HHS Public Access

Author manuscript

Nat Rev Cancer. Author manuscript; available in PMC 2015 March 19.

Published in final edited form as:

Nat Rev Cancer. 2014 September ; 14(9): 598–610. doi:10.1038/nrc3792.

FAK in cancer: mechanistic findings and clinical applications

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Preface

Focal adhesion kinase (FAK) is a cytoplasmic protein tyrosine kinase that is over-expressed and activated in several advanced-stage solid cancers. FAK promotes tumor progression and metastasis through effects on cancer cells, as well as stromal cells of the tumor microenvironment. FAK's kinase-dependent and –independent functions control cell movement, invasion, survival, gene expression, and cancer stem cell self-renewal. Small molecule FAK inhibitors decrease tumor growth and metastasis in several preclinical models and possess initial clinical activity in patients with limited adverse events. We discuss FAK signaling effects on both tumor and stromal cell biology that provide rationale and support for future therapeutic opportunities.

Keywords

focal adhesion kinase; tumor growth; metastasis; tumor microenvironment; FAK inhibitors

Introduction

Focal Adhesion Kinase (FAK) is a multi-functional regulator of cell signaling within the tumor microenvironment[1–3]. During development and in various tumors, FAK promotes cell motility, survival and proliferation through kinase-dependent and -independent mechanisms. In the past few years, phase I and II clinical trials have been initiated with FAK inhibitors; yet, some of FAK's functions in tumorigenesis remain under investigation.

Chromosomal region 8q24.3, which encompasses *PTK2* (encoding FAK), is linked to ovarian cancer susceptibility[4]. Large databases such as The Cancer Genome Atlas show that FAK mRNA levels are elevated in serous ovarian tumors (~37%)[5] and invasive breast cancers (~26%)[6] with correlations to poor overall patient survival[7, 8]. Increased FAK mRNA levels are also found in several other human malignancies (Figure 1A)[3]. Studies with tumor tissue arrays find that FAK activation, as determined by phosphospecific antibody recognition of the FAK tyrosine (Y) 397 auto-phosphorylation site, increases with tumor progression[3]. However, unlike classical oncogenes such as Ras or PI3-kinase (PI3K), only a few missense mutations within *PTK2* are found in tumors[5]. Instead, elevated FAK activity is

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Competing interests statement

The authors declare no competing financial interests.

associated with *PTK2* amplification, consistent with a model whereby increased FAK dimerization induced by higher FAK levels contributes to catalytic activation[9].

Here, we discuss advances in understanding FAK signaling connections in tumor and stromal cells. We cover the intricate roles of FAK in tumor invasion, growth, and metastasis. We highlight genetic mouse models used to elucidate new roles for FAK in endothelial cells (ECs) and discuss how stromal FAK signaling contributes to tumor progression. Finally, we summarize new translational developments using small molecule FAK inhibitors.

FAK regulation

Control of FAK expression

Nuclear factor κB (NFκB) and p53 are well-characterized transcription factors that activate and repress the *PTK2* promoter, respectively[10, 11]. Other transcription factors such as Nanog[12], Argonaute2 (Ago2)[13], and PEA3[14] also increase *PTK2* promoter activity. Nanog promotes FAK expression in colon carcinoma cells and as part of a signaling loop, Nanog activity is increased by FAK phosphorylation[12]. Ago2, a part of the cellular RNA interference machinery, is amplified in hepatocellular carcinoma and induces FAK transcription[13]. Ago2-silencing reduces FAK levels and concomitantly blocks tumorigenesis and metastasis in mice. Elevated PEA3 and FAK levels correlate with metastatic stages in human oral squamous cell carcinoma[14]. PEA3 induces FAK expression *in vitro* and silencing of either PEA3 or FAK reduces metastasis of human melanoma xenografts. Given the complexity and size of the *PTK2* promoter region, it is likely that transcription factor combinatorial effects regulate *PTK2* transcription.

FAK is also subject to alternative splicing as *PTK2* with deletion of exon 33 (FAK amino acids 956–982), identified in a subset of breast and thyroid patient samples, results in enhanced cell motility and invasion[15]. However, this deletion likely disrupts FAK linkage to integrins and it is unclear how truncated FAK may function. *PTK2* with deletion of exon 26, also occurring in breast cancer, removes a FAK C-terminal domain caspase cleavage site and results in increased FAK protein stability and anti-apoptotic signaling[16]. Interestingly, alternative splicing or increased FAK mRNA expression does not always translate into elevated FAK protein levels[17]. FAK mRNA turnover mediated by microRNA-7 blocks orthotopic breast carcinoma growth and lung metastasis in mice, and microRNA-7 expression in breast cancer patient samples inversely correlates to cancer stage[18]. At the protein level, FAK is subject to proteasomal or calpain-mediated degradation[19]. Poly-ubiquitination by the E3 ligase mitsugumin 53 (also known as TRIM72) promotes FAK proteasomal degradation during myogenesis, but this has not been tested in tumor cells[20]. However, in general, FAK protein levels are elevated in advanced stage solid tumors. Together, these results support the notion that elevated FAK expression is connected to several tumor-associated phenotypes.

Regulation of FAK activity

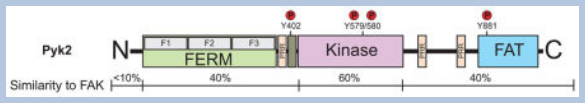
FAK is a cytoplasmic tyrosine kinase that associates with receptors at the plasma membrane and with distinct protein complexes in the nucleus[21]. Elucidating the regulatory mechanism(s) of how FAK associates with these distinct signaling complexes is key to

understanding FAK biological function. FAK domain structure (Figure 1B) consists of an N-terminal FERM (band 4.1-ezrin-radixin-moesin) domain, a central kinase region, proline-rich domains, and a C-terminal focal adhesion targeting domain[2, 19]. Proline-rich tyrosine kinase 2 (Pyk2), a FAK ortholog with ~45% amino acid sequence identity, can compensate for some FAK functions after FAK loss in knockout (KO) mouse models (Box 1)[22–24].

Box 1

Commonalities and differences between Pyk2 and FAK

Proline-rich tyrosine kinase-2 (Pyk2) shares a similar domain organization with focal adhesion kinase (FAK) (see figure), with 60% sequence identity in the central kinase domain, and the conserved arrangement of proline-rich regions (PRRs) and tyrosine phosphorylation sites. Phosphorylation of Pyk2 Y402 and Y881 create Src-homology-2 (SH2) binding sites for Src and growth-factor-receptor-bound-2 (GRB2), respectively. Pyk2 and FAK contain C-terminal focal adhesion (FA) targeting (FAT) domains that bind to paxillin[19, 136]. However, Pyk2 shows peri-nuclear distribution and, unlike FAK, is not strongly localized to FAs. Substitution of the Pyk2 C-terminal domain with that of FAK facilitates co-localization of a Pyk2-FAK chimera to β1 integrin-containing FA, indicating that there are significant binding differences between FAK and Pyk2[19]. For instance, the FAK FAT domain uniquely binds the integrin-associated protein talin[3]. Although Pyk2 can be activated by integrins, this is highly dependent on Src activation. Upon FAK KO in fibroblasts, increased Pyk2 levels induce an intrinsic mechanism promoting cell survival[23]. Consistent with findings in ovarian carcinoma cells, this is in part mediated through nuclear translocation and selective regulation of the p53 tumor suppressor by Pyk2. Phosphorylation of Pyk2 Y881 has been proposed as a prognostic marker for non-small-cell lung cancer progression[137]. In this cancer type, Pyk2 is positively correlated to the expression of cancer stem cell markers, indicating a possible mode of action. In glioblastoma cells, Pyk2 is regulated by a specific microRNA, miR-23b, that does not target FAK[138] and supports the idea of differential Pyk2 and FAK functions. Pyk2 signaling upon FAK knockout has also been linked to increased Rho GTPase activation[22], facilitation of angiogenesis[24], regulation of macrophage motility[101], and control of tumorigenic outgrowth mediated by mammary cancer stem cells[139]. Due to these functions, Pyk2 activity could compromise the outcome of FAK-targeted therapy; indeed FAK-selective inhibitors have been shown to enhance Pyk2 tyrosine phosphorylation in endothelial cells[24].



