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FAK signaling in human cancer as a target for therapeutics

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ABSTRACT

Focal adhesion kinase (FAK) is a key regulator of growth factor receptor- and integrin-mediated signals, governing fundamental processes in normal and cancer cells through its kinase activity and scaffolding function. Increased FAK expression and activity occurs in primary and metastatic cancers of many tissue origins, and is often associated with poor clinical outcome, highlighting FAK as a potential determinant of tumor development and metastasis. Indeed, data from cell culture and animal models of cancer provide strong lines of evidence that FAK promotes malignancy by regulating tumorigenic and metastatic potential through highly-coordinated signaling networks that orchestrate a diverse range of cellular processes, such as cell survival, proliferation, migration, invasion, epithelial–mesenchymal transition, angiogenesis and regulation of cancer stem cell activities. Such an integral role in governing malignant characteristics indicates that FAK represents a potential target for cancer therapeutics. While pharmacologic targeting of FAK scaffold function is still at an early stage of development, a number of small molecule-based FAK tyrosine kinase inhibitors are currently undergoing pre-clinical and clinical testing. In particular, PF-00562271, VS-4718 and VS-6063 show promising clinical activities in patients with selected solid cancers. Clinical testing of rationally designed FAK-targeting agents with implementation of predictive response biomarkers, such as merlin deficiency for VS-4718 in mesothelioma, may help improve clinical outcome for cancer patients. In this article, we have reviewed the current knowledge regarding FAK signaling in human cancer, and recent developments in the generation and clinical application of FAK-targeting pharmacologic agents.

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Abbreviations: ATP, Adenosine triphosphate; CDK, cyclin-dependent kinase; JNK, c-jun N-terminal kinase; DRT, detergent-resistant membrane; EC, endothelial cell; EGF, epidermal growth factor; EGFR, epidermal growth factor receptor; EMT, epithelial–mesenchymal transition; ECM, extracellular matrix; Erk, extracellular signal-regulated kinase; FRNK, FAK-related non-kinase; FRET, fluorescence resonance energy transfer; 5FU, fluorouracil; FAK, Focal adhesion kinase; FAT, focal adhesion targeting; FERM, four-point one-ezrin–radixin–moesin; GAP, GTPase-activating protein; GEF, guanine nucleotide exchange factor; GTP, guanosine-5′-triphosphate; HGF, hepatocyte growth factor; IAP, inhibitory apoptosis protein; MDCK, Madin–Darby canine kidney; MPM, malignant pleural mesothelioma; MMP, matrix metalloproteinase; MTD, maximum tolerated dosage; MAPK, mitogen-activated protein kinase; MMTV, mouse mammary tumor virus; NSCLC, non-small cell lung cancer; NF-κB, nuclear factor kappa-light-chain-enhancer of activated B cells; OS, overall survival; PDAC, pancreatic ductal adenocarcinoma; PyMT, polyoma middle T; PFS, progression free survival; Ras, rat sarcoma; ROCK, Rho-associated protein kinase; PR2D, recommended phase II dose; WRC, scar/WAVE regulatory complex; shRNA, small hairpin ribonucleic acid; siRNA, small interfering ribonucleic acid; SCC, squamous cell carcinoma; SFK, Src family kinase; SH2, Src homology 2; SH3, Src homology 3; IC_{50}, the half maximal inhibitory concentration; 3D, three-dimensional; TGF, transforming growth factor; TKI, tyrosine kinase inhibitor; VEGF, vascular endothelial growth factor.

