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Commentary & View

The role of focal adhesion kinase in tumor initiation and progression

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Focal adhesion kinase (FAK) is a nonreceptor tyrosine kinase that acts as a primary regulator of focal adhesion signaling to regulate cell proliferation, survival and migration. While FAK is known to directly influence many fundamental adhesion and growth factor signaling pathways important in cancer and FAK is overexpressed in multiple human cancers studies addressing a causal role for FAK in tumor initiation and progression using transgenic models of human cancer had not been performed. Recently, using tissue-specific FAK-knockout in mouse models of human cancer, the consequences of FAK ablation in carcinoma were demonstrated by multiple independent research groups. Strong consensus evidence indicates that epithelial cells are able to transform in the absence of FAK, but do not undergo a malignant conversion to invasive carcinoma, and as such, metastasis is impaired. This is likely the consequence of decreased Src and p130Cas activation in concert with misregulated actin cytoskeleton dynamics and Rho GTPase signaling. Hence, FAK, as well as the FAK-regulating/regulated signaling network, are viable candidates for cancer metastasis therapies.

Epithelial carcinogenesis involves a complex set of genetic events that promote proliferation, survival and invasion.[1] However, a central role for the extracellular microenvironment, and in particular the epithelial-extracellular matrix (ECM) interaction, in mediating tumorigenesis, invasion and metastasis is now being recognized.[2] For instance, integrins, which can regulate cell-ECM adhesions, play a role in regulating the malignant phenotype and growth of epithelial cells.[3,4] Moreover, targeted deletion of β_1-integrin in the mouse mammary epithelium inhibits tumorigenesis.[5] Hence, integrin-mediated adhesion has been shown to regulate mammary carcinoma formation and progression, raising important questions regarding the roles of integrin-associated signaling molecules in human cancer.

Focal adhesions (FAs) are sites of integrin-clustering that link the actin cytoskeleton to the ECM, functioning primarily to provide physical attachment to the ECM and transduce force between the cell and the ECM. In particular, the FA component focal adhesion kinase (FAK), a nonreceptor tyrosine kinase, acts as a primary regulator of FA signaling by performing a scaffolding function for protein-protein interactions, phosphorylating multiple substrates, and regulating cross-talk between integrin and growth factor signaling to regulate cell proliferation, survival and migration.[6,7] For instance, phosphorylation of FAK at Y397 creates a high-affinity site that is recognized by several Src homology 2 (SH2) domain-containing proteins such as Src, Shc, PI3K and GRB7, and FAK phosphorylation at Y925 by Src links FAK via Grb2 to the Ras pathway (reviewed in refs. 6 and 7). Furthermore, FAK can directly bind to and promote Src-mediated phosphorylation of p130Cas, which is necessary for formation of the p130Cas-Crk-DOCK180 complex and Rac activation, which also promotes migration.[8] Hence, FAK directly regulates many fundamental adhesion and growth factor signaling pathways important in human cancer, suggesting that FAK may play a significant role in tumor formation and metastasis.

Additional evidence that FAK has a significant role in human cancer can be seen from several pathological studies reporting FAK protein overexpression in cancers of the breast, colon, ovary, pancreas, prostate and others.[9] Furthermore, FAK overexpression correlates with more aggressive and invasive breast carcinomas[9] and it was recently shown that the FAK-encoding *PTK2* gene is amplified in human breast cancer, with high levels of FAK mRNA expression predicting significantly shorter metastasis-free survival.[10] Despite these studies, the specific role of FAK in tumor initiation and progression has not been well understood. This is largely because, until recently, causal and mechanistic studies of FAK in vivo have been limited by an embryonically lethal phenotype associated with whole animal FAK knockout. However, the emergence of Cre/LoxP technology has recently allowed a previously unobtainable examination of the consequences of tissue specific FAK ablation in models of human cancer.