The best-characterized mechanism promoting FAK activation involves integrin receptor clustering upon cell binding to extracellular matrix (ECM) proteins which may involve FAK dimerization[9]. This leads to FAK auto-phosphorylation at Y397, binding of Src-family kinases to the phosphorylated site, Src-mediated phosphorylation of the FAK kinase domain activation loop (Y576/577), and formation of an activated FAK-Src complex[1–3]. Indirect

interactions between the FAK C-terminal domain and integrins at focal adhesions (FAs) mediate the integrin/FAK linkage, as over-expression of a FAK C-terminal fragment blocks integrin-mediated FAK activation[19]. Alternatively, the activation loop within the FAK kinase domain is also directly phosphorylated by the RET receptor, thereby enhancing FAK kinase activity[25]. FAK phosphorylation at Y397 can also occur in trans[9].

Recent studies show that FAK FERM domain plays a prominent role in the intra-molecular regulation of FAK activity by binding to the kinase domain and blocking FAK Y397 site accessibility and auto-phosphorylation[21, 26, 27]. Studies with a fluorescent biosensor revealed FAK FERM conformational changes upon phosphoinositide lipid interaction and upon cell binding to ECM[27, 28]. FAK FERM domain interaction with membrane-associated proteins such as tetraspan TM4SF5 or growth factor receptors can also induce FAK activation[29, 30]. FAK activity is also increased via FAK FERM domain alterations induced by changes in pH and increased cell-ECM tension[31–34]. Local pH increases promote FERM conformational changes *via* His58 deprotonation, mediated in part by plasma membrane sodium/hydrogen transporters like NHE1[32]. Increased intracellular pH commonly occurs in cancer and increased matrix stiffness or forces associated with collagen fiber cross-linking trigger increase FAK Y397 phosphorylation and tumor progression in mouse breast cancer models[32, 33]. It is likely that context-dependent stimuli trigger FAK activation through steps involving the FERM domain, FAK dimerization, or other yet to be determined mechanisms.

FAK in tumor cells

FAK is at the intersection of various signaling pathways promoting cancer growth and metastasis (Figure 2). This includes kinase-dependent control of cell motility[19], invasion[35], cell survival[11, 36], and transcriptional events promoting epithelial-mesenchymal transition (EMT)[37–39]. Additionally, kinase-independent scaffolding functions of FAK can influence cell survival or breast cancer stem cell proliferation[11, 40, 41]. Understanding the balance between kinase-dependent and -independent functions is key to the interpretation of FAK-related phenotypes. Conditional tissue-specific FAK floxed mouse models and chemical FAK inhibitors have allowed the delineation of several FAK-associated signaling pathways (Table 1). For example, several groups have used polyoma virus middle T antigen (PyMT)-driven breast tumor models combined with tissue-specific FAK knockout through the mouse mammary tumor virus (MMTV) promoter (MMTV-PyMT model)[42–45] to assess FAK function in tumor progression.

FAK promotes invasive cell phenotypes

Tumor cell invasion into the surrounding microenvironment is a key step in cancer progression, allowing cancer cells to form metastases at secondary locations. This requires transition to a motile phenotype through changes in FA and cytoskeletal dynamics, and alterations in matrix metalloproteinase (MMP) expression or activation to facilitate ECM invasion. EMT, which is driven by a transcriptional program, supports the progression to these invasive properties.

Canonical FAK signaling is linked to FA formation and turnover[2, 19, 46]. FAK recruitment and activation at nascent FAs involves binding to the Rho guanine nucleotide exchange

factor (GEF) ARHGEF28 (p190RhoGEF or Rgnef), and a FAK-ARHGEF28 signaling complex promotes local invasion of orthotopic colon carcinomas in mice[47, 48]. FAK-ARHGEF28 dimerization and the associated increase in FAK activation is thereby linked to elevated tyrosine phosphorylation of paxillin, an adaptor protein involved in FA maturation[49]. FAK also recruits the integrin activating adapter protein talin to nascent FAs, occurring independently of direct talin binding to β integrins[50].

FAK not only promotes FA formation-maturation, but also drives FA turnover through control of targeted FA protein proteolysis. Mutations that disrupt FAK-talin binding inhibit proteolytic talin cleavage, preventing efficient FA turnover[50]. Protein cleavage is mediated by proteases like calpain-2 or caspase-8 containing multi-protein complexes[51]. Additionally, FAK proline-rich sites facilitate interaction with the actin-binding protein cortactin, whose phosphorylation by FAK contributes to FA turnover[52]. In head and neck cancer, blocking of an integrin/FAK/cortactin/JNK1 signaling cascade through specific antibodies against β1 integrins renders cells sensitive to radiotherapy and delays xenograft growth[53]. Interestingly, FAK depletion decreases the abundance of tyrosine-phosphorylated proteins at FAs, while simultaneously increasing their levels at invadopodia in a Src-dependent manner[54]. However, increased invadopodia formation is not sufficient to promote tumor cell invasion in the absence of FAK, indicating that increased FAK-mediated cell motility underlies an invasive cell phenotype.

Dynamic rearrangement of the actin cytoskeleton is another integral component of cell movement and cell protrusion. FAK-associated proteins such as talin and cortactin bind actin and link FAs to changes in actin dynamics[19]. Additionally, catalytically inactive FAK associates with Arp2/3 through its FERM domain and enhances f-actin polymerization in cooperation with N-WASP[55, 56]. These events are proposed to occur prior to integrin-mediated FAK activation. In this model, the FAK FERM domain may function as scaffold to direct Arp2/3 activity to cell protrusions preceding mature FA formation.

MMP expression and activation at cell protrusions facilitates matrix invasion of motile cells. FAK activity increases MMP-9 expression and spontaneous breast carcinoma metastasis in a syngeneic and orthotopic mouse model[57]. Other studies show that MMP regulation and surface presentation in cancer cells involves multiple downstream pathways such as p130Cas[58] and the PI3K/Akt/mTOR cascade[59]. Although mTOR effects on MMPs are not clearly understood, the FAK-p130Cas complex targets MMP-14 to FAs and promotes MMP-14 membrane surface presentation[58]. Knockdown of FAK or p130Cas does not alter the generation of pancreatic carcinoma protrusions, but prevents ECM degradation. During *in vivo* metastasis of human breast cancer cell xenografts in mice, MMP-14 function also has been proposed to require FAK signaling through an alternative pathway involving activation of Krüppel-like factor 8[60].

EMT-like transcriptional programs have been shown to drive tumor cell motility and invasion, and FAK signaling contributes to this EMT profile change[61]. FAK re-expression in FAK-null cells drives Snail1-induced EMT[37]. FAK scaffolding increases endophilin A2 phosphorylation leading to alterations in EMT markers, including MMPs, and affecting PyMT-induced breast tumor progression[38]. Indirect factors such as micro-RNA-7-mediated

reduction of FAK expression results in loss of mesenchymal markers along with increased E-cadherin expression in breast tumor models[18]. Additionally, FAK may also act in a proximal manner to affect the dynamics of E-cadherin internalization in tumor cells[39]. Over-expression of a FAK mutant unable to be phosphorylated on multiple sites in colon carcinoma cells blocks Src-induced E-cadherin internalization[62] and pharmacological inhibition of FAK activity increases cell-cell adhesion strength in part by stabilizing E-cadherin surface expression[63]. Together, these results implicate FAK in both the cell surface and transcriptional regulation of E-cadherin levels in tumor cells.

FAK drives tumor survival and growth

FAK promotes cell survival through kinase-dependent and -independent linkages. Kinase-dependent pathways include signaling through the PI3K/Akt cascade (Figure 2)[3]. Integrins and other extracellular stimuli induce FAK-survival signals to prevent anoikis and other types of cell death[3]. In ovarian cancer, tumor ascites prevents death-inducing signals via activation of an integrin αvβ5/FAK/Akt signaling pathway[64]. Moreover, FAK activity was shown to promote anchorage-independent survival of murine ovarian carcinoma cells, independent of effects on Src kinase activity[8].