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1. Introduction

It has been over two decades since focal adhesion kinase (FAK) was first identified as a highly phosphorylated substrate of the viral Src oncogene product (v-Src) localized to the integrin cluster of focal adhesions (Kanner et al., 1990; Schaller et al., 1992). Subsequent identification of potential links between FAK and human cancer of various types (Weiner et al., 1993) led to a plethora of studies, unraveling the molecular mechanisms by which FAK contributes to cancer development and progression. FAK is ubiquitously expressed and functions as a non-receptor cytoplasmic tyrosine kinase as well as a scaffold protein, mediating and regulating specific signals initiated at sites of integrin-mediated cell-extracellular matrix (ECM) attachment (Frame et al., 2010; Schaller, 2010), as well as those triggered by activated growth factor receptors (Saito et al., 1996; Brunton et al., 1997; Chen et al., 1998). Examination of human cancers has identified that enhanced expression of FAK transcripts (Weiner et al., 1993), protein (Owens et al., 1995; Okamoto et al., 2003; Park et al., 2010) and increased FAK activity (Hess et al., 2005) are positively correlated with metastasis and often associated with poorer clinical outcomes (Pylayeva et al., 2009). Based on these pre-clinical findings, attempts to develop FAK-targeting cancer therapeutics have primarily focused on impairing its kinase activity and scaffold function using pharmacological agents, and a number of FAK-directed small molecule inhibitors are currently undergoing clinical testing in cancer patients (Table 1).

In this article, we first review our current understanding of FAK-mediated signaling and how this contributes to cancer development and progression, and then describe the current landscape of FAK-directed cancer therapeutic strategies under pre-clinical and clinical development.

2. Structural features and activation of FAK

2.1. Structural features

The human gene encoding FAK, termed *PTK2*, is localized at chromosome 8q24.3, a region characterized by frequent aberrations in human cancers (Pylayeva et al., 2009; Schaller, 2010). FAK comprises four major domains; a central kinase domain, flanked by a N-terminal four-point-one, ezrin, radixin, moesin (FERM) domain, proline rich regions and a focal adhesion targeting (FAT) C-terminal domain (Fig. 1). Through these multi-domain structural features, FAK functions as both a protein tyrosine kinase and scaffold. Key tyrosine-phosphorylated residues located across these domains play pivotal roles in regulating the molecular functions of FAK by serving as binding sites for the recruitment of signaling proteins, as well as by regulating its catalytic activity (Fig. 1). For example, the phosphorylated Y925 residue serves as a docking site for the Src homology 2 (SH2) domain-containing adaptor protein, Grb2 (Schlaepfer et al., 1994), an important link to the Rat sarcoma (RAS)-mitogen-activated protein kinase (MAPK) signaling pathway, mediating angiogenic and proliferative signals (Fig. 2). In addition, the phosphorylation of two tyrosine residues 576 and 577 located within the activation loop, regulates the catalytic activity of FAK, with phosphorylation of both tyrosine residues being required to establish full catalytic activity (Calalb et al., 1995). Tyrosine 397 serves as the major site of autophosphorylation (Schaller et al., 1994), as well as the binding site for various interacting partners including Src family kinases (SFKs) (Schaller et al., 1994; Polte & Hanks, 1995) and p85 (Chen et al., 1996) (Fig. 1).

2.1.1. N-terminal FERM domain

The FERM domain is localized in the N-terminal region of approximately 30 mammalian proteins, including non-receptor tyrosine kinases such as FAK and JAK, myosins such as MYO7, MYO10 and MYO15, phosphatases such as PTPE1, and ERMs and talins (Frame et al., 2010). In the context of FAK, the FERM domain undertakes several important functions. First, as discussed later, displacement of the FAK FERM domain from the kinase domain represents a critical event in FAK activation (Chen et al., 2001; Serrels et al., 2007; S. T. Lim et al., 2008a,b; Frame et al., 2010; X. L. Chen et al., 2012). This can be initiated by binding of a FERM interacting partner, such as ezrin (Poullet et al., 2001; Frame et al., 2010). Second, the FERM domain undertakes a scaffolding role, mediating protein–protein and protein–lipid interactions, and thereby triggering downstream signaling cascades. For example, upon integrin engagement, the FAK FERM domain binds to the PH domain of the non-receptor tyrosine kinase ETK, which in turn, promotes cell motility (Chen et al., 2001). The FAK FERM domain also couples upstream growth factor receptors such as epithelial growth factor receptor (EGFR) and c-Met at the plasma membrane to promote cell migration or invasion. Transient expression of the wild type FAK FERM domain promoted EGFR-stimulated migration of FAK-null fibroblasts, but not to the same extent as full-length FAK, suggesting that targeting of the C-terminal domain to sites of integrin engagement also contributes to this response (Sieg et al., 2000). With regard to the EGFR, the steroid

Table 1
FAK targeting drugs in clinical trials.