In one of the first studies to examine the consequences of FAK ablation in native cells within their resident microenvironment, McLean and co-workers[11] generated conditional "floxed" FAK knockout ($FAK^{flox/flox}$) mice with epidermis-specific FAK deletion and induced papilloma formation (that progresses to invasive squamous cell carcinomas in some animals) with the 7,12-dimethylbenzanthracene (DMBA; which promotes an activating

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Ras gene mutation) and 12-O-tetradecanoylphorbol-13-acetate (TPA). In this chemically-induced skin tumor model, loss of FAK reduced papilloma formation and blocked malignant conversion to squamous cell carcinomas.[11] However, it remained to be seen whether FAK had a causal role in tumorigenesis in cancers that were genetically, and not chemically, initiated.

Recently, four manuscripts[10,12-14] have addressed the role of FAK in breast cancer using the MMTV-polyoma middle-T (PyVT) transgenic model of human breast cancer, which recapitulates the multi-step progression of human breast cancer by advancing from hyperplasia to an adenoma/mammary intraepithelial neoplasia (MIN) mixed phenotype, and then to early and late carcinoma with reliable pulmonary metastasis.[15,16] In each study, tissue-specific ablation of FAK (with mammary-specific Cre expression driven by the MMTV promoter) significantly delayed mammary tumor formation, but did not inhibit tumor formation entirely. Consequently, because work by Muller and co-workers[5] demonstrated that mammary tumor formation in β_1-integrin knockout mice was solely the result of inefficient Cre-mediated recombination (i.e., the lack of β_1-integrin deletion in those cells), it was necessary in each study to determine whether or not transformed epithelial cells in tumors that arose in $FAK^{flox/flox}$ mice (carrying the PyVT and Cre recombinase transgenes) had undergone efficient Cre-mediated recombination, and were truly FAK-null. To address this issue, each study examined FAK expression in early adenoma/MIN regions or in late stage tumors arising in $FAK^{flox/flox}$ mice carrying the PyVT and Cre recombinase transgenes. In total, the presence or absence of FAK in transformed cells was examined by PCR analysis for Cre-mediated recombination,[12] microarray analysis for FAK mRNA levels,[12] western blotting for FAK protein in tumor lysates,[10,12,14] and staining for Cre-mediated recombination[10,13] or FAK protein.[10,12-14] Examination of early adenoma/MIN lesions for the presence of cells that had undergone Cre-mediated recombination showed that epithelial oncogenic transformation could occur in the absence of FAK.[13] In contrast, Pylayeva and co-workers[10] concluded that FAK is required for tumor initiation. However, approximately 15% of the adenoma/MIN lesions they examined stained positive for Cre-mediated recombination,[10] suggesting the possibility of FAK-null lesions. In palpable tumor masses, analysis by Provenzano and colleagues[12] showed that FAK protein levels were decreased and Cre-mediated recombination had taken place in 22 out of 22 $FAK^{flox/flox}$ tumor samples examined, that FAK mRNA levels were significantly decreased in independent samples, and that FAK was absent in cytokeratin-positive epithelial cells composing the tumor mass. These findings were supported by Luo et al.[14] who showed decreased FAK protein levels in $FAK^{flox/flox}$ tumors by western blotting and immunohistochemistry. Importantly, the primary candidate for compensation following FAK loss, the FAK-related kinase Pyk2, showed no increase in expression or activity.[10,12,14] Hence, three[12-14] of the four[10,12-14] studies convincingly demonstrated that FAK is not required for mammary tumorigenesis, providing strong consensus evidence that mammary epithelial tumors can form in the absence of FAK.

