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FAK in cancer: mechanistic findings and clinical applications

Florian J. Sulzmaier, Christine Jean and David D. Schlaepfer

Abstract | Focal adhesion kinase (FAK) is a cytoplasmic protein tyrosine kinase that is overexpressed and activated in several advanced-stage solid cancers. FAK promotes tumour progression and metastasis through effects on cancer cells, as well as stromal cells of the tumour microenvironment. The kinase-dependent and kinase-independent functions of FAK control cell movement, invasion, survival, gene expression and cancer stem cell self-renewal. Small molecule FAK inhibitors decrease tumour growth and metastasis in several preclinical models and have initial clinical activity in patients with limited adverse events. In this Review, we discuss FAK signalling effects on both tumour and stromal cell biology that provide rationale and support for future therapeutic opportunities.

Focal Adhesion Kinase (FAK) is a multifunctional regulator of cell signalling within the tumour microenvironment[1–3]. During development and in various tumours, FAK promotes cell motility, survival and proliferation through kinase-dependent and kinase-independent mechanisms. In the past few years, Phase I and II clinical trials have been initiated with FAK inhibitors; however, some of the functions of FAK in tumorigenesis remain under investigation.

Chromosomal region 8q24.3, which encompasses protein tyrosine kinase 2 (*PTK2*; which encodes FAK), is linked to susceptibility to ovarian cancer[4]. Large databases such as The Cancer Genome Atlas show that FAK mRNA levels are increased in serous ovarian tumours (~37%)[5] and invasive breast cancers (~26%)[6], and these increased levels are correlated with poor overall patient survival[7,8]. Increased FAK mRNA levels are also found in several other human malignancies[3] (FIG. 1a). Studies with tumour tissue arrays find that FAK activation, as determined by phosphospecific antibody recognition of the FAK Y397 autophosphorylation site, increases with tumour progression[3]. However, unlike classical oncogenes such as RAS or PI3K, only a few missense mutations within *PTK2* are found in tumours[5]. Instead, increased FAK activity is associated with *PTK2* amplification, and this is consistent with a model whereby increased FAK dimerization that is induced by higher FAK levels contributes to catalytic activation[9].

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

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FAK regulation

Control of FAK expression. Nuclear factor-κB (NF-κB) and p53 are well-characterized transcription factors that activate and repress the *PTK2* promoter, respectively[10,11]. Other transcription factors, such as NANOG[12], argonaute 2 (AGO2)[13], and PEA3 (also known as ETV4)[14] also increase *PTK2* promoter activity. NANOG promotes FAK expression in colon carcinoma cells and NANOG activity is increased through phosphorylation by FAK as part of a signalling loop[12]. AGO2, a part of the cellular RNA interference machinery, is amplified in hepatocellular carcinoma and induces FAK transcription[13]. Silencing of AGO2 reduces FAK levels and concomitantly blocks tumorigenesis and metastasis in mice. Increased levels of PEA3 and FAK 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), which was identified in samples from patients with breast cancer or thyroid cancer, results in increased cell motility and invasion[15]. However, this deletion probably disrupts FAK

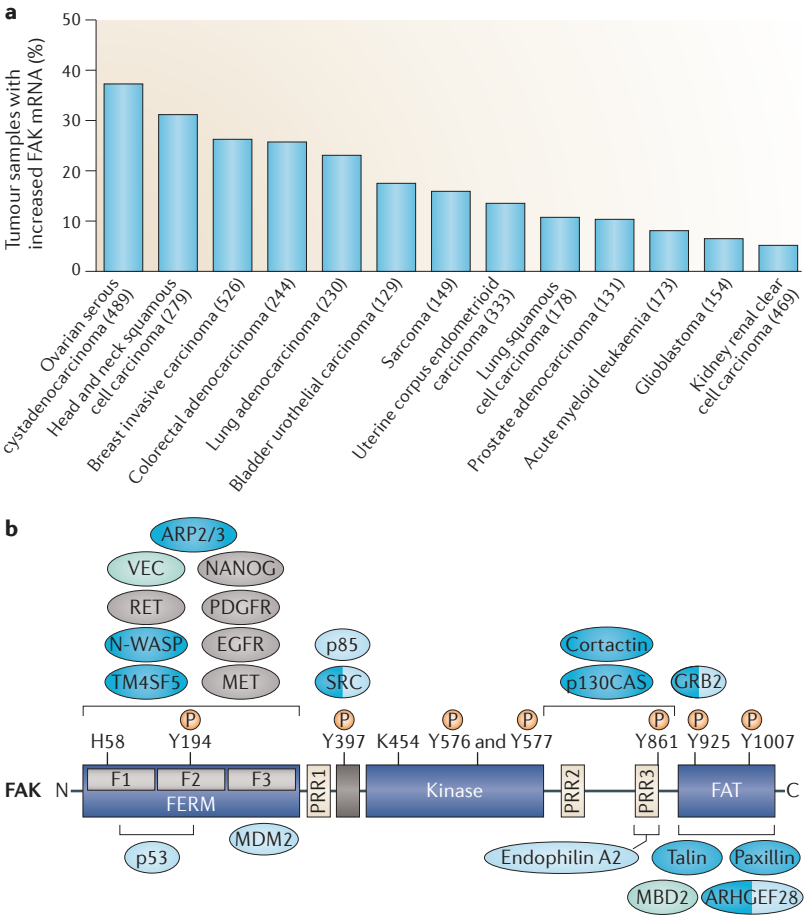


Figure 1 | **Focal adhesion kinase (FAK) expression in cancer and FAK domain structure. a** | Percentage of tumour samples with increased levels of FAK mRNA. The Cancer Genome Atlas was queried using the cBioPortal. Search criteria included mRNA expression data (Z-scores for all genes) and tumour data sets with mRNA data. Numbers of tumours analysed are shown (in parentheses) on the x-axis. **b** | FAK consists of a central kinase domain flanked by a protein band 4.1–ezrin–radixin–moesin (FERM) homology domain on the amino-terminal side and a carboxy-terminal focal adhesion targeting (FAT) domain. Both terminal domains are separated from the kinase domain by a linker region that contains proline-rich regions (PRRs). Important tyrosine phosphorylation (P) sites are indicated; Y397, K454 and H58 have crucial roles in FAK activation. FAK binding partners are shown at their interaction sites within FAK. Binding of these proteins affects outcomes such as cell motility (dark blue), cell survival (light blue) or both functions (dark blue/light blue). Roles involving FAK activation are shown in grey, and important contributions to the tumour environment are shown in green. EGFR, epidermal growth factor receptor; GRB2, growth factor receptor-bound 2; MBD2, methyl-CpG-binding domain 2; N-WASP, neural Wiskott–Aldrich syndrome protein; PDGFR, platelet-derived growth factor receptor; TM4SF5, transmembrane 4 L6 family member 5; VEC, vascular endothelial cadherin.