Name	Target protein(s)	Company	Patients	Trial phase	Status/conclusion	NIH number
PF-00562271	FAK, Pyk2	Pfizer/Verastem	Solid cancers	Phase I	Completed	NCT00666926
PF-04554878/VS-6063	FAK, Pyk2	Pfizer/Verastem	Solid cancers	Phase I	Completed	NCT0787033
			Mesothelioma	Phase II	Recruiting	NCT0187060
			NSCLC	Phase II	Recruiting	NCT01951690
			Ovarian	Phase IB/II	Recruiting	NCT01778803
VS-4718	FAK	Verastem	Non-hematologic	Phase I	Recruiting	NCI01849744
GSK2256098	FAK	GlaxoSmithKline	Healthy subjects	Phase I	Completed	NCT00996671
			Solid tumors	Phase I	Recruiting	NCT01138033
			Solid tumors inc. mesothelioma	Phase I	Recruiting	NCT01938443
BI 853520	FAK	Boehringer Ingelheim	Advanced solid cancers	Phase I	Recruiting	NCT01335269
			Advanced solid cancers	Phase I	Completed	NCT01905111

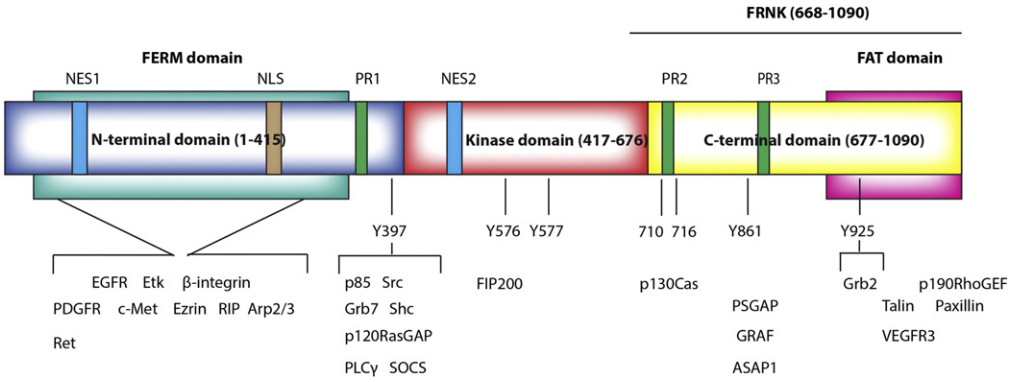

Fig. 1. Schematic representation of FAK, highlighting its multidomain nature, binding partners and key phosphorylation sites. The N-terminal domain (amino acids 1–415) contains the FERM domain and nuclear export sequence 1 (NES1) and nuclear localization sequence (NLS). Binding to specific receptors such as EGFR, PDGFR, c-Met and Ret occurs via the FERM domain. The Y397 autophosphorylation site serves as a binding site for various proteins such as Src, Shc and the regulatory subunit of PI3K, p85. The central kinase domain (417–676) contains the critical Y576 and Y577 residues. Phosphorylation of both of these residues is required for full kinase activity. The C-terminal domain (677–1090) contains two proline-rich regions (PR2 (710–716) and PR3) as well as the FAT domain. PR2 and the FAT domain mediate interaction with various regulators and effectors. Phosphorylation of the Y861 site enhances FAK binding to p130Cas.

