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Disrupting the Scaffold to Improve Focal Adhesion Kinase–Targeted Cancer Therapeutics

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Abstract

Focal adhesion kinase (FAK) is emerging as a promising cancer target because it is highly expressed at both the transcriptional and translational level in cancer and is involved in many aspects of tumor growth, invasion, and metastasis. Existing FAK-based therapeutics focus on inhibiting the kinase's catalytic function and not the large scaffold it creates that includes many oncogenic receptor tyrosine kinases and tumor suppressor proteins. Targeting the FAK scaffold is a feasible and promising approach for developing highly specific therapeutics that disrupt FAK signaling pathways in cancer.

It has been 20 years since the first report of substantially increased expression of the gene encoding focal adhesion kinase (FAK) in human cancer (1). During this time, many studies attempted to determine the biological reasons why so much of the FAK protein is found in tumor cells, whereas so little is found in their normal cell counterparts. Clearly, FAK is involved in nearly every aspect of cancer: invasion, metastasis, angiogenesis, epithelial mesenchymal transition (EMT), maintenance of cancer stem cells, and globally promoting tumor cell survival (2–10). As our understanding of FAK has evolved, it is clear that this protein is not only a kinase, but probably—and more importantly—a scaffold for a number of different signaling proteins. It seems intuitive that a signaling complex containing oncogenic proteins, such as the epidermal growth factor receptor (EGFR), human epidermal growth factor receptor 2 (HER-2), MET (the hepatocyte growth factor receptor, encoded by *c-Met*), and Src (short for sarcoma), and tumor suppressor proteins such as the transcription factor p53 and neurofibromin-1 (NF-1) places FAK at the center of cancer cell growth and regulation (11–15). These observations have stimulated the development of molecular therapeutics that target FAK, but most of these drugs have been kinase enzyme inhibitors, the darling tools of pharmaceutical companies to inhibit cytoplasmic tyrosine kinases like FAK (16). However, this approach has been hampered by difficulties in targeting the adenosine triphosphate (ATP)–binding site of the FAK enzyme as well as by off-target effects from the multiple consensus sequences contained in the kinase domain. Nonetheless, clinical trials have commenced using FAK inhibitors. Preliminary results in phase I studies have shown a limited tumor response with substantial toxicity to normal cells, such as in the gastrointestinal tract (17, 18).

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The problem with the development of FAK as a cancer target is that its nonkinase scaffolding function has largely been ignored. Concerns about the complexity of the FAK molecule and its interactome, as well as the pharmaceutical dogma about the feasibility of targeting and disrupting critical protein-protein interactions, have left the development of scaffold-targeted molecular therapeutics practically untouched. At the same time, there is a growing body of literature that demonstrates the importance of FAK scaffolding in the development, maintenance, and dissemination of cancer (19-22). These data suggest that the FAK interactome and the FAK intrinsic enzymatic activity have related but also independent contributions to its multitude of functions in promoting cancer.

The scaffolding portion of the FAK protein consists of long N- and C-terminal segments to which many proteins bind (Fig. 1). The amino terminus contains a four-point-one, ezrin, radixin, moesin (FERM) domain that has multiple functions: It provides the scaffold for several oncogenic receptor tyrosine kinases and tumor suppressor proteins, physically interacts with other parts of the FAK protein, and spatially organizes this complex interactome. It has been speculated that the FERM domain can physically open its conformation to enable derepression of the FAK kinase domain. In addition, it has been shown that the N terminus of FAK is cleaved and shuttled to the nucleus, where it interacts with nuclear proteins, including p53, a transcription factor for various genes involved in many cellular processes (23, 24). With so many signaling molecules binding to FAK, it has been difficult to determine the exact relevance and directionality of each interaction. However, several important concepts have emerged about the role of the FAK scaffold in cancer.

First, FAK has been shown to integrate signals from integrins and many of the major oncogenes that bind to its scaffold (9, 20). Studies show that the binding of MET, EGFR, or platelet-derived growth factor receptor (PDGFR) to FAK directly phosphorylated the FAK FERM domain at Tyr¹⁹⁴ (25). This event is critical for the activation of FAK at its major autophosphorylation site at Tyr³⁹⁷ that enables Src and other proteins with Src homology 2 (SH2) domains to bind and further activate FAK (26). Furthermore, stimulation of cell motility by growth factors, such as PDGF or EGF, that signal through these receptors did not require FAK kinase activity (20). FAK and EGFR also induced cooperative signals that suppressed apoptosis and enhanced cell survival in breast cancer cells through activation of both the ERK (external signal-related kinase) and AKT [also referred to as the phosphoinositide 3-kinase (PI3K)] pathways (12). Together, these data demonstrate how receptor tyrosine kinases activate FAK and can initiate or enhance cancer-promoting signals independently of the kinase activity of FAK.