Integrin receptor clustering
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.

linkage to integrins, and it is unclear how truncated FAK may function. *PTK2* with deletion of exon 26, which also occurs in breast cancer, removes a caspase cleavage site in the FAK carboxy-terminal domain and results in increased FAK protein stability and anti-apoptotic signalling[16]. Interestingly, alternative splicing or increased FAK mRNA expression does not always translate into increased FAK protein levels[17]. FAK mRNA turnover that is mediated by microRNA-7 (miR-7) blocks orthotopic breast carcinoma growth and lung metastasis in mice, and miR-7 expression in samples from patients with breast cancer inversely correlates with cancer stage[18]. At the protein level, FAK is subject to proteasomal or calpain-mediated degradation[19]. Polyubiquitylation by the E3 ligase mitsugumin 53 (MG53; also known as TRIM72) promotes proteasomal degradation of FAK during myogenesis, but this has not been tested in tumour cells[20]. However, in general, FAK protein levels are increased in advanced-stage solid tumours. Together, these results support the notion that increased expression of FAK is connected to several tumour-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 (or mechanisms) of how FAK associates with these distinct signalling complexes is crucial for understanding the biological function of FAK. The structure of FAK (FIG. 1b) consists of an amino-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; also known as PTK2B), a FAK orthologue with ~45% amino acid sequence identity, can compensate for some FAK functions after FAK loss in knockout mouse models (BOX 1)[22–24].

The most well-characterized mechanism that promotes FAK activation involves integrin receptor clustering upon the binding of cells to extracellular matrix (ECM) proteins, in a process that involves FAK dimerization[9]. This leads to FAK autophosphorylation at Y397, binding of SRC-family kinases to the phosphorylated site, SRC-mediated phosphorylation of the FAK kinase domain activation loop (Y576 and Y577), and formation of an activated FAK–SRC complex[1–3]. Indirect interactions between the FAK C-terminal domain and integrins at focal adhesions mediate the integrin–FAK linkage, as overexpression of a FAK C-terminal fragment blocks integrin-mediated activation of FAK[19]. Alternatively, the activation loop within the FAK kinase domain is also directly phosphorylated by the RET receptor, thereby increasing FAK kinase activity[25]. Phosphorylation of FAK at Y397 can also occur in *trans*[9].

Recent studies show that the FAK FERM domain has a prominent role in the intramolecular regulation of FAK activity by binding to the kinase domain and blocking the accessibility to FAK Y397 and auto-phosphorylation[21,26,27]. Studies with a fluorescent biosensor revealed FAK FERM conformational changes upon interaction with phosphoinositide lipids and upon cell binding to ECM[27,28] (see Note added in proof). FAK FERM domain interaction with membrane-associated proteins such as tetraspanin transmembrane 4 L6 family member 5 (TM4SF5) or growth factor receptors can also induce the activation of FAK[29,30]. FAK activity is also increased via FAK FERM domain alterations that are induced by changes in pH and increased cell–ECM tension[31–34]. Local increases in pH promote FERM conformational changes via deprotonation of H58, which is mediated in part by plasma membrane sodium and hydrogen exchangers such as NHE1 (REF. 32). Increased intracellular pH commonly occurs in cancer, and increased matrix stiffness or forces

Focal adhesions
Multi-protein complexes that regulate cellular attachment by linking the actin cytoskeleton to components of the extracellular matrix via transmembrane receptors termed integrins.

Epithelial-to-mesenchymal transition
(EMT). A cellular mechanism that allows polarized epithelial cells to acquire a mesenchymal phenotype that is characterized by increased cell migration and invasion and the ability to survive in adhesion-independent conditions.

Floxed mouse models
Transgenic insertion of loxP sites flanking a gene of interest. Induced expression of Cre recombinase catalyses recombination between the loxP repeats and mediates the deletion of the gene of interest.

MMTV–PyMT model
A mouse model with conditional expression of the polyomavirus middle T antigen (PyMT) under the control of the mouse mammary tumour virus (MMTV) promoter, inducing the formation of mammary tumours.

Guanine nucleotide exchange factor
(GEF). A protein that promotes the exchange of GDP for GTP on a GTPase, thereby facilitating its activation.

that are associated with collagen fibre crosslinking trigger increased FAK Y397 phosphorylation and tumour progression in mouse models of breast cancer[32,33]. It is likely that context-dependent stimuli trigger FAK activation through steps that involve the FERM domain, FAK dimerization or other mechanisms that are yet to be determined.

FAK in tumour cells

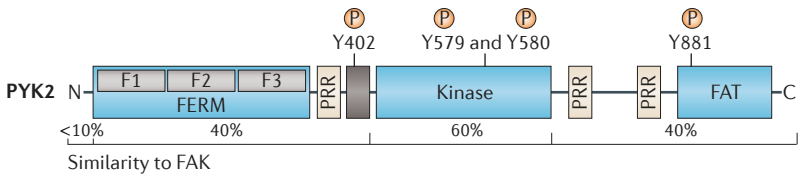
FAK is at the intersection of various signalling pathways that promote cancer growth and metastasis (FIG. 2). This includes kinase-dependent control of cell motility[19], invasion[35], cell survival[11,36] and transcriptional events promoting epithelial-to-mesenchymal transition (EMT)[37–39]. Additionally, 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 kinase-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 signalling pathways (TABLE 1). For example, several groups have used polyomavirus middle T antigen (PyMT)-driven breast tumour models combined with tissue-specific FAK-knockout through the mouse mammary tumour virus (MMTV) promoter (the MMTV–PyMT model)[42–45] to assess the function of FAK in tumour progression.

FAK promotes invasive cell phenotypes. Tumour 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 focal adhesion and cytoskeletal dynamics, and alterations in the expression or activation of matrix metalloproteinases (MMPs) to facilitate ECM invasion. EMT, which is driven by a transcriptional programme, supports the progression to these invasive properties.

Canonical FAK signalling is linked to the formation and turnover of focal adhesions[2,19,46]. FAK recruitment and activation at nascent focal adhesions involves binding to the RHO guanine nucleotide exchange factor (GEF) ARHGEF28 (also known as p190RHOGEF and RGNEF), and a FAK–ARHGEF28 signalling 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 increased tyrosine phosphorylation of paxillin, an adaptor protein that is involved in the maturation of focal adhesions[49]. FAK also recruits the integrin-activating adaptor protein talin to nascent focal adhesions, and this occurs independently of direct binding of talin to β-integrins[50].