FAK signaling is also associated with ovarian cancer resistance to paclitaxel-induced cell death[65]. Pharmacological FAK inhibition enhances chemosensitivity in taxane-resistant cells by decreasing YB-1 transcription factor phosphorylation in an Akt-dependent manner[65]. Additionally, phosphorylation of FAK Y861 by protein tyrosine kinase 6 has been postulated to initiate an Akt-dependent anti-anoikis cascade[66]. Simplistically, increased FAK activity-mediated survival signals *in vitro* correspond to increased tumor growth. However, it remains undetermined whether mutational activation of the PI3K or Akt signaling pathways, commonly occurring in tumors, may bypass effects of FAK inhibition. Additionally, FAK functions downstream of G-protein-linked receptors for stress hormones like norepinephrine, and prevents ovarian cancer cell anoikis[7].

FAK activity also enhances cell cycle progression (Figure 2)[46] and recent studies have linked this to a matrix stiffness-sensitive signaling linkage between FAK, p130Cas, Rac GTPase, and the actin binding protein lamellipodin[67]. However, studies with transgenic mice show that kinase-dead (KD) FAK-expressing fibroblasts and ECs grow normally[68, 69]. Thus, FAK kinase activity is not essential for proliferation of all cell types. Yet, tumor cells require FAK activity in the processes of extravasation and proliferation of micro-metastases in foreign tissue environments[35, 70]. Although conditional FAK knockout (KO) in the intestinal epithelium of transgenic mice shows that FAK is dispensable for normal intestinal homeostasis, these mice require FAK for intestinal regeneration following DNA damage[71]. Reduced cyclin D1 levels associated with decreased epithelial proliferation also occur in the colonic epithelium of FAK knockout mice during mucosal wound healing[72]. Additionally, double deletion of FAK and the tumor suppressor adenomatous polyposis coli (APC) suppresses tumor formation induced by APC loss[71]. Although it has been proposed that FAK inhibition (as opposed to FAK KO) may suppress colorectal cancer tumor formation, the mechanisms connecting FAK activity to cell proliferative responses remain unresolved.

A reduction in cyclin D1 levels upon FAK loss is associated with decreased ERK activity in mammary epithelial cells[73]. Similarly, FAK deletion in the mouse PyMT breast tumor model decreases Src-mediated p130Cas activation and signaling to ERK[43]. *Ex vivo* knockout and subsequent transient re-expression of FAK in cells isolated from PyMT-induced breast tumors showed that FAK Y397 auto-phosphorylation, catalytic activity, and the integrity of FAK proline-rich region 2 (Figure 1B) are needed for cell proliferation as well as anoikis resistance[45]. In human breast carcinoma cell lines, FAK knockdown prevented tumor growth driven by oncogenic mutations in the Ras and PI3K signaling pathways[45]. These studies support the notion that FAK serves as a regulator for cell intrinsic signals promoting proliferation.

The location of FAK signaling complexes controlling cell survival and growth is also varied. Studies with FAK-KD mutants and pharmacological inhibitors support a kinase-independent scaffolding role in the nucleus[38, 40, 68]. *In vitro*, FAK nuclear translocation occurs after oxidative stress[74] and upon pharmacological FAK inhibitor treatment[75] (Figure 2). Nuclear FAK restricts p53 tumor-suppressive functions by promoting Mdm2 E3 ligase-dependent ubiquitination and degradation of p53[11, 40]. This prevents p53 transcriptional activity, reducing the levels of targets like the p21CIP1 cell cycle inhibitor[76]. FAK/p53 regulation may also release p53-induced inhibition of the *PTK2* promoter, increasing FAK mRNA transcription. Interestingly, in a squamous cell carcinoma mouse model, FAK loss increases cell resistance to DNA damage after ionizing radiation, associated with p53-mediated induction of DNA repair[77]. Although this study raises the issue whether FAK inhibition in combination with radiation may be clinically disadvantageous, alternative conclusions find that endothelial FAK knockout acts to sensitize tumors to DNA-damaging therapy[78].

FAK control of cancer stem cells

Cancer stem cells or tissue-specific progenitor cells can facilitate tumor growth, and in certain cancers, FAK signaling has been linked to the maintenance of these cell types. In the MMTV-PyMT mouse model, conditional embryonic FAK deletion suppresses mammary cancer stem cell (MaCSC) generation[44]. FAK loss reduces number and size of mammospheres and MaCSC surface markers. In the PyMT breast tumor model, FAK effects on MaCSC-associated markers are linked to FAK scaffolding effects on endophilin A2[38]. Subsequent studies showed that FAK regulates MaCSC and normal progenitor cell activities *via* both kinase-dependent and -independent mechanisms[41]. In a conditional FAK-KD knockin mouse, loss of FAK kinase activity impairs luminal progenitor proliferation and reduces the MaCSC number, but does not affect FAK scaffolding functions required for basal mammary stem cell self-renewal[41]. This suggests that pharmacological FAK inhibition may be effective in only the subset of human breast cancer subtypes arising from luminal progenitor cells. However, this needs to be tested further as orthotopic tumor growth and spontaneous metastasis of basal-like murine 4T1 and human MDA-MB-231 breast carcinoma grafts is prevented by FAK inhibition in mice[79]. Inhibiting FAK also induces apoptosis in precursor B cells with a deletion in the Ikaros transcription factor[80]. Ikaros loss prevents B cell differentiation and locks precursor cells in a state of high adhesion-dependent proliferation, a process associated with B cell acute lymphoblastic leukemia[80]. Precursor B cells rely on integrin/FAK signaling as major driver of cell proliferation,

survival, and self-renewal. Thus, FAK may be a key signaling protein downstream of integrins in the control of stem cell proliferation.

FAK in the stromal microenvironment

Signals between tumors and cells in the surrounding microenvironment can drive tumor progression. At sites of micro-metastases, tumor cells need to adapt to a new microenvironment and/or modify it. As mentioned above, non-cellular microenvironment cues, such as matrix composition or stiffness, cytokines, growth factors, integrins and pH changes trigger FAK activity that influences various aspects of tumor growth and metastasis. In this section, we will focus on the cellular microenvironment component, as FAK signaling plays important roles within vascular and non-vascular stromal cells in the tumor microenvironment.

Endothelial FAK in the control of progression

Several transgenic mouse models support the importance of FAK expression and activity in ECs during vascular development and tumor angiogenesis (Table 1). EC proliferation and survival are fundamental events promoting angiogenesis. Global, as well as EC-specific FAK-KO[81–83], FAK-KD[68, 76], or deletion of residues surrounding FAK Y397[84] result in early embryonic lethality associated with multiple vascular defects, such as hemorrhage and edema. *In vitro*, primary ECs from these mice exhibit defects in survival, proliferation, sprouting, migration, and tubulogenesis.

EC FAK is considered a therapeutic target in vascular disease[85, 86]. In tumor-associated ECs, FAK mRNA and protein are elevated[87] and FAK Y397 phosphorylation is increased[88]. Stimulated changes in EC migration are a fundamental component of angiogenesis and FAK activation downstream of growth factor, integrin, and cytokine receptors contributes to EC motility[86]. Pharmacological FAK inhibition prevents EC motility and tubulogenesis *in vitro*, aortic sprouting *ex vivo*, and growth factor-stimulated angiogenesis in mice[24, 89]. In a proteomic screen analyzing invading versus non-invading ECs in 3D-collagen matrices, pro-angiogenic factors promote the association of RACK1 and vimentin with FAK during endothelial invasion[90]. This linkage is hypothesized to mediate changes in EC shape and FA formation, initial steps in tumor neovascularization.