Interestingly, although FAK-null epithelial cells were able to transform and compose a tumor mass, these cells possessed a growth defect (with no change in apoptosis within mammary tumors[13]) and did not undergo malignant conversion to invasive carcinoma.[12,13] Ki-67 staining, a marker of proliferation potential, in both early hyperplastic regions[13] and late-stage tumors[12] was significantly decreased in FAK-null cells when compared to control cells, consistent with data showing decreased tumor mass and volume in $FAK^{flox/flox}$ animals.[12,14] This may be the result of decreased Ras-MAPK pathway activation since ERK phosphorylation[12] and cyclin D1 protein expression[14] were significantly diminished in FAK-null tumor cells, consistent with data linking FAK to the G_1 phase of the cell cycle and cyclin D1 expression in fibroblasts[17] and glioblastoma cells.[18] Furthermore, global analysis of microarray data from control and FAK-null glands and tumors demonstrated that the transcriptome in FAK-null tumors more closely resembled FAK-positive tumors than either normal or FAK-null mammary glands,[12] suggesting that FAK regulates a subset of essential genes. More specifically, in $FAK^{flox/flox}$ tumors many of the events associated with epithelial transformation were present and numerous genes were still upregulated relative to the normal gland, but the levels of many of these genes were upregulated to a lesser degree in FAK-null tumors than in FAK competent cells. In particular, loss of FAK resulted in a significant decrease in expression of transcripts associated with the G_2/M phases of the cell cycle when comparing tumors from $FAK^{flox/flox}$ and wild-type control backgrounds. Computational analysis of these genes (and genes associated with metastasis) showed strong enrichment for regulation by a conserved group of transcription factors regulated in the FAK signaling network, including p53;[12] which is of particular relevance to human cancer. p53 is a known tumor suppressor and regulator of both the G_1/S and G_2/M transitions.[19] Recently, Lim et al.[20] demonstrated that loss of FAK resulted in activated p53 and impaired cell proliferation, while nuclear localization of FAK protein inactivated p53 via Mdm2-dependent p53 ubiquitination. Furthermore, since the PTK2 (FAK) gene is a p53 target[21] and FAK overexpression and p53 mutation correlate strongly in human breast cancer patients,[22] FAK-p53 cross-regulation may be a primary axis to regulate fundamental cell behavior during development and disease.

Perhaps of more direct clinical relevance than decreased proliferation in FAK-null cells is the observation that transformed FAK-null epithelial cells were minimally invasive and metastasis was impaired.[12,13] As discussed above, FAK deletion in skin papilloma blocked malignant conversion to squamous cell carcinomas.[11] Furthermore, expression of FRNK, a dominant-negative C-terminal region of FAK, in MTLn3 mammary adenocarcinoma cells significantly reduced lung metastasis in the syngeneic rat model.[23] In the mammary epithelial-specific knockout tumor models, Provenzano et al.[12] demonstrated that tumors in $FAK^{flox/flox}$ animals present with a largely benign, non-invasive, phenotype when compared to FAK-positive epithelial tumors in control animals that had progressed to invasive carcinomas. Moreover, pulmonary metastasis was reduced over 50-fold (with the majority of animals having 0–1 lung metastasis).[12] And Muller and

colleagues demonstrated that, in their system, cells which metastasized to the lung in $FAK^{flox/flox}$ animals resulted from epithelial populations that did not undergo Cre-mediated recombination and therefore competently expressed FAK protein, supporting the conclusion that FAK-null cells do not efficiently metastasize.[13] Combined these studies suggest that loss of FAK inhibits conversion to an invasive phenotype that can undergo local invasion through the stroma and ultimately enter the vasculature (i.e., intravasation) in order to form metastases in distant tissues. However, tail vein injection experiments using primary $FAK^{flox/flox}$ tumor cells expressing fluorescent protein for whole animal bioluminescent imaging (with and without Cre-mediated FAK excision) demonstrated that FAK-null cells do accumulate in the lungs at day 0, but that majority of these cells were no longer present after 24 hours, in contrast to FAK-expressing control cells.[10] Further examination of these cells in the lung capillary bed showed that a significantly higher fraction of the FAK deficient tumor cells underwent apoptosis when compared to control cells, and that surviving FAK-null cells were unable to project extensions across the vessel wall and leave the vasculature (i.e., extravasation).[10] Hence, it seems likely that FAK plays a key role in both local invasion through the collagenous stroma, leading to intravasation, as well as migration/invasion out of the vasculature and into the ECM environment of the distant tissue. Combined, these data suggest that in vivo, FAK is a primary regulator of cell motility during multiple stages in the metastatic process and therefore FAK, as well as the FAK-regulating/regulated signaling network, are viable candidates to target for cancer metastasis therapies.