FAK not only promotes focal adhesion formation and maturation but also drives focal adhesion turnover through control of targeted focal adhesion protein proteolysis. Mutations that disrupt FAK–talin binding inhibit proteolytic talin cleavage, thereby preventing efficient focal adhesion turnover[50]. Protein cleavage is mediated by proteases such as calpain 2 and caspase 8-containing multiprotein complexes[51]. Additionally, proline-rich sites in FAK facilitate interaction with the actin-binding protein cortactin, the phosphorylation of which by FAK contributes to focal adhesion turnover[52]. In head and neck cancer, the blockade of an integrin–FAK–cortactin–JUN N-terminal kinase 1 (JNK1) signalling 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 focal adhesions, while simultaneously increasing their levels

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 the figure), with 60% sequence identity in the central kinase domain, as well as the conserved arrangement of proline-rich regions (PRRs) and tyrosine phosphorylation (P) 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 carboxy-terminal focal adhesion targeting (FAT) domains that bind to paxillin[19,136]. However, PYK2 shows perinuclear distribution and, unlike FAK, is not strongly localized to focal adhesions. Substitution of the PYK2 C-terminal domain with that of FAK facilitates colocalization of a PYK2–FAK chimaera to β1 integrin-containing focal adhesions, indicating that there are substantial binding differences between FAK and PYK2 (REF. 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 knockout in fibroblasts, increased PYK2 levels induce an intrinsic mechanism that promotes cell survival[23]. Consistent with findings in ovarian carcinoma cells, this is in part mediated through nuclear translocation and selective regulation of the p53 tumour 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 expression is positively correlated with 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 this supports the idea of differential PYK2 and FAK functions. PYK2 signalling 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]. Owing 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]. FERM, band 4.1–ezrin–radixin–moesin.

Invadopodia
Specialized membrane protrusions (also known as an invasive pseudopodia) in which active extracellular matrix degradation takes place.

ARP2/3
A seven-subunit protein complex that is involved in regulation of the actin cytoskeleton; it mediates the nucleation of branched actin filaments.

Neural Wiskott–Aldrich syndrome protein
(N-WASP). A protein that promotes actin polymerization by stimulating the activity of the ARP2/3 complex.

at invadopodia in a SRC-dependent manner[54]. However, increased invadopodia formation is not sufficient to promote tumour 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 to actin and link focal adhesions 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 Neural Wiskott–Aldrich syndrome protein (N-WASP)[55,56]. These events are proposed to occur before integrin-mediated activation of FAK. In this model, the FAK FERM domain might function as a scaffold to direct ARP2/3 activity to cell protrusions before the formation of mature focal adhesions.

Expression and activation of MMPs at cell protrusions facilitates matrix invasion of motile cells. FAK activity increases the expression of MMP9 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 (also known as BCAR1)[58] and the PI3K–AKT–mTOR cascade[59]. Although the effects of mTOR on MMPs are not clearly understood, the FAK–p130CAS complex targets MMP14 to focal adhesions and promotes the presentation of MMP14 at the membrane surface[58]. Knockdown of FAK or p130CAS does not alter the generation of pancreatic carcinoma protrusions, but it prevents ECM degradation. MMP14 function has also been proposed to require FAK signalling through an alternative pathway involving activation of Krüppel-like factor 8 during *in vivo* metastasis of human breast cancer cell xenografts in mice[60].