Orthotopic glioma implantation in adult mice with EC-specific FAK deletion results in tumor vascular normalization associated with reduced vascular permeability (VP), partial restoration of EC-EC and astrocyte-EC interactions[91]. Similarly, EC FAK deletion in melanoma and lung carcinoma-bearing mice results in tumor growth inhibition by impairing VEGF-induced angiogenesis[92]. Surprisingly, increased melanoma and lung carcinoma growth occurs in FAK heterozygous compared to wildtype mice[93]. This FAK heterozygous phenotype was associated with increased angiogenesis, but it is unclear whether this represents compensatory mechanisms or reduced FAK activity. In contrast, pharmacological FAK inhibition prevents EC sprouting in a dose-dependent manner[24] and FAK inhibitors are potent anti-angiogenic agents[89]. Furthermore, FAK inhibition reduces tumor angiogenesis in animal models of human colon[94], ovarian[8, 88, 95] and hepatocellular carcinoma[96], supporting a stimulating role of FAK activity in angiogenesis.

During development, EC specific FAK-KO results in decreased EC proliferation and survival, and increased apoptosis[81, 83]. FAK-KO results in reduced VEGF-stimulated Akt phosphorylation, associated with reduced EC proliferation and increased cell death[92]. It remains unclear, whether FAK plays differential roles in maintaining basal EC proliferation and survival, as opposed to stimulated events occurring during angiogenesis. In adult mice with a developed vasculature, EC FAK-KO does not trigger apoptosis, in part because Pyk2 is expressed and compensates for FAK loss in promoting cell survival[23, 24] (Box 1). However, as mice with double FAK- and Pyk2-KO within ECs have not been described, it remains undetermined whether FAK-KD expression in a Pyk2-null background may function to promote EC survival and, if so, whether this would be dependent on a FAK-KD scaffolding or nuclear function. These experiments would provide important fundamental insights, as increased FAK nuclear translocation occurs upon treatment of ECs with FAK inhibitors[75]. Future studies need to determine the specific molecular roles of kinase-inhibited FAK in potentially promoting EC survival, but preventing angiogenesis.

The vasculature of tumors is often disorganized, tortuous, and leaky[86]. These changes are associated with alterations in EC adherens junctions (AJs) that maintain vascular barrier function. During development, global or EC-specific FAK-KD expression results in disorganized EC patterning and defective blood vessel morphogenesis[68, 76] (Table 1). In human ECs, knockdown of FAK enhances AJ stability, associated with enhanced cell membrane localization of vascular endothelial cadherin (VE-cadherin)[97]. Although it was reported that conditional deletion of FAK in mouse endothelium disrupts lung barrier function in part through RhoA activity deregulation[82], this phenotype has not been observed in two other EC FAK-KO mouse models[24, 91]. However, in other cell types, elevated RhoA activity occurs upon FAK-KO[22, 98] and has been linked to alterations in Pyk2 and ARHGEF28 signaling[22]. Additionally, loss of FAK activity disrupted AJ formation during development[76], but it remains unclear whether this is distinct from the observed embryonic lethal phenotype. In adult mice, pharmacological or genetic inhibition of FAK activity does not alter basal vascular barrier formation, but instead prevents paracellular permeability increases by VEGF[69]. These results support both kinase-dependent and -independent connections of FAK to AJ regulation.

VEGF promotes VP via tension-independent FAK activation, rapid FAK localization to EC cell-cell junctions, binding of the FAK FERM domain to VE-cadherin, and direct FAK phosphorylation of β-catenin, facilitating VE-cadherin-β-catenin dissociation and EC AJ breakdown[69]. In glioma studies, FAK expression is essential for tumor-induced VP in the brain of mice[91]. The signaling pathway promoting FAK activation downstream of VEGFR is different from that triggered by integrins, as VEGF-stimulated FAK activation and binding to VE-cadherin is regulated by a conformational change within the FAK FERM domain[69]. However, the molecular mechanisms underlying this regulation remain undetermined. Subsequent studies revealed that FAK kinase activity is required for Src translocation to AJs, and that FAK controls VE-cadherin Y658 phosphorylation, required to promote VEGF-stimulated VP and tumor cell extravasation[88]. Notably, FAK-KD expression and VE-cadherin Y658F mutation in ECs prevents tumor cell transmigration across EC barriers. Importantly, EC-specific FAK-KD expression in mice decreases VEGF-enhanced

tumor cell extravasation *in vivo*, and EC FAK-KD expression prevents spontaneous orthotopic melanoma metastasis without affecting primary tumor growth[88]. While the mechanism for this anti-metastatic effect is not known, tumor- and VEGF-associated VE-cadherin internalization within ECs depends on FAK activity. Mechanistically, these results are consistent with roles of EC FAK in modulating expression of VCAM-1, a protein mediating the adhesion of immune cells to ECs[75], and E-selectin[99]. The latter is proposed as a mechanism to prepare vascular microenvironment sites for the seeding of metastatic disease (Figure 3).

Overall, these studies reveal new roles for EC-specific FAK activity in the control of metastasis. Further studies are needed to determine whether anti-tumor growth effects of FAK inhibitors are primarily mediated through signaling inhibition within tumor cells or through effects on EC function. FAK inhibition may possess beneficial effects for patients in the control of tumor and other vascular pathologies by preventing VP and angiogenesis without negative effects on EC survival.

FAK promotion of tumor growth via effects in non-vascular stromal cells

In addition to FAK signaling within tumor and ECs, non-vascular stromal FAK functions also contribute to multiple aspects of tumor progression (Figure 4). Neutrophils and macrophages are major effectors of immune responses in conditions that induce inflammation, including cancer. Myeloid-specific (lysozyme M Cre) FAK-KO in mice decreases the capability of neutrophils to kill pathogens and triggers accelerated spontaneous death[100]. Other studies question whether FAK is expressed in neutrophils, and have used a similar myeloid-specific FAK-KO model to study macrophage function[101]. Primary FAK-KO mouse bone marrow macrophages have impaired directional chemotaxis *in vitro* and exhibit decreased monocyte recruitment to inflammatory sites *in vivo*. Pyk2 loss or combined FAK/Pyk2 knockdown have similar effects to FAK-KO, supporting overlapping signaling roles in macrophages[101]. Tumor-associated macrophages (TAMs) are key contributors to tumor progression and cancer-related inflammation. In a mouse model of pancreatic ductal adenocarcinoma, FAK inhibitor administration does not alter tumor angiogenesis, necrosis or apoptosis, but results in fewer TAMs within tumors and decreased primary tumor size[102]. In breast cancer mouse models, pharmacologic FAK inhibition decreases tumor growth, associated with diminished leukocyte and macrophage tumor infiltration[79, 103]. Other studies have used MX1-Cre to delete FAK in hematopoietic cells[104, 105] and PF4-Cre to delete FAK in megakaryocyte lineages[106]. Resulting mouse and cell phenotypes are variable and have not yet been integrated into tumor studies (Table 1). Since potential “off-target” effects have been proposed to account for the inhibitory effects of FAK inhibitors on platelet spreading[107], and most hematopoietic cells express Pyk2, combined Pyk2-FAK-KO or FAK-KD transgenic models are needed to further our understanding of tumor-immunological roles for FAK signaling.

Cancer-associated fibroblasts (CAFs) influence tumor progression through mechanisms that are not fully understood. In a breast carcinoma model, tumor-secreted lysyl-oxidase-like-2 (LoxL2) activates fibroblasts and promotes α-smooth muscle actin expression in a FAK-dependent manner *via* Akt activation[108]. This signaling was blocked *in vitro* by

pharmacologic inhibition of FAK but not of Src[108]. Since LoxL2 catalyzes matrix cross-linking, the effects on FAK may also be mediated through increased tissue tension[33]. Accordingly, FAK expression and activity are elevated in fibroblasts from lung fibrosis patients. Interestingly, FAK inhibition in a bleomycin-induced fibrosis mouse model shows marked abrogation of lung fibrosis[109]. Although early studies suggested that FAK expression might inhibit fibroblast differentiation and α-SMA expression, the potential role of compensatory Pyk2 levels in FAK-null fibroblasts was not addressed[110]. Finally, similar to TAMs, FAK inhibition in a pancreatic ductal adenocarcinoma model decreased CAF recruitment and tumor size[102]. Together, these studies support the notion that FAK promotes pro-tumor CAF functions.