receptor co-activator variant Src3Δ4 serves as a signaling linkage between the EGFR and FAK FERM domain critical for cell motility (Long et al., 2010). In addition, the hepatocyte growth factor (HGF)-stimulated assembly of a c-Met/FAK complex via the FAK FERM domain results in a 15-fold increase in FAK activation and promotes the invasion of Madin–Darby canine kidney (MDCK) cells through matrigel (Chen & Chen, 2006). Interestingly, upon the FAT domain-induced recruitment of FAK to focal adhesions, the high local concentration of a phospholipid, PtdIns(4,5)P2, at the plasma membrane may also function as an activator of FAK through the displacement of the FERM domain (Lietha et al., 2007; Cai et al., 2008; Frame et al., 2010; Goni et al., 2014).

The FAK FERM domain also regulates the subcellular localization of FAK. A nuclear function of FAK was initially suggested when N-terminal fragments of FAK were found localized in the nucleus (Lobo & Zachary, 2000; Jones et al., 2001), where they mediated survival signals (Lobo & Zachary, 2000). More recent studies reported that, upon cellular stress such as staurosporine treatment or the disruption of cell adhesions, the nuclear localization sequence (NLS) located within

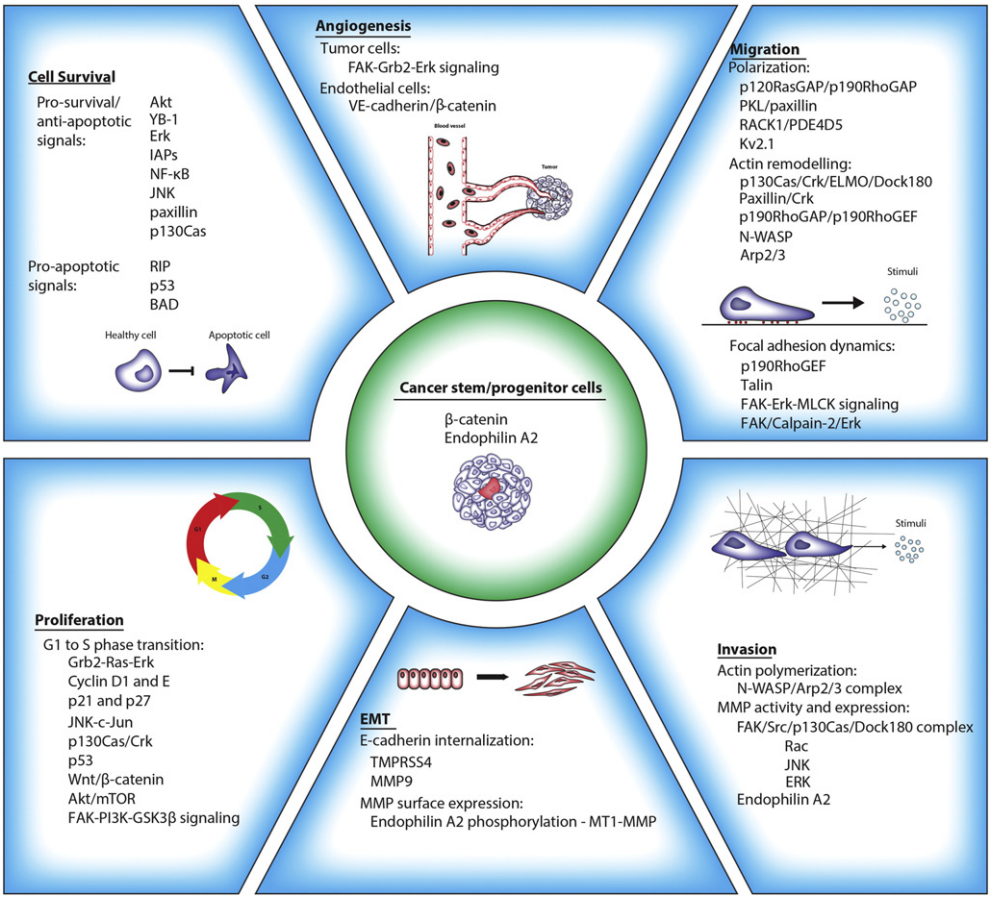

Fig. 2. Summary of FAK signaling targets and pathways that regulate specific processes critical for tumorigenesis and cancer progression.