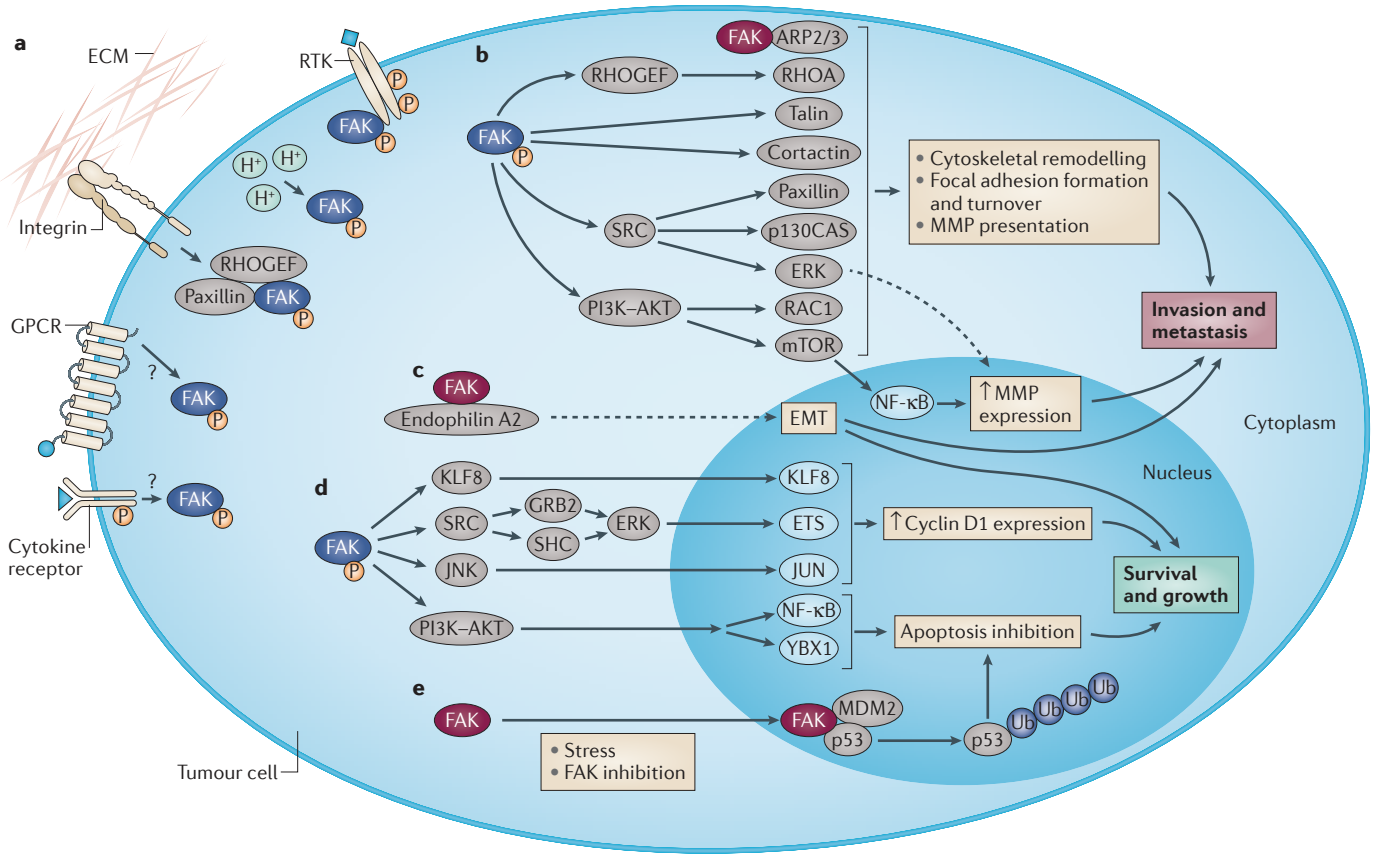


Figure 2 | **Connections of focal adhesion kinase (FAK) to tumour growth and metastasis.** FAK drives cancer growth and metastasis through kinase-dependent (blue) or kinase-independent (red) functions. **a** | FAK is activated by receptor tyrosine kinases (RTKs), intracellular pH changes (H^+), integrins, G protein-coupled receptors (GPCRs) and cytokine receptors. The exact mechanisms are not always clear (indicated by '?'). Oxidative stress and FAK catalytic inhibition increase nuclear localization of FAK. **b** | Active FAK increases cell motility through effects on ARP2/3, RHO guanine nucleotide exchange factors (RHOGEFs), talin or cortactin, and SRC- or PI3K-mediated signalling. This drives cytoskeletal remodelling, focal adhesion formation and turnover, and expression and cell-surface presentation of matrix metalloproteinases (MMPs), enhancing cell invasion and tumour metastasis. **c** | Kinase-independent scaffolding of endophilin A2 induces the expression of epithelial-to-mesenchymal transition (EMT) markers. FAK affects survival and proliferation through kinase-dependent and kinase-independent roles to promote tumour growth. Dashed arrows indicate steps that remain uncharacterized. **d** | FAK induces cell cycle progression through cyclin D1, involving Krüppel-like factor 8 (KLF8), SRC–ERK, or JUN N-terminal kinase (JNK) signalling. Signalling through PI3K–AKT mediates inhibition of apoptosis through transcriptional effects by nuclear factor-κB (NF-κB) or Y-box-binding protein 1 (YBX1). **e** | Nuclear FAK functions as a scaffold for p53 and MDM2 in a kinase-independent manner, increasing p53 polyubiquitylation (Ub) and degradation, thereby promoting cell survival. ECM, extracellular matrix; GRB2, growth factor receptor-bound 2; P, phosphorylation; SHC, SRC homology 2 domain-containing.

Table 1 | **Mouse models available to characterize the functions of FAK in cancer**

Model	Type	Affected cells	Promoter	Inducer	Phenotype	Refs
$FAK^{flox/flox}$ knockout	Cell lineage-specific knockout	Endothelial cells	PDGFβ	Cre-ERT	Impact neovascularization, tumour growth and angiogenesis	92
			SCL	Cre-ERT		24,82
			TIE2	Cre-ERT		81,83
		Haematopoietic cells	MX1	Cre-ERT	Increased HSC pool, impaired cytokine-induced growth survival, and anti-apoptotic signalling in myeloid and erythroid lineages	104, 105
		Keratinocytes	K14	Cre-ERT	Reduced papilloma formation and/or progression	114, 117
		Mammary epithelial cells	MMTV	Cre-ERT	Reduced breast tumour formation and progression; suppression of mammary cancer stem cells	45, 73,74
		Megakaryocytes and 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 heterozygote	Global deletion of one allele	All	None	None	Increased tumour angiogenesis	93
FAK-KD knock-in	Cell lineage-specific knock-in	Endothelial cells	SCL and TIE2	Cre-ERT	Disruption of adherens junction formation during development; reduced vascular permeability and VEGF-induced tumour cell extravasation and metastasis	69, 76,88

Cre-ERT, Cre recombinase bound to a modified oestrogen receptor; FAK, focal adhesion kinase; HSC, haematopoietic stem cell; K14, keratin 14; KD, kinase dead; MMTV, mouse mammary tumour virus; MX1, myxovirus resistance 1; PDGFβ, platelet-derived growth factor-β; PF4, platelet factor 4; SCL, stem cell leukaemia; VEGF, vascular endothelial growth factor.

EMT-like transcriptional programmes have been shown to drive the motility and invasion of tumour cells, and FAK signalling contributes to this change towards an EMT profile[61]. FAK re-expression in FAK-null cells drives SNAIL1-induced EMT[37] (see Note added in proof). FAK scaffolding increases endophilin A2 phosphorylation that leads to alterations in EMT markers, including MMPs, and this affects PyMT-induced progression of breast tumours[38]. Indirect factors such as miR-7-mediated reduction of FAK expression result in the loss of mesenchymal markers as well as increased E-cadherin expression in breast tumour models[18]. Additionally, FAK might also function in a proximal manner to affect the dynamics of E-cadherin internalization in tumour cells[39]. Overexpression of a FAK mutant that is unable to be phosphorylated on multiple sites in colon carcinoma cells blocks SRC-induced internalization of E-cadherin[62], and pharmacological inhibition of FAK activity increases cell–cell adhesion strength, in part by stabilizing the expression of E-cadherin at the cell surface[63]. Together, these results implicate FAK in both the cell surface and transcriptional regulation of E-cadherin levels in tumour cells.

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

FAK signalling is also associated with resistance of ovarian cancer to paclitaxel-induced cell death[65]. Pharmacological FAK inhibition enhances chemosensitivity in taxane-resistant cells by decreasing phosphorylation of the transcription factor Y-box-binding protein 1 (YBX1) 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 tumour growth. However, it remains undetermined whether mutational activation of the PI3K or AKT signalling pathways, which commonly occurs in tumours, may bypass effects of FAK inhibition. Additionally, FAK functions downstream of G protein-linked receptors for stress hormones such as noradrenaline and prevents anoikis of ovarian cancer cells[7].

Anoikis
Cell death (apoptosis) that is induced by the loss of cell matrix adhesion and a physiological mechanism to prevent cell displacement.

Mammospheres
A collection of cells arising from a single cell of mammary origin through clonal growth in culture.

FAK activity also enhances cell cycle progression[46] (FIG. 2) and recent studies have linked this to a matrix stiffness-sensitive signalling linkage between FAK, p130CAS, RAC GTPase and the actin binding protein lamellipodin (also known as RAPH1)[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. However, tumour cells require FAK activity in the processes of extravasation and proliferation of micro-metastases in foreign tissue environments[35,70]. Although conditional FAK knockout 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 that are 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 tumour suppressor adenomatous polyposis coli (APC) suppresses tumour formation that is induced by APC loss[71]. Although it has been proposed that FAK inhibition (as opposed to FAK knockout) may suppress colorectal cancer tumour 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 tumour model decreases SRC-mediated p130CAS activation and signalling to ERK[43]. *Ex vivo* knockout and subsequent transient re-expression of FAK in cells isolated from PyMT-induced breast tumours showed that FAK Y397 autophosphorylation, catalytic activity, and the integrity of FAK proline-rich region 2 (FIG. 1b) are needed for cell proliferation as well as anoikis resistance[45]. In human breast carcinoma cell lines, FAK knockdown prevented tumour growth driven by oncogenic mutations in the RAS and PI3K signalling pathways[45]. These studies support the notion that FAK functions as a regulator for cell intrinsic signals that promote proliferation.