FAK is well recognized as a central regulator of cell motility on 2D substrates (reviewed in refs. 6 and 7), however, its role in regulating cell migration and invasion in 3D ECM environments, particularly those associated with invasion and metastasis, is less understood. In contrast to cell migration on 2D surfaces, migration within 3D microenvironments requires that cells progress through the extracellular matrix (ECM) using highly diverse cell morphologies and actively remodel the matrix.[24,25] Microarray analysis of FAK-null epithelial cells in mammary tumors revealed decreased expression of a number of transcripts for proteins that are either components of or regulate FAs, Rho-family GTPases, and the actin cytoskeleton.[12] Thus, the loss of invasiveness in FAK-null epithelial cells may be due in part to misregulated actin dynamics that impair cell migration. Furthermore, src phosphorylation[12] as well as src-mediated p130Cas phosphorylation at Y249[12] and 410,[10] which promote p130Cas-Crk binding and enhanced cell migration, was decreased in FAK-null mammary tumor cells. Interestingly, in these cells, transcripts for the adaptor protein Crk, as well as Dock180, Elmo and Rac1 were also downregulated,[12] suggesting that part of the migration defect in FAK-null mammary epithelial cells may be due to suppression of the p130Cas-Crk-Dock180/Elmo complex and Rac activation. Furthermore, alignment of FAK-regulated tumor cell transcriptome data[12] with data from Wang et al.[26] which identified a set of genes differentially expressed in an invasive metastatic sub-population of PyVT mammary tumor model cells, identified 98 genes that are regulated by FAK and predictive of human breast cancer patient metastasis-free outcome. Hence, continued work to understand the mechanistic role of FAK in regulating 3D matrix adhesions and motility events in 3D cell microenvironments is needed.

In addition to FAK's known role in regulating focal adhesion signaling in 2D culture, FAK is also known to play a role in mechanosensing and mechanotransduction.[27] Interestingly, regions of local invasion have altered ECM architectures that influence local matrix stiffness[28] and FAK overexpression correlates with more aggressive and invasive human breast carcinomas.[9] As such, it is reasonable to hypothesize that the increased stiffness associated with breast adenomas and carcinomas[29,30] and increased matrix stiffness from increased collagen density and alignment is conducive to FAK activation, and that this in turn promotes invasive tumor cell behavior. Furthermore, additional alterations to the ECM (change in composition, crosslinking etc.,) that increase matrix stiffness, or activation of oncogenes that increase cellular contractility, which can promote integrin-clustering and/or matrix re-organization to produce a stiffer microenvironment, could lead to aberrant hyperactivation of FAK. And chronic increases in FAK activity may help drive cells that are positioned near the cell-matrix interface toward conversion to an invasive sub-population with metastatic potential.

Hence, although FAK has been well studied in 2D culture, largely in the context of 2D focal adhesions, a better understanding of FAK's role in 3D environments both in vitro and in vivo is required. Understanding the architecture of the 3D matrix adhesion and how it does or does not differ from the 2D hallmark focal adhesion, as well as, a better understanding of the peripheral signaling network regulated by FAK in 3D adhesions is needed. In particular, in addition to information regarding how FAK is regulated through molecular interactions, a better understanding of how the composition, architecture and stiffness of the microenvironment influence 3D matrix adhesions, and FAK's role in regulating subsequent adhesion-mediated signaling will provide great insight into cell growth, survival and motility in relevant 3D microenvironments. Furthermore, while recent studies have, for the first time, began to elucidate the role of FAK in tumor initiation and progression, much work remains to be completed to understand the molecular mechanisms by which tumor cells transform in the absence of FAK, but possess a deficit in proliferation and lack the ability to be invasive and produce metastasis.

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