FAK also influences the tumor microenvironment indirectly. FAK signaling within breast carcinoma cells regulates VEGF expression that, as described above, promotes VP and angiogenesis[111]. In acute myeloid leukemia, FAK expression and activity are important in the production of interleukin-6, -8, SDF-1 and angiopoietin-1, factors crucial for mesenchymal stromal stem cell maintenance[112]. FAK inhibition decreases TNFα-induced breast cancer cell interleukin-6 production and is correlated with reduced tumor-associated splenomegaly and tumor-associated CD45+ cells in a syngeneic model[79]. Overall, there is emerging evidence for the importance of FAK signaling as regulator of EC, hematopoietic cell, platelet, macrophage, and fibroblast signaling in the tumor microenvironment. Importantly, phenotypes associated with FAK inhibition show that there are multiple points of regulation for FAK function not only in tumor cells but also in the tumor microenvironment.

FAK in clinical applications

FAK functions drive various tumor-promoting signaling pathways (Figure 2)[3, 94, 113, 114] and small molecule FAK inhibitors are emerging as promising chemotherapeutics, as FAK inhibition in mouse models prevents tumor growth, metastasis, vascular permeability, and angiogenesis[8, 69, 79, 88, 89, 95, 102, 103, 115]. Despite similarities between FAK- and Src-associated signaling pathways[116, 117], unique FAK substrates have been identified and combined treatment with FAK and Src inhibitors shows enhanced anti-tumor activity in non-small cell lung cancer models[118]. FAK inhibitors have also exhibited enhanced activity in combination with cytotoxic drugs[65, 95] or agents targeting angiogenesis, like the receptor tyrosine kinase inhibitor sunitinib[96]. This supports the notion that FAK inhibition will yield distinct responses and may act as a chemotherapy sensitizer.

However, the ability of Pyk2 to take over certain FAK functions after FAK deletion (Box 1) has to be factored into the design of FAK inhibitor therapies and dual FAK/Pyk2 inhibitors may yield different phenotypes. Similarly, FAK's kinase-independent functions (Figure 2) have to be taken into consideration when designing or testing FAK kinase inhibitor therapy approaches. FAK's scaffolding functions are not blocked, but possibly enhanced by FAK inhibition[40], leading to potentially unpredictable therapy outcomes. Compounds in development against FAK can be sub-divided into ATP-competitive kinase inhibitors (KI), molecules blocking FAK catalytic activity by alternative means (aKI), and compounds targeting FAK scaffolding (scaffold inhibitors, SI) (Table 2).

Small molecule ATP-competitive kinase inhibitors

Small molecules inhibitors that bind within the active site of kinases compete with relatively high levels of ATP present in cells. Inhibitors are designed to make binding interactions with residues surrounding the ATP binding pocket of kinases. The best characterized cellular-active and selective nanomolar affinity FAK inhibitors are comprised of pyrimidine (TAE-226, PF 573,228, PF 562,271) or pyridine (VS-4718, previously known as PND-1186) ATP site hinge binders[119–122]. Despite highly conserved elements within the tyrosine kinase ATP binding pocket, FAK inhibitor selectivity is achieved through stabilization of the FAK kinase activation loop "DFG" motif into a helical conformation[121, 123]. The unusual kinase domain conformation and presence of Gly-563 preceding the DFG motif may confer loop flexibility as well as FAK selectivity[124]. Other pre-clinical FAK inhibitors, as discussed later, do not possess this type of target selectivity and no peer-reviewed information is available for the clinical-stage FAK inhibitor GSK-2256098.

In cell culture and animal models, these FAK inhibitors effectively decrease FAK Y397 auto-phosphorylation, prevent cell movement, but do not necessarily induce cell apoptosis in adherent culture conditions[120, 122]. Certain breast and ovarian tumor cells are resistant to growth inhibition at micromolar FAK inhibitor levels in 2D tissue culture conditions, but become sensitive to nanomolar FAK inhibitor concentrations when grown in a 3D anchorage-independent cell spheroid environment[8, 122]. This has been linked to cell type-specific dependence on integrin-ECM signals within spheroids. Particularly in mesothelioma cells with inactivating mutations in the NF2 gene, which encodes the Merlin tumor suppressor protein, survival and proliferation signals are mediated through cell-ECM rather than cadherin cell-cell contact signals[125]. Low Merlin protein levels are therefore predicted to serve as a biomarker for FAK inhibitor sensitivity in mesothelioma (Trial ID: NCT01870609) and possibly also in ovarian cancer[126].

Alternative approaches to inhibit FAK function

New allosteric FAK inhibitors that bind to distinct kinase domain sites and do not directly compete with ATP binding are being developed[127, 128]. These compounds have the potential for high FAK specificity, but have not been rigorously tested in pre-clinical models. Several studies have identified small molecules *via* molecular docking analyses that may disrupt different FAK scaffolding protein-protein interactions. These include compounds of limited complexity (molecular weight < 300) termed C4, Y11, Y15, and R2 (Table 2). Proposed mechanisms are that C4 blocks FAK C-terminal domain interactions, Y11 and Y15 block access to the FAK Y397 site, and R2 blocks FAK interaction with p53[129–131]. These compounds act at micromolar concentrations in cells and show anti-tumor activity in xenograft mouse models. Although they have been shown to enhance the anti-tumor activity of other chemotherapeutics, questions remain about target selectivity.

FAK inhibitors in clinical trials

Pfizer (PF 562,271, Trial ID: NCT00666926) and GSK (GSK-2256098, Trial ID: NCT00996671) initiated phase I clinical trials with FAK inhibitors in 2008 and 2009, respectively. Both trials found that the compounds are tolerated with low adverse events.

Notably, in the Pfizer trial, some patients exhibited stable disease while being treated with FAK inhibitor[132]. However, PF 562,271 (now termed VS-6062) displayed non-linear pharmacokinetics and was discontinued. PF-04554878, a later generation ATP site hinge binder, showed more favorable pharmacokinetics, and a phase I trial identified some ovarian, colorectal, and bile duct tumor patients exhibiting stable disease (Trial ID: NCT00787033)

Following the acquisition of the FAK inhibitor rights, Verastem initiated new trials (phase I and II) with PF-04554878 (now named VS-6063 or defactinib) and one phase I clinical study with PND-1186 (acquired from Poniard, now termed VS-4718) (Table 2). A phase II trial in patients with KRAS mutant non-small cell lung cancer (Trial ID: NCT01951690) is testing responses to VS-6063 treatment based on the status of tumor-associated INK4a/Arf and p53 mutations. This study is based upon the finding that FAK inhibitor sensitivity may be associated with the inactivation of INK4a/Arf as part of a RhoA GTPase feedback pathway that leads to FAK activation[133]. Additionally, as VS-6063 was found to enhance taxane-sensitivity of ovarian carcinoma cells[65], a phase I/Ib study (Trial ID: NCT01778803) is evaluating the safety and effectiveness of VS-6063 as combinatorial treatment with paclitaxel. Finally, the inverse relationship between Merlin expression and FAK inhibitor sensitivity[125] provides rationale for trials with VS-6063 (Trial ID: NCT01870609) and GSK2256098 (Trial ID: NCT01938443) in mesothelioma, where a large number of patients possess mutations in the Merlin gene. Results from these trials will show if Merlin status can provide a useful biomarker to predict patient response to FAK inhibitor therapy.

FAK's future as a therapeutic target

One of the strongest rationales for FAK inhibition is that effects are mediated through alterations in both tumor and stromal cell biology. Known pathways are the prevention of cell motility, invasion, survival, and proliferation that are being driven by oncogenes and a variety of cell surface receptors. In addition to cancer, FAK inhibition may yield clinical benefits for vascular pathologies such as edema and in limiting inflammation. FAK inhibition in the prevention of vascular permeability may prevent tumor metastasis, could enhance chemotherapy drug delivery, and may help overcome chemo-resistance in patients.