The location of FAK signalling complexes that control 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 treatment with pharmacological FAK inhibitors[75] (FIG. 2). Nuclear FAK restricts p53 tumour-suppressive functions by promoting MDM2 E3 ligase-dependent ubiquitylation and degradation of p53 (REFS 11,40). This prevents p53 transcriptional activity, reducing the levels of targets such as the p21 (also known as CIP1) cell cycle inhibitor[76]. FAK–p53 regulation may also release p53-induced inhibition of the *PTK2* promoter, increasing FAK mRNA transcription. Interestingly, in a mouse model of squamous cell carcinoma, FAK loss increases cell resistance to DNA damage after ionizing radiation, and this is associated with p53-mediated induction of DNA repair[77]. Although this study raises the issue of whether FAK inhibition in combination with radiation may be clinically disadvantageous, alternative conclusions find that endothelial FAK knockout functions to sensitize tumours to DNA-damaging therapy[78].

Control of cancer stem cells by FAK. Cancer stem cells or tissue-specific progenitor cells can facilitate tumour growth and, in certain cancers, FAK signalling 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 the number and size of mammospheres and MaCSC surface markers. In the PyMT breast tumour model, the effects of FAK on MaCSC-associated markers are linked to FAK scaffolding effects on endophilin A2 (REF. 38). Subsequent studies showed that FAK regulates the activities of MaCSCs and normal progenitor cells via both kinase-dependent and kinase-independent mechanisms[41]. In a conditional FAK-KD-knock-in mouse, loss of FAK kinase activity impairs luminal progenitor proliferation and reduces the number of MaCSCs, but it does not affect FAK scaffolding functions that are required for self-renewal of basal mammary stem cells[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 tumour 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 transcription factor Ikaros[80]. Ikaros loss prevents B cell differentiation and locks precursor cells in a state of high adhesion-dependent proliferation, a process that is associated with B cell acute lymphoblastic leukaemia[80]. Precursor B cells rely on integrin–FAK signalling as a major driver of cell proliferation, survival and self-renewal. Thus, FAK may be a key signalling protein downstream of integrins in the control of stem cell proliferation.

FAK in the stromal microenvironment

Signals between tumours and cells in the surrounding microenvironment can drive tumour progression. At sites of micrometastases, tumour cells need to adapt to a new microenvironment and/or modify it. As mentioned above, non-cellular microenvironmental cues, such as matrix composition or stiffness, cytokines, growth factors, integrins and pH changes trigger FAK activity that influences various aspects of tumour growth and metastasis. In this section, we will focus on the cellular microenvironment component, as FAK signalling has important roles within vascular and non-vascular stromal cells in the tumour 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 tumour angiogenesis (TABLE 1). EC proliferation and survival are fundamental events that promote angiogenesis. Global and/or EC-specific knockout of FAK[81–83], expression of FAK-KD[68,76], or deletion of

residues surrounding FAK Y397 (REF. 84) result in early embryonic lethality that is associated with multiple vascular defects, such as haemorrhage and oedema. *In vitro*, primary ECs from these mice exhibit defects in survival, proliferation, sprouting, migration and tubulogenesis.

EC FAK is considered to be a therapeutic target in vascular disease[85,86]. In tumour-associated ECs, FAK mRNA and protein levels are increased[87], as is FAK Y397 phosphorylation[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 inhibition of FAK prevents EC motility and tubulogenesis *in vitro*, aortic sprouting *ex vivo*, and growth factor-stimulated angiogenesis in mice[24,89]. In a proteomic screen analysing invading versus non-invading ECs in three-dimensional collagen matrices, pro-angiogenic factors promoted the association of receptor of activated protein kinase C1 (RACK1; also known as GNB2L1) and vimentin with FAK during endothelial invasion[90]. This linkage is hypothesized to mediate changes in EC shape and focal adhesion formation, which are initial steps in tumour neovascularization.

Orthotopic glioma implantation in adult mice with EC-specific FAK deletion results in tumour vascular normalization that is associated with reduced vascular permeability, as well as partial restoration of EC–EC and astrocyte–EC interactions[91]. Similarly, FAK deletion in ECs in melanoma- or lung carcinoma-bearing mice results in tumour growth inhibition by impairing vascular endothelial growth factor (VEGF)-induced angiogenesis[92]. Surprisingly, increased melanoma and lung carcinoma growth occurs in FAK-heterozygous mice compared to wild-type mice[93]. This FAK-heterozygous phenotype was associated with increased angiogenesis, but it is unclear whether this represents compensatory mechanisms or reduced FAK activity. By contrast, pharmacological inhibition of FAK prevents EC sprouting in a dose-dependent manner[24] and FAK inhibitors are potent anti-angiogenic agents[89]. Furthermore, FAK inhibition reduces tumour 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 knockout results in decreased EC proliferation and survival, and increased apoptosis[81,83]. FAK-knockout results in reduced VEGF-stimulated phosphorylation of AKT, which is associated with reduced EC proliferation and increased cell death[92]. It remains unclear whether FAK has 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-knockout does not trigger apoptosis, in part because PYK2 is expressed and compensates for loss of FAK in promoting cell survival[23,24] (BOX 1). However, as mice with double FAK- and PYK2-knockout 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 tumours is often disorganized, tortuous and leaky[86]. These changes are associated with alterations in EC adherens junctions 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 the stability of adherens junctions, and this is 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 deregulation of RHOA activity[82], this phenotype has not been observed in two other EC FAK-knockout mouse models[24,91]. However, in other cell types, increased RHOA activity occurs upon FAK knockout[22,98] and has been linked to alterations in PYK2 and ARHGEF28 signalling[22]. Additionally, loss of FAK activity disrupted adherens junction 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 increases in paracellular permeability by VEGF[69]. These results support both kinase-dependent and kinase-independent connections of FAK to adherens junction regulation.

