progression in a mouse skin carcinogenesis model, and this was linked to a FAK survival function. It will be important to understand the molecular events that underlie elevated FAK expression in HNSCC and other cancers. In their study, Canel et al. (1) provided new insight into this question, reporting a lack of correlation between FAK protein level and gene copy number in an analysis of 33 HNSCC tumors. Thus, although FAK gene copy number was found increased in some HNSCC cell lines (44), it seems that increased FAK expression in HNSCC tumors results from changes occurring at the level of gene transcription, RNA processing, translation, and/or protein stability. Toward understanding mechanisms of FAK overexpression in cancer, the promoter region of the human FAK gene was recently characterized and found to have potential for regulation by nuclear factor-κB and p53 (45).

Another important question is whether FAK overexpression is predictive of patient outcome. In their study, Canel et al. (1) addressed this question by analysis of 87 HNSCC cases and found no significant correlation between FAK overexpression and tumor recurrence or patient survival. Similarly, FAK expression was of no prognostic significance in a recent study of node-negative breast cancer (46). However, in a study of hepatocellular carcinoma, FAK overexpression was found to be a significant indicator of patient survival (47). Because FAK overexpression does not necessarily equate to elevated FAK signaling, more predictive power may be obtained through an analysis focusing on FAK activation. In this regard, it is significant that Canel et al. (1) further analyzed a small subset of HNSCC tumors and observed a >10-fold range in the ratio of immunoblot signals obtained using antibodies recognizing total FAK versus the phosphorylated Tyr397 site. In addition to FAK, it could be informative to assess the activation states of Src and downstream substrates, such as CAS and paxillin, using phosphospecific antibodies or mass spectrometric approaches.

Based on its common overexpression in cancer and known roles in promoting cell survival and migration/invasion, FAK has been suggested as a potential target for cancer therapy, but the argument in favor of this is not compelling (7). Previous success stories with tyrosine kinase inhibitors have targeted enzymes with activated/deregulated tyrosine kinase activity resulting from mutation. However, there is no evidence that FAK is mutated in cancer, or that FAK kinase activity, per se, is a critical event in the development of any cancer. Another concern with FAK as a therapeutic target is that FAK kinase activity is not absolutely essential for its signaling functions, including Tyr397 phosphorylation and Src recruitment. If the Src kinase domain is the primary mediator of responses downstream of the FAK/Src complex, as evidence suggests, then targeting integrin-mediated tyrosine kinase signaling using Src inhibitors may be a more effective strategy.

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The Signaling and Biological Implications of FAK Overexpression in Cancer

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