Interestingly, a recent report suggests that EC FAK inactivation may enhance the effects of DNA damaging cancer treatments like doxorubicin or radiation therapy[78]. In this study, EC FAK loss prevented doxorubicin-stimulated NF-kB activation and the production of various cytokines, which act to protect tumor cells from DNA damage-driven apoptosis. Further studies are needed to determine if this connection is generalizable or dependent upon the loss of FAK activity. Nevertheless, FAK inhibitors may be promising drugs for combinatorial therapies including DNA damaging agents, an approach that may increase efficacy of these agents and overcome chemoresistance.

Lastly, FAK inhibitors may possess single agent activity in cancers where FAK expression and activity are amplified or where tumor cells become dependent on FAK-associated signals[126, 134, 135]. Examples include Ewing sarcoma[134] or ovarian serous carcinoma[135], where treatment with the FAK inhibitor PF-562,271 blocked *in vitro* and *in vivo* tumor

growth in pre-clinical studies. Future research into the FAK-associated pathways will elucidate new chemotherapy combinations and biomarkers for patient stratification.

Acknowledgments

We apologize to those authors whose work on FAK signaling has advanced the field, but we were unable to cite due to journal limitations. Studies in the Schlaepfer laboratory are funded by National Institutes of Health (NIH) grants CA102310 and CA180769. F. Sulzmaier is supported by NIH training grant (T32-CA121938). C. Jean is supported by an American Heart Association fellowship (12POST11760014). We thank Jonathan Pachter, Head of Research at Verastem Inc., for insightful discussions of ongoing FAK inhibitor clinical trials. We also thank Christine Lawson, Nichol L. Miller and Isabelle Tancioni for discussions and help in revising the manuscript.

Abbreviations

AJ	adherens junction
EC	endothelial cell
ECM	Extracellular matrix
EMT	epithelial-mesenchymal transition
FA	focal adhesion
FERM	band 4.1-ezrin-radixin-moesin
GEF	guanine-nucleotide exchange factor
MMP	matrix metalloproteinase
KD	kinase-dead
KO	knock out
VP	vascular permeability

Glossary of terms

Integrin receptor clustering (p.6)	The formation of multimeric membrane integrin clusters upon binding to extracellular matrix ligands, inducing the formation of multi-protein complexes at cytoplasmic integrin tails to drive focal adhesion formation and cytoskeletal rearrangement
Focal Adhesion (p.6)	A multi-protein complex regulating cellular attachment by linking the actin cytoskeleton to components of the extracellular matrix via transmembrane receptors termed integrins
Epithelial-mesenchymal transition (EMT) (p.7)	A cellular mechanism that allows polarized epithelial cells to acquire a mesenchymal phenotype characterized by increased cell migration and invasion and the ability to survive in adhesion independent conditions
Floxed mouse models (p.7)	Transgenic insertion of loxP sites flanking a gene of interest. Induced expression of Cre recombinase catalyzes

	recombination between the loxP repeats and mediates the deletion of the gene of interest
MMTV-PyMT model (p. 7)	A mouse model with conditional expression of the polyoma virus middle T-antigen under the control of the mouse mammary tumor virus promoter, inducing the formation of mammary tumors
Guanine nucleotide exchange factor (GEF) (p.7)	A protein that promotes the exchange of GDP for GTP on a GTPase, thereby facilitating its activation
Invadopodia (p.8)	Specialized membrane protrusions (also known as an invasive pseudopodia) where active extracellular matrix degradation takes place
Arp2/3 (p.8)	A seven-subunit protein complex involved in regulation of the actin cytoskeleton; mediates the nucleation of branched actin filaments
N-WASP (Neural Wiskott-Aldrich syndrome protein) (p.8)	Promotes actin polymerization by stimulating the activity of the Arp2/3 complex
Anoikis (p.10)	Cell death (apoptosis) induced by the loss of cell-matrix adhesion and a physiological mechanism to prevent cell displacement
Mammospheres (p.11)	A collection of cells arising from a single cell of mammary origin through clonal growth in culture
Vascular normalization (p.13)	The process of restoring normal vasculature form the classical cancer-associated tortuous and leaky vessels. This phenomenon involves increased vascular pericyte coverage and decreased VP and hypoxia, and results in decreased metastasis and increased blood perfusion, rendering vessels more efficient for oxygen and drug delivery
Tumor cell extravasation (p.15)	The crucial step in tumor metastasis where tumor cells exit the vasculature to penetrate target organs. This requires tumor cell adhesion to the endothelium, spreading out across endothelial cells, and penetration of the basement membrane
ATP site hinge (p.18)	A segment that connects the two lobes of a kinase domain. Hinge and kinase lobes form an interface creating the ATP binding pocket

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Biographies

David D. Schlaepfer, Ph.D. is a Professor in The Moores UCSD Cancer Center in La Jolla, CA and has studied FAK for over 20 years. His group uses mouse models to study tumor-stromal interactions, mechanisms of metastasis, pre-clinical analyses of small molecule FAK inhibitors, and signaling linkages that promote ovarian tumor recurrence and progression.

Florian J. Sulzmaier, Ph.D. is a postdoctoral fellow at The Moores UCSD Cancer Center in La Jolla, CA. He completed his Ph.D. at the University of Hawaii at Manoa, studying kinase signaling pathways that control cell metastasis in cancers of the central nervous system.

Florian is currently investigating the role of FAK in tumor epithelial-mesenchymal transition.

Christine Jean, Ph.D. is a postdoctoral fellow at The Moores UCSD Cancer Center in La Jolla, CA. She completed her Ph.D. in Toulouse France her interests revolve around the role of the microenvironment in modulating tumor growth and metastasis. She is currently investigating mechanisms of endothelial FAK signaling on tumor progression.

Key Points

- Focal Adhesion Kinase (FAK) is a non-receptor protein tyrosine kinase that drives tumor growth and metastasis through kinase-dependent and –independent pathways.
- FAK promotes metastasis by regulating processes involved in tumor cell motility and invasion, including control of focal adhesion and cytoskeletal dynamics, as well as the regulation of matrix metalloproteinase (MMP) surface expression.
- Tumor growth is enhanced through pro-proliferative and anti-apoptotic functions of FAK.
- FAK is connected to cancer stem cell and progenitor cell maintenance through kinase-dependent and -independent functions. FAK signals contribute to the malignant outgrowth of these cells.
- FAK favors tumor progression via the regulation of signaling pathways within cells of the tumor microenvironment, such as endothelial cells, hematopoietic cells, platelets, macrophages, and fibroblasts.
- FAK activity promotes endothelial cell migration, proliferation, survival and stimulates tumor angiogenesis. FAK-mediated regulation of endothelial cell permeability can influence tumor metastasis.
- FAK expression and activity in tumor and endothelial cells is frequently upregulated and correlated with a poor patient prognosis.
- Several molecules targeting FAK kinase activity or its kinase-independent scaffolding function are under investigation in pre-clinical trials. Promising drug candidates in phase I or II clinical trials are small molecule ATP-competitive inhibitors.