Second, interference with scaffold protein interactions has a dramatic effect on cancer cell survival and progression. This was elegantly demonstrated in a report examining the role of the FAK scaffold in the phosphorylation of endophilin A2 by Src (27). Endophilin A2 binds to FAK at the Pro⁸⁷⁸ and Pro⁸⁸¹ (Pro^{878/881}) region, and its subsequent phosphorylation promotes the epithelial-mesenchymal transition (EMT) and self-renewal of mammary cancer stem cells (MaCSCs). When FAK knock-in mice were generated with a P878/881A mutation in a mouse mammary tumor virus-based model of human breast cancer, FAK scaffolding was disrupted, the phosphorylation of endophilin A2 was reduced, and mammary tumor growth and metastasis was decreased, presumably through suppression of EMT and MaCSC proliferation. Intriguingly, this interaction also appeared to be specific for mammary tumor cells, because it was not required for normal mammary gland development. These data further emphasize the attractiveness of the FAK scaffold as a drug target in cancer, because the scaffolding functions and requirements appear to be different between normal and transformed cells (Fig. 2). In another example, transduction of the C-terminal fragment of FAK (FAK-related nonkinase, or FRNK) in breast cancer cells caused

dephosphorylation and degradation of FAK and induction of apoptosis, whereas its transduction in normal mammary epithelial cells caused dephosphorylation but not degradation of FAK, affecting only the polarity of the normal cells without affecting cell adhesion or viability (28). Thus, targeting precise interactions at the FAK scaffold has the potential to inhibit individual signaling pathways within a tumor and to take advantage of the distinct FAK-mediated signaling requirements that exist in cancer cells compared with normal cells.

Finally, an additional model for the FAK scaffold function called sequestration suggests that FAK binds and inactivates a number of proapoptotic proteins, including p53, receptor-interacting protein (RIP), and NF-1, inhibiting them from performing their normal function of inducing death in aberrant cells (29). For example, binding of endogenous p53 by FAK not only inactivates its tumor-suppressive functions but also derepresses the FAK promoter, resulting in an amplification loop that increases the expression of the gene encoding FAK. This raises the possibility that developing drugs to disrupt these interactions may reactivate p53 and its downstream targets, similar to the effects seen with other methods using small-molecule inhibitors to disrupt the interaction between p53 and its negative regulator MDM2 (30).

A final consideration for targeting the FAK scaffold concerns the ability to successfully develop therapeutics that are capable of disrupting these interactions to a degree that causes cancer cell death, either alone or in combination with other therapies. For example, as this approach to FAK targeting has been contemplated, concerns have been raised that the sites of protein-protein interactions are too large for small-molecule antagonists and that the effects of disrupting one interaction will be compensated immediately by another interaction in the cancer cell. Although this approach is in its infancy, it is clear that these interactions can be disrupted by peptides and small molecules with resultant cancer cell death. For example, vascular endothelial growth factor receptor 3 (VEGFR-3) interacts with the FAK C terminus, and a peptide from this region disrupts the interaction between these proteins and causes apoptosis in breast cancer cells (31). Similarly, a small molecule inhibitor identified by *in silico* modeling of this also successfully disrupts these interactions and inactivates VEGFR-3, providing proof-of-principle for this approach to cancer therapeutics (32). Finally, one of the most attractive sites on the FAK scaffold is the Tyr³⁹⁷ autophosphorylation site. Because of the unique biology of its interaction with Src and related SH-2 proteins (19), this region provides a new target to inhibit both FAK and Src and provides advantages for strict kinase enzymatic inhibitors that target the ATP-binding site (33). The small-molecule inhibitor that targets this site was recently shown to have efficacy against glioblastoma both alone and in combination with cytotoxic therapy (34). Thus, the FAK scaffold can be successfully antagonized to disrupt its signaling transduction and cause cytotoxicity in cancer cells.

In conclusion, FAK is a promising cancer target because it is highly expressed in most cancer cells and is associated with promoting oncogenic signals and inactivating tumor suppressive signals. Although current efforts have focused primarily on the kinase function, the FAK scaffold provides a new area for developing highly specific therapeutics against its interactome and can be personalized to an individual tumor phenotype. This approach may be useful in targeting some of the cancer-specific properties of FAK signaling while possibly sparing some of the FAK pathways in normal cells.

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Pharmaceuticals, LLC. University of Florida and Roswell Park Cancer Institute have patents pending and awarded that are based on their work developing FAK inhibitors.

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growth and synergizes with temozolomide. Mol. Cancer Ther. 2013; 12:162–172. [PubMed: 23243059]

Fig. 1. Components of the FAK scaffold that promote tumor cell survival

FAK interacts with many oncogenic tyrosine kinases, tumor suppressor genes, and tumor-related proteins across its broad N- and C-terminal domains.

Fig. 2. Advantages of targeting the FAK scaffold in cancer cells versus normal cells
 Inhibitors that target the FAK scaffold have the potential to specifically target oncogenic-related proteins and reactivate tumor-suppressing proteins in cancer cells while sparing normal cell counterparts that do not rely on these signals for survival.