the FERM domain (Fig. 1) triggers the nuclear transportation of FAK, promoting cell proliferation and survival independent of FAK activation (S. T. Lim et al., 2008a). Export of FAK from the nucleus might be regulated by two nuclear export sequences (NES), one located in the FERM domain and the other in the catalytic domain (Fig. 1) (Schaller, 2010). Such pro-survival actions of nuclear FAK are largely induced through the direct interaction of the N-terminal domain of FAK with the N-terminal domain of p53 and subsequent p53 inhibition (Golubovskaya et al., 2005), as well as via the promotion of Mdm2-dependent p53 ubiquitination and turnover (S. T. Lim et al., 2008a).

2.1.2. C-terminus

Two proline-rich regions within the C-terminal domain of FAK provide the binding sites for Src homology 3 (SH3) domain-containing proteins such as p130Cas, which exerts a motility signal through the activation of Rac (Fig. 1) (Polte & Hanks, 1995; Hsia et al., 2003). In addition, ASAP1 binds to the C-terminus of FAK in order to regulate cytoskeletal dynamics and focal adhesion assembly (Randazzo et al., 2000; Liu et al., 2002). The FAK C-terminus also links FAK to the N-terminal region of VEGFR3 (Garces et al., 2006). When FAK recruitment to focal adhesions was inhibited using a 12-amino acid peptide corresponding to the VEGFR3 binding site, this resulted in decreased cellular proliferation, as well as increased detachment and apoptosis, of breast cancer cells in vitro (Garces et al., 2006).

The FAT domain within the C-terminus of FAK directs FAK to the focal adhesion complex, promoting its co-localization with integrins through an interaction with integrin-associated proteins such as paxillin (Tachibana et al., 1995; Scheswohl et al., 2008) and talin (Chen et al., 1995). The FAT domain also associates with a number of regulators of Rho GTPases, such as p190RhoGEF (Zhai et al., 2003; Y. Lim et al., 2008), GRAF (Schaller, 2010) and PSGAP (Ren et al., 2001).

2.2. Regulation of FAK activation

Several types of signaling events initiate FAK activation. The well-documented example involves engagement of integrins with the ECM and the subsequent co-clustering of proteins such as talin and paxillin with the cytoplasmic tail of integrins (Mitra & Schlaepfer, 2006; Lawson et al., 2012). This, in turn, leads to the recruitment of FAK to sites of integrin clustering via interactions with integrin-associated proteins, leading to FAK activation. Other examples of signaling stimuli promoting FAK activation include stimulation by specific growth factors such as epithelial growth factor (EGF), hepatocyte growth factor (HGF), vascular endothelial growth factor (VEGF) and platelet derived growth factor (PDGF) (Rankin & Rozengurt, 1994; Abedi & Zachary, 1997), activation of particular G-protein-coupled receptors (Salazar & Rozengurt, 2001), and the binding of interacting partners of the FAK FERM domain such as ezrin in an integrin-independent manner (Poullet et al., 2001).

The crystal structure of a fragment of FAK harboring both the FERM and kinase domains in the auto-inhibited state first revealed the precise structural basis of intramolecular FAK regulation (Tsujioka et al., 1999). The first step in FAK activation involves displacement of the FERM domain from the kinase domain, presumably reflecting binding of a phospholipid or peptide ligand to the FERM domain, allowing rapid autophosphorylation of Y397 (Frame et al., 2010). This then creates a high affinity binding site for the SH2 domain of Src, or other SFKs, leads to exposure of the activation loop, and prevents further interactions between the FERM and kinase domains. Src then trans-phosphorylates additional sites on FAK. These include Y576 and Y577 on the kinase domain activation loop, leading to full activation (Calalb et al., 1995). The phosphorylated activation loop also precludes the inhibitory docking of the FERM domain.