VEGF promotes vascular permeability 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, thereby facilitating dissociation of VE-cadherin–β-catenin and breakdown of EC adherens junctions[69]. In glioma studies, FAK expression is essential for tumour-induced vascular permeability in the brain of mice[91]. The signalling pathway that promotes FAK activation downstream of the VEGF receptor (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 translocation of SRC to adherens junctions and that FAK controls VE-cadherin Y658 phosphorylation, which is required to promote VEGF-stimulated vascular permeability and tumour cell extravasation[88]. Notably, FAK-KD expression and VE-cadherin Y658F mutation in ECs prevents tumour cell transmigration across EC barriers. Importantly, EC-specific FAK-KD expression in mice decreases VEGF-enhanced tumour cell extravasation *in vivo*, and EC FAK-KD expression prevents spontaneous orthotopic melanoma metastasis without affecting primary tumour growth[88]. Although the mechanism for this anti-metastatic effect is not known, tumour- and VEGF-associated VE-cadherin

Vascular normalization
The process of restoring normal vasculature from the classical cancer-associated tortuous and leaky vessels. This phenomenon involves increased vascular pericyte coverage and decreased vascular permeability and hypoxia, and it results in decreased metastasis and increased blood perfusion, rendering vessels more efficient for oxygen and drug delivery.

Tumour cell extravasation
The crucial step in tumour metastasis in which tumour cells exit the vasculature to penetrate target organs. This requires tumour cell adhesion to the endothelium, spreading out across endothelial cells, and penetration of the basement membrane.

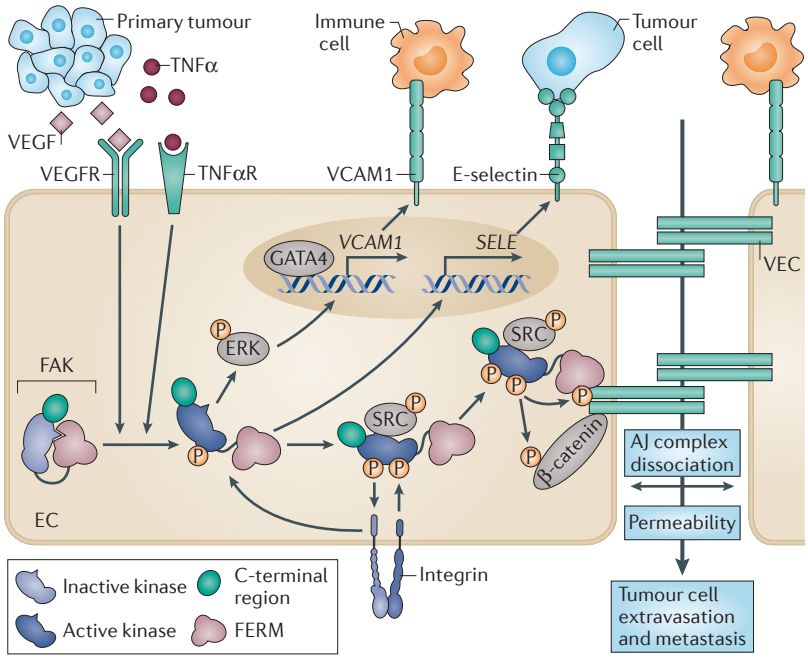


Figure 3 | **Regulation of vascular permeability and extravasation processes by endothelial cell (EC) focal adhesion kinase (FAK).** Tumour cells induce conformational FAK activation through multiple mechanisms via the secretion of growth factors and the activation of EC-specific receptors. FAK signalling promotes activation of the ERK signalling cascade, which leads to GATA4-dependent transcriptional expression of vascular cell adhesion molecule 1 (VCAM1)[75], a surface protein that can facilitate the adhesion of immune cells to ECs. EC FAK also promotes the expression of E-selectin (encoded by *SELE*), which favours tumour cell adhesion to ECs and the lodging of metastatic cancer cells within sites of vascular hyperpermeability[99]. FAK activation can occur downstream of integrin receptor binding to matrix proteins promoting formation of a FAK–SRC multiprotein complex. In response to vascular endothelial growth factor (VEGF) signals, FAK promotes the localization of SRC to adherens junctions (AJs) — key sites that maintain vascular barrier integrity. FAK binding via the band 4.1–ezrin–radixin–moesin (FERM) domain to the vascular endothelial cadherin (VEC) cytoplasmic tail, and FAK-dependent phosphorylation (P) of VEC on Y658 and β-catenin on Y142 promotes VEC and β-catenin dissociation and VEC internalization and degradation. Loss of VEC from the cell surface leads to increased vascular permeability, allows for tumour cell transmigration across EC barriers and leads to increased tumour cell metastasis[69,88]. TNFα, tumour necrosis factor-α; TNFαR, TNFα receptor; VEGFR, VEGF receptor.

internalization within ECs depends on FAK activity. Mechanistically, these results are consistent with roles of EC FAK in modulating the expression of vascular cell adhesion molecule 1 (VCAM1; a protein that mediates the adhesion of immune cells to ECs[75]) and E-selectin[99]. The role of EC FAK in modulating the expression of E-selectin is proposed as a mechanism to prepare vascular microenvironment sites for the seeding of metastatic disease (FIG. 3).

Overall, these studies reveal new roles for EC-specific FAK activity in the control of metastasis. Further studies are needed to determine whether antitumour growth effects of FAK inhibitors are primarily mediated through signalling inhibition within tumour cells or through effects on EC function. FAK inhibition may have beneficial effects for treating cancers and vascular pathologies by preventing vascular permeability and angiogenesis without negative effects on the survival of ECs.

Promotion of tumour growth by FAK via effects in non-vascular stromal cells. In addition to FAK signalling within tumour cells and ECs, non-vascular stromal FAK functions also contribute to multiple aspects of tumour progression (FIG. 4). Neutrophils and macrophages are major effectors of immune responses in conditions that induce inflammation, including cancer. Myeloid-specific (lysozyme M-Cre) FAK knockout 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-knockout model to study macrophage function[101]. Primary FAK-knockout 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 and PYK2-knockdown have similar effects compared to FAK knockout, and this supports the idea of overlapping signalling roles in macrophages[101]. Tumour-associated macrophages (TAMs) are key contributors to tumour progression and cancer-related inflammation. In a mouse model of pancreatic ductal adenocarcinoma, the administration of a FAK inhibitor does not alter tumour angiogenesis, necrosis or apoptosis, but it results in fewer TAMs within tumours and decreased primary tumour size[102]. In mouse models of breast cancer, pharmacological FAK inhibition decreases tumour growth and this is associated with diminished infiltration of leukocytes and macrophages into tumours[79,103]. Other studies have used myxovirus resistance 1 (MX1)-Cre to delete FAK in haematopoietic cells[104,105] and platelet factor 4 (PF4)-Cre to delete FAK in megakaryocyte lineages[106]. Resulting mouse and cell phenotypes are variable and have not yet been integrated into tumour studies (TABLE 1). As potential 'off-target' effects have been proposed to account for the inhibitory effects of FAK inhibitors on platelet spreading[107], and as most haematopoietic cells express PYK2, combined PYK2–FAK-knockout or FAK-KD transgenic models are needed to further our understanding of the roles for FAK signalling in immune cells during tumour progression.