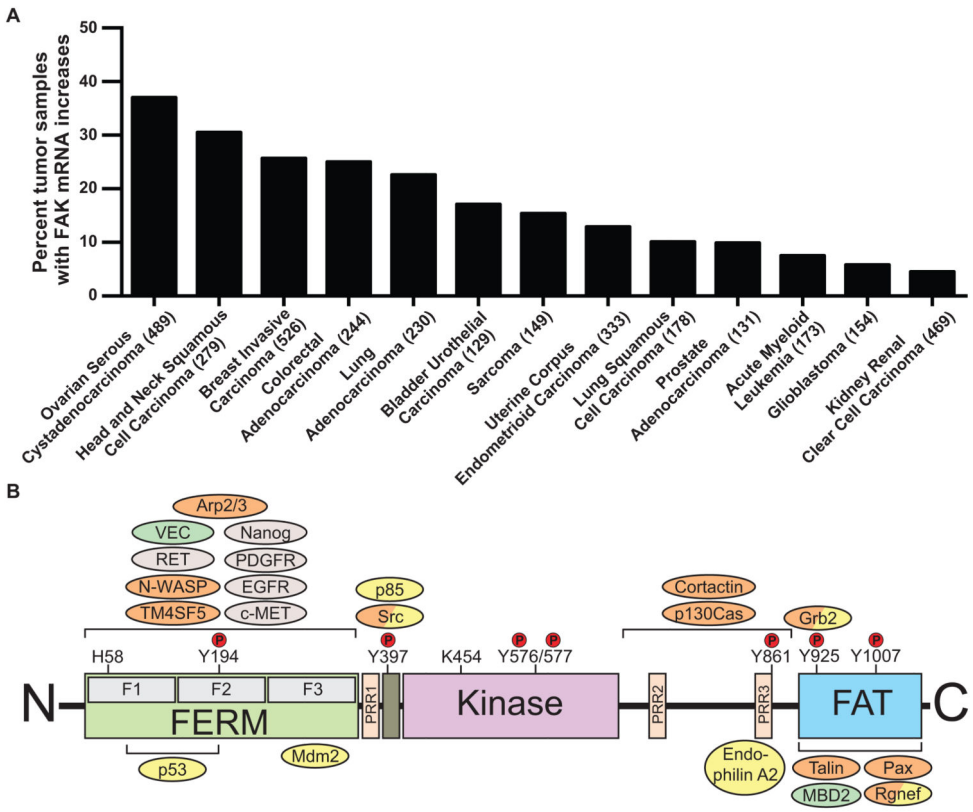

Figure 1. FAK expression in cancer and FAK domain structure

(A) Percent of tumor samples with elevated focal adhesion kinase (FAK) mRNA. The Cancer Genome Atlas was quiered using the cBioPortal (www.cbioportal.org). Search criteria included mRNA expression data (Z-scores for all genes) and tumor datasets with mRNA data. Numbers of tumors analyzed (n) is shown on the X axis. (B) FAK consists of a central kinase domain flanked by a protein4.1-ezrin-radixin-moesin (FERM) homology domain on the N-terminal side and a C-terminal focal adhesion targeting (FAT) domain. Both terminal domains are separated from the kinase domain by a linker region containing proline-rich regions (PRR). Important tyrosine (Y) phosphorylation (P) sites are indicated; Y397, K454 and H58 play crucial roles in FAK activation. FAK binding partners are shown at their interaction sites within FAK. Binding of these proteins affects outcomes like cell motility (orange), cell survival (yellow) or both functions (orange/yellow). Roles involving FAK activation are shown in grey, important contributions to the tumor environment in green.

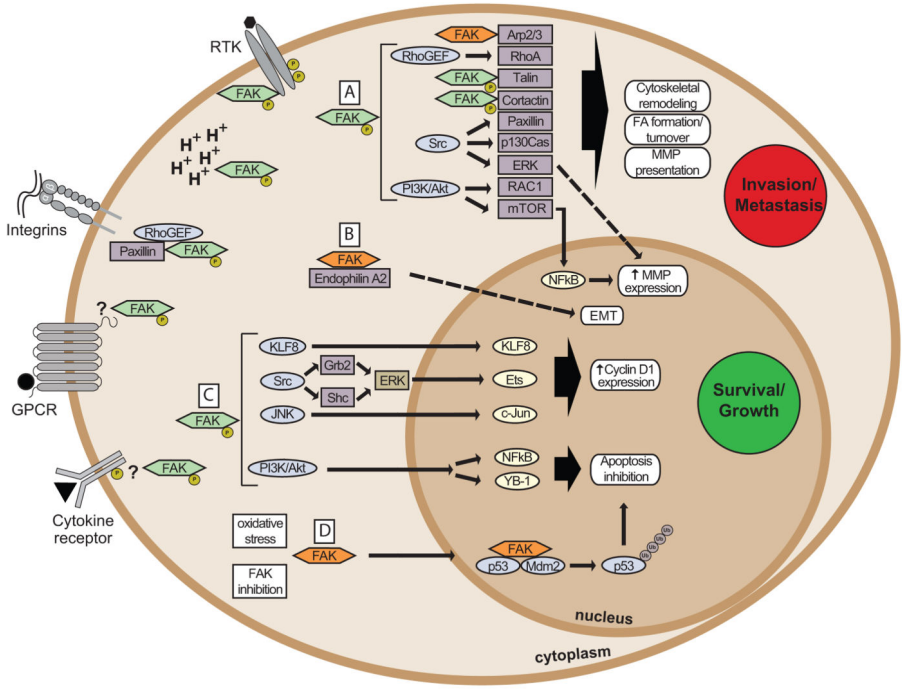

Figure 2. FAK connections to tumor growth and metastasis

FAK drives cancer growth and metastasis through kinase-dependent (green) or -independent (orange) functions. FAK is activated by receptor tyrosine kinases (RTK), intracellular pH changes (H+), integrins, G-protein coupled receptors (GPCR) and cytokine receptors. The exact mechanisms are not always clear (indicated by '?'). Oxidative stress and FAK catalytic inhibition increase FAK nuclear localization. (A) Active FAK increases cell motility through effects on Arp2/3, Rho guanine nucleotide exchange factors (RhoGEF), talin or cortactin, and Src- or PI3-kinase (PI3K) mediated signaling. This drives cytoskeletal remodeling, focal adhesion (FA) formation and turnover, and expression and cell-surface presentation of matrix metalloproteinases (MMPs), enhancing cell invasion and tumor metastasis. (B) Kinase-independent scaffolding of endophilin A2 induces the expression of endothelial-mesenchymal transition (EMT) markers. FAK affects survival and proliferation through kinase-dependent and –independent roles to promote tumor growth. (C) FAK induces cell cycle progression through cyclin D1 involving Krüppel-like factor 8 (KLF8), Src–ERK, or JNK signaling. Signaling through PI3K/Akt mediates inhibition of apoptosis through transcriptional effects by NFkB or YB-1. (D) Nuclear FAK acts as a scaffold for p53 and Mdm2 in a kinase-independent manner, increasing p53 poly-ubiquitination (Ub) and degradation, thereby promoting cell survival.

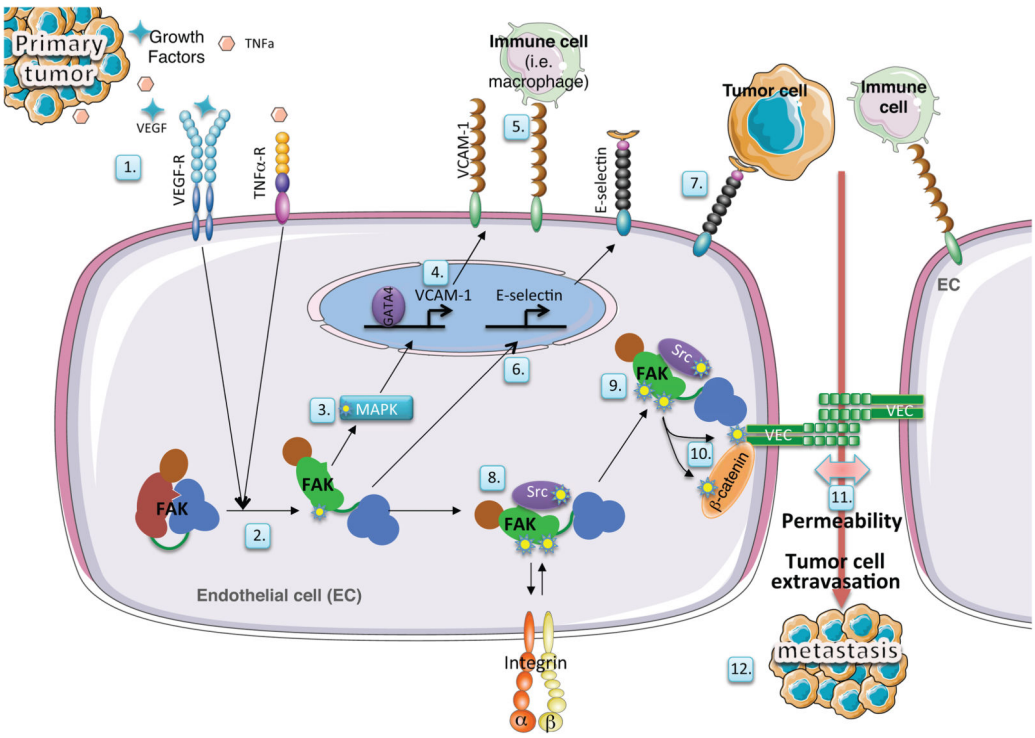

Figure 3. Regulation of vascular permeability and extravasation processes by endothelial cell FAK