Recent studies provide additional mechanistic insights into FAK activation, one of which establishes a phospholipid, phosphatidylinositol 4,5 bisphosphate (PI(4,5)P2), as an important signaling messenger linking integrin signaling to FAK regulation (Goni et al., 2014). Integrin-mediated local production of PI(4,5)P2 promotes binding of PI(4,5)P2 to a basic region (K216AKTLRK222) of the regulatory FAK FERM domain, inducing FAK clustering in focal adhesions (Goni et al., 2014). FAK subsequently transits into a partially open conformation where the autophosphorylation site Y397 is exposed without releasing the autoinhibitory interaction of FERM-kinase domains, but this is sufficient to facilitate autophosphorylation and subsequent Src recruitment to Y397 (Goni et al., 2014). Src-phosphorylation of the activation loop then releases the FERM/kinase domain tether leading to a fully active conformation. Using a variety of complementary approaches including structural and biophysical analyses, Brami-Cherrier et al. identified that autophosphorylation of Y397 requires FAK dimerization, mediated via FERM:FERM and FERM:FAT interactions, and occurs in trans. FERM:FAT interaction involves binding of FAT to a basic patch on the FERM domain. Interestingly, paxillin contributes to positive regulation of FAK activity, by clustering FAK at focal adhesions and reinforcing FERM:FAT association (Brami-Cherrier et al., 2014).

Different types of cellular stimuli impact upon FAK activation. For example, elevated intracellular pH positively regulates FAK (Choi et al., 2013). Here, deprotonation of the His58 FAK residue at high intracellular pH initiates conformational changes that may enhance accessibility of Y397 for autophosphorylation (Choi et al., 2013). Collagen fiber crosslinking and tissue stiffening enhance FAK Y397 phosphorylation in vitro and in vivo, and this is associated with tumor progression in mouse models of breast cancer (Levental et al., 2009). Furthermore, phosphorylation of Y194 in the FERM domain by the Met receptor tyrosine kinase promotes FAK activation through disrupting intramolecular autoinhibition (Chen et al., 2011).

FAK activity is also susceptible by regulation by protein tyrosine phosphatases, and this can occur indirectly or directly. Studies with PTPα-deficient fibroblasts indicate that PTPα phosphatase is required for maximal integrin-stimulated FAK tyrosine phosphorylation, reflecting the role of this phosphatase as a positive regulator of Src activity (Zeng et al., 2003). Furthermore, EphA2 associates with FAK and recruits the protein tyrosine phosphatase SHP2, leading to dephosphorylation of FAK and paxillin and dissociation of the FAK–EphA2 complex (Miao et al., 2000).

FAK is also regulated by association with specific protein binding partners. The delayed rectifier Kv2.1 potassium channel associates with FAK via the LD-like motif at the N-terminus of the channel, and acts to positively regulate Y397 and Y576/577 phosphorylation (Wei et al., 2008). In addition, Abbi et al. (2002) reported that FIP200 functions as an inhibitor for FAK through direct binding to the kinase domain of FAK and subsequent inhibition of FAK catalytic activity in vitro and autophosphorylation in vivo. The association of endogenous FIP200 with FAK was decreased upon integrin-mediated cell adhesion, concomitant with FAK activation. Overexpression of FIP200 inhibited cell spreading, cell migration and cell cycle progression, highlighting the functional consequences of FIP200-mediated FAK inhibition.

A recent fluorescence resonance energy transfer (FRET)-based FAK biosensor study revealed the dynamic spatioregulation of FAK activation in subcellular compartments (Seong et al., 2011). Elevated FRET signals were observed in detergent-resistant membrane (DRM) versus non-DRM regions in response to both cell adhesion and PDGF stimulation. This suggests that membrane microdomains may function to concentrate FAK molecules and manifest their activation. Furthermore, the authors demonstrated differential molecular hierarchy between FAK and Src in response to different stimuli. Cell adhesion-induced FAK activation occurred independent of Src kinase activity yet it still needed the scaffolding function of Src, and FAK acted upstream of Src to activate the latter kinase. However, Src activity was required for PDGF-stimulated FAK activation (Seong et al., 2011).