Cancer-associated fibroblasts (CAFs) influence tumour progression through mechanisms that are not fully understood. In a breast carcinoma model, tumour-secreted lysyl oxidase-like 2 (LOXL2) activates fibroblasts and promotes the expression of α-smooth muscle actin (α-SMA) in a FAK-dependent manner via AKT activation[108]. This signalling was blocked *in vitro* by pharmacological inhibition of FAK but not of SRC[108]. As LOXL2 catalyses matrix crosslinking, the effects on FAK might also be mediated through increased tissue tension[33]. Accordingly, FAK expression and activity are increased in fibroblasts from patients with lung fibrosis. Interestingly, FAK inhibition in a mouse model of bleomycin-induced fibrosis results in 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

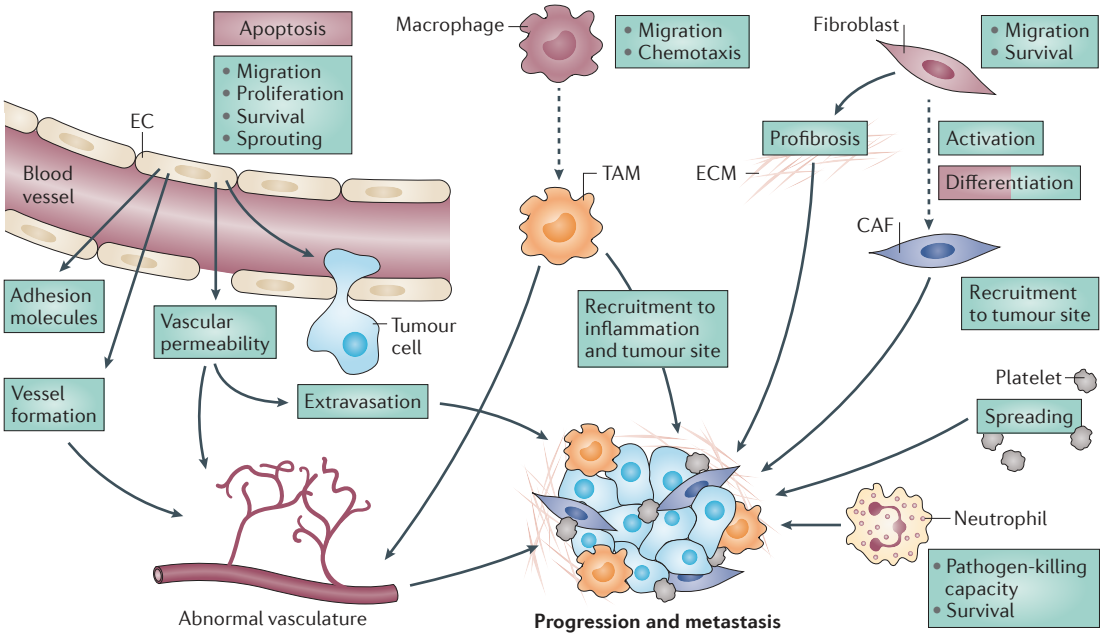


Figure 4 | **Tumour microenvironmental impact of FAK signals.** FAK is an important regulator of endothelial cell (EC), neutrophil, platelet, macrophage and fibroblast signalling in the tumour microenvironment, leading to the increase (green boxes) or decrease (red boxes) of stromal cell functions. In 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. Moreover, as described in detail in FIG. 3, EC FAK is a key regulator of vascular permeability and tumour intravasation and extravasation leading to metastasis. FAK stimulates macrophage and fibroblast migration. FAK promotes the differentiation (dashed arrow) of macrophages. This occurs in a stimulus-specific manner, in which FAK activation either promotes or reverses (red/green box) fibroblast differentiation into cancer-associated fibroblasts (CAFs). For both macrophages and fibroblasts, FAK activity positively affects cell recruitment to the tumour site. FAK promotes spreading, adhesion and survival of stromal cells, with concomitant regulation of extracellular matrix (ECM) synthesis or remodelling to promote tumour progression. See main text for details. TAM, tumour-associated macrophage.

was not addressed[110]. In addition, similar to TAMs, FAK inhibition in a pancreatic ductal adenocarcinoma model decreased CAF recruitment and tumour size[102]. Together, these studies support the notion that FAK promotes pro-tumour functions of CAFs.

FAK also indirectly influences the tumour microenvironment. FAK signalling within breast carcinoma cells regulates VEGF expression that, as described above, promotes vascular permeability and angiogenesis[111]. In acute myeloid leukaemia, FAK expression and activity are important in the production of interleukin-6 (IL-6), IL-8, stromal cell-derived factor 1 (SDF1) and angiopoietin 1 — factors that are crucial for mesenchymal stromal stem cell maintenance[112]. FAK inhibition decreases tumour necrosis factor-α (TNFα)-induced IL-6 production in breast cancer cells and is correlated with reduced tumour-associated splenomegaly and reduced numbers of tumour-associated $CD45^+$ cells in a syngeneic model[79]. Overall, there is emerging evidence for the importance of FAK signalling as a regulator of EC, haematopoietic cell, platelet, macrophage and fibroblast signalling in the tumour microenvironment. Importantly, phenotypes that are associated with FAK inhibition show that there are multiple points of regulation for FAK function, not only in tumour cells but also in the tumour microenvironment.

FAK in clinical applications

FAK functions drive various tumour-promoting signalling pathways[3,94,113,114] (FIG. 2), and small molecule FAK inhibitors are emerging as promising chemotherapeutics, as FAK inhibition in mouse models prevents tumour growth, metastasis, vascular permeability and angiogenesis[8,69,79,88,89,95,102,103,115]. Despite similarities between FAK- and SRC-associated signalling pathways[116,117], unique FAK substrates have been identified, and combined treatment with FAK and SRC inhibitors shows enhanced antitumour activity in non-small-cell lung cancer models[118]. FAK inhibitors have also shown enhanced activity in combination with cytotoxic drugs[65,95] or agents that target angiogenesis, such as the receptor tyrosine kinase inhibitor sunitinib[96]. This supports the notion that FAK inhibition will yield distinct responses and may function 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, the kinase-independent functions of FAK (FIG. 2) have to be taken into consideration when designing or testing approaches to FAK kinase inhibitor therapy. The scaffolding functions of FAK are not blocked by FAK inhibition, but they are possibly enhanced by it[40], thereby leading to

ATP site hinge
A segment that connects the two lobes of a kinase domain. Hinge and kinase lobes form an interface that creates the ATP-binding pocket.

potentially unpredictable therapy outcomes. Compounds in development against FAK can be subdivided into ATP-competitive kinase inhibitors, molecules blocking FAK catalytic activity by alternative means, and compounds targeting FAK scaffolding (scaffold inhibitors) (TABLE 2).