Tumor cells, via the secretion of growth factors and the activation EC-specific receptors (1) induce conformational FAK activation through multiple mechanisms. (2) FAK signaling promotes ERK/MAPK signaling cascade activation (3) leading to GATA-4-dependent transcriptional expression of VCAM-1 (4)[75], a surface protein that can facilitate immune cell adhesion to ECs (5). EC FAK also promotes E-selectin expression (6) favoring tumor cell adhesion to ECs (7) and the lodging of metastatic cancer cells within sites of vascular hyper-permeability[99]. FAK activation can occur downstream of integrin receptor binding to matrix proteins consisting of a FAK-Src multi-protein complex (8). In response to VEGF signals, FAK promotes the localization of Src to adherens junctions; key sites that maintain vascular barrier integrity (9). FAK binding *via* FERM domain to the VE-cadherin (VEC) cytoplasmic tail, and FAK-dependent pY658 VEC and pY142 β-catenin phosphorylation (10) promotes VEC/β-catenin dissociation and VEC internalization/degradation. Loss of VEC from the cell surface leads to increased vascular permeability (11), allows for tumor cell transmigration across EC barriers, and leads to increased tumor cell metastasis (12)[69, 88].

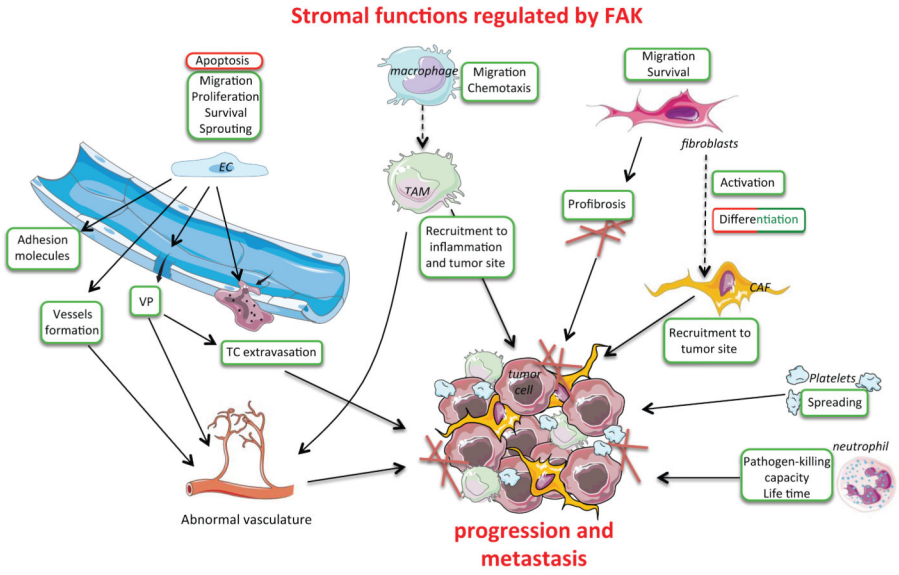

Figure 4. Tumor microenvironmental impact of FAK signals

FAK is an important regulator of EC, neutrophil, platelet, macrophage and fibroblast signaling in the tumor microenvironment leading to the increase (green boxes) or decrease (red boxes) of stromal cell functions. In ECs, FAK inhibits apoptosis and increases proliferation. EC FAK also contributes to the formation of abnormal vasculature *via* the increase of cell migration, survival, and vascular permeability (VP). Moreover, as described in detail in Figure 3, EC FAK is a key regulator of vascular permeability and tumor intra/extravasation leading to metastasis. FAK stimulates macrophage and fibroblast migration. FAK promotes the differentiation (dotted arrow) of macrophages. This occurs in stimulus-specific fashion where FAK activation either promotes or reverses (red/green box) fibroblast differentiation into CAF. For both macrophages and fibroblasts, FAK activity positively impacts cell recruitment to the tumor site. FAK promotes spreading, adhesion, and survival of stromal cells; with concomitant regulation ECM synthesis/remodeling to promote tumor progression. References are available in the corresponding text. VP: vascular permeability, TC: tumor cell, EC: endothelial cell, TAM: tumor-associated macrophage and CAF: cancer-associated fibroblast.

Table 1

Mouse models available to characterize FAK function in cancer

Shown are characteristics of common mouse models for the dissection of FAK function.

Model	Type	Affected cells	Promoter	Inducer	Phenotype	Reference
FAK $^{flox/flox}$ knock-out	Cell lineage-specific k.o.	Endothelial cells	PDGFb	Cre-ERT	Impact neovascularization, tumor growth and angiogenesis	(92)
		Endothelial cells	SCL	Cre-ERT		(24)(82)
		Endothelial cells	Tie2	Cre-ERT		(81)(83)
		Hematopoietic cells	MX1	Cre-ERT	Increased HSC pool, impaired cytokine-induced growth survival, and anti-apoptotic signaling in myeloid and erythroid lineages	(104)(105)
		Keratinocytes	K14	Cre-ERT	Reduced papilloma formation/progression	(114)(117)
		Mammary epithelial cells	MMTV	Cre-ERT	Reduced breast tumor formation and progression, suppression of mammary cancer stem cells	(45)(73)(74)
		Megakaryocytes, Platelets	Pf4	Cre-ERT	Increased bleeding time, megakaryopoiesis and decreased platelet spreading	(106)
		Myeloid cells	Lysozyme M	Cre-ERT	Modified neutrophil and macrophage functions	(100)(101)
		Prostate cells	Probasin	Cre-ERT	Reduced androgen-independent formation of neuroendocrine carcinoma. No change in progression to adenocarcinoma.	(115)
FAK hetero-zygote	Global deletion of one allele	All	None	none	Increased tumor angiogenesis	(93)
FAK-KD knock-in	Cell lineage-specific k.i.	Endothelial cells	SCL, Tie2	Cre-ERT	Disruption of AJ formation during development. Reduced VP and VEGF-induced tumor cell extravasation/ metastasis	(69)(76)(88)

k.o. knock-out, k.i. knock-in, VP: vascular permeability, HSC: hematopoietic stem cell.

Table 2

Anti-cancer compounds targeting FAK

Shown are compounds currently in pre-clinical (PC) and clinical trials (I, Ib or II).

Name	Alt. Name	Type	Specificity	Phase	Trial#	Reference
GSK2256098		N.A.	N.A.	I	NCT01938443, NCT01138033, NCT00996671	N.A.
NVP-TAC544		KI	FAK	PC	none	(24)
PF 573,228	PF-228	KI	FAK	PC	none	(120)
TAE226	NVP-226	KI	FAK/Pyk2	PC	none	(119)
VS-4718	PND-1186	KI	N.A.	I	NCT01849744	(122)
VS-6062	PF 562,271 PF-271	KI	FAK/Pyk2	I	NCT00666926	(121)(132)
VS-6063	PF-04554878 defactinib	KI	N.A.	I/Ib, II	NCT01951690, NCT00787033, NCT01943292, NCT02004028, NCT01778803	(65)(132)
1H-Pyrrolo(2,3-b)pyridine		aKI	N.A.	PC	none	(128)
Compound 1 and 2		aKI	N.A.	PC	none	(127)
Y15	Compound 14	aKI	FAK	PC	none	(130)
C4	chloropyramine hydrochloride	SI	N.A.	PC	none	(130)
R2	Roslins	SI	N.A.	PC	none	(131)
Y11		SI	FAK	PC	none	(129)

KI: ATP-competitive kinase inhibitors, aKI: molecules blocking enzymatic activity by alternative means, SI: protein scaffold inhibitors. N.A. Data not available.