Small molecule ATP-competitive kinase inhibitors. Small molecule kinase inhibitors are designed to bind residues that surround the ATP-binding pocket of kinases. As this pocket is similar in many different kinases, there is potential for off-target effects. The most well-characterized cellular-active and selective nanomolar affinity FAK inhibitors are comprised of pyrimidine (TAE226, PF-573,228 and 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 G563 preceding the DFG motif may confer loop flexibility as well as FAK selectivity[124]. Other preclinical FAK inhibitors, as discussed below, do not possess this type of target selectivity, and no peer-reviewed information is available for the clinical-stage FAK inhibitor GSK2256098.

In cell culture and animal models, these FAK inhibitors effectively decrease FAK Y397 autophosphorylation and prevent cell movement, but do not necessarily induce cell apoptosis in adherent culture conditions[120,122]. Certain breast and ovarian tumour cells are resistant to growth inhibition at micromolar levels of FAK inhibitors in two-dimensional tissue culture conditions but become sensitive to nanomolar FAK inhibitor concentrations when grown in a three-dimensional 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 neurofibromin 2 (*NF2*) gene, which encodes the Merlin tumour suppressor protein, survival and proliferation signals are mediated through cell–ECM rather than cadherin cell–cell contact signals[125]. Low levels of Merlin protein are therefore predicted to serve as a biomarker for FAK inhibitor sensitivity in mesothelioma (ClinicalTrials.gov identifier: 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 preclinical 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 (relative molecular mass <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 (REFS 129–131). These compounds function at micromolar concentrations in cells and show antitumour activity in xenograft mouse models. Although they have been shown to enhance the antitumour activity of other chemotherapeutics, questions remain about target selectivity.

Table 2 | **Anticancer compounds targeting FAK that are currently in preclinical and clinical trials**

Name	Alternative names	Type	Specificity	Phase	Trial identifiers*	Refs
GSK2256098		KI	FAK	I	NCT01938443, NCT01138033 and NCT00996671	N/A
NVP-TAC544		KI	FAK	Preclinical	None	24
PF-573,228	PF-228	KI	FAK	Preclinical	None	120
TAE226	NVP-226	KI	FAK and PYK2	Preclinical	None	119
VS-4718	PND-1186	KI	FAK and PYK2	I	NCT01849744	122
VS-6062	PF-562,271 and PF-271	KI	FAK and PYK2	I	NCT00666926	121,132
VS-6063	PF-04554878 and defactinib	KI	N/A	I/Ib and II	NCT01951690, NCT00787033, NCT01943292, NCT02004028 and NCT01778803	65,132
1H-Pyrrolo(2,3-b) pyridine		aKI	N/A	Preclinical	None	128
Compound 1 and 2		aKI	N/A	Preclinical	None	127
Y15	Compound 14	aKI	FAK	Preclinical	None	130
C4	Chloropyramine hydrochloride	SI	N/A	Preclinical	None	130
R2	Roslins	SI	N/A	Preclinical	None	131
Y11		SI	FAK	Preclinical	None	129

aKI, molecules blocking enzymatic activity by means other than ATP-competitive kinase inhibition (KI); FAK, focal adhesion kinase; N/A, data not available; PYK2, proline-rich tyrosine kinase 2; SI, protein scaffold inhibitors. *See ClinicalTrials.gov.

FAK inhibitors in clinical trials. Pfizer (PF-562,271 (now termed VS-6062); ClinicalTrials.gov identifier: NCT00666926) and GSK (GSK2256098; ClinicalTrials.gov identifier: 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 the FAK inhibitor[132]. However, PF-562,271 showed nonlinear pharmacokinetics and was discontinued. PF-04554878 (now named VS-6063 or defactinib), a later generation ATP site hinge binder, showed more favourable pharmacokinetics, and a Phase I trial identified some patients with ovarian, colorectal or bile duct tumours who exhibited stable disease (ClinicalTrials.gov identifier: NCT00787033)

Following the acquisition of the rights to the FAK inhibitor, Verastem initiated new trials (Phase I and II) with PF-04554878 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 (ClinicalTrials.gov identifier: NCT01951690) is testing responses to VS-6063 treatment on the basis of the status of tumour-associated cyclin-dependent kinase inhibitor 2A (*CDKN2A*; which encodes INK4A and ARF) and *TP53* mutations. This study is based on the finding that FAK inhibitor sensitivity may be associated with the inactivation of INK4A and ARF as part of a RHOA GTPase feedback pathway that leads to FAK activation[133]. Additionally, as VS-6063 was found to enhance the sensitivity of ovarian carcinoma cells to taxane[65], a Phase I/Ib study (ClinicalTrials.gov identifier: NCT01778803) is evaluating the safety and effectiveness of VS-6063 as combinatorial treatment with paclitaxel. Furthermore, the inverse relationship between Merlin expression and FAK inhibitor sensitivity[125] provides rationale for trials with VS-6063 (ClinicalTrials.gov identifier: NCT01870609) and GSK2256098 (ClinicalTrials.gov identifier: NCT01938443) in mesothelioma, in which a large number of patients have mutations in *NF2*. Results from these trials will show whether *NF2* status can provide a useful biomarker to predict patient response to FAK inhibitor therapy.

The future of FAK as a therapeutic target

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

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

In addition, FAK inhibitors may have single agent activity in cancers in which FAK expression and activity are amplified or in which tumour cells become dependent on FAK-associated signals[126,134,135]. Examples include Ewing sarcoma[134] or ovarian serous carcinoma[135], in which treatment with the FAK inhibitor PF-562,271 blocked *in vitro* and *in vivo* tumour growth in preclinical studies. Future research into the FAK-associated pathways will elucidate new chemotherapy combinations and biomarkers for patient stratification.

Note added in proof

In the sub-section "Regulation of FAK activity": Phosphatidylinositol-4,5-bisphosphate [PI(4,5)P2] binding to FAK FERM induces FAK clustering and partial FAK activation[140].

In the sub-section: "FAK promotes invasive cell phenotypes": Loss of the tumour suppressor DIX domain-containing protein 1 (DIXDC1) triggers SNAIL1- and FAK-dependent EMT[141].

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Competing interests statement
The authors declare no competing interests.

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