The FAK/Src complex phosphorylates and/or recruits a plethora of downstream signaling targets including p130Cas, paxillin, PLCγ, SOCS, GRB7, Shc, p120RasGAP, and the p85 subunit of PI3K, initiating specific cellular signaling pathways and responses (Schlaepfer et al., 1999).

Furthermore, Src phosphorylates FAK at Y861, which is associated with an increase in SH3 domain-mediated binding of p130Cas to the FAK C-terminal proline-rich regions (Polte & Hanks, 1995). Activated Src also phosphorylates FAK at Y925, which creates a binding site for the SH2 domain of the Grb2 adaptor protein, and promotes the association of FAK with a VEGF-stimulated $\alpha v\beta 5$ integrin signaling complex in endothelial cells, a critical angiogenic characteristic (Eliceiri et al., 2002).

3. FAK regulation of cell survival and the cell cycle in tumorigenesis

3.1. Cell survival

FAK plays an integral role in tumorigenesis by promoting sustained proliferative and survival signals (Fig. 2, left panels). An association between FAK and cellular transformation was first established by Guan and Shalloway, who reported enhanced tyrosine phosphorylation of FAK in v-Src-transformed cells (Guan & Shalloway, 1992). For normal cells, disruption of integrin-mediated cell-ECM adhesion and the corresponding detachment from the substratum confers deleterious effects on cell survival via induction of a form of apoptosis, known as anoikis. However, in cancer cells, enhanced FAK signaling can override anoikis and promote cell survival in the absence of adhesion signals (Reddig & Juliano, 2005). Both autophosphorylation of Y397 and the kinase activity are required for this effect in MDCK cells in suspension or three-dimensional (3D) culture conditions (Frisch et al., 1996). These findings have subsequently been extended to ovarian carcinoma (Ward et al., 2013), pancreatic adenocarcinoma (Duxbury et al., 2004), squamous cell carcinoma (SCC) (Serrels et al., 2012), mouse mammary tumor (Tanjoni et al., 2010; Walsh et al., 2010), and human basal breast cancer cells (Hochgrafe et al., 2010) where pharmacologic blockade of FAK activity and/or RNAi-based silencing of FAK expression selectively prevented anchorage-independent 3D growth of these cells without affecting their adherent-dependent proliferation in monolayer culture. This functional role of FAK provides one mechanism whereby FAK promotes tumor xenograft growth (Duxbury et al., 2004; Roberts et al., 2008; Tanjoni et al., 2010; Walsh et al., 2010; Stokes et al., 2011; Serrels et al., 2012; Ward et al., 2013).

The molecular basis of FAK-mediated anoikis-resistance in cancer cells has been reported to primarily involve promotion of anti-apoptotic and pro-survival signals (Frisch & Ruoslahti, 1997; Giancotti & Ruoslahti, 1999) (Fig. 2 top left hand panel). Signalling from the FAK/Src complex to Akt1 and extracellular signal-regulated kinase 1/2 (Erk1/2) has been implicated in FAK-mediated suppression of anoikis (Bouchard et al., 2007) and both the tyrosine kinase PTK6 (Zheng et al., 2013), and the cytokine TGFβ1 (Horowitz et al., 2007), act upstream of FAK and PI3K/Akt to confer anoikis-resistance. Further, impaired fibronectin signals induce anoikis of SCC cells by suppressing integrin αv-mediated phosphorylation of FAK and Erk (Kamarajan & Kapila, 2007). Crosstalk between FAK and a number of its downstream signaling components, including JNK (Almeida et al., 2000), p53 (Ilic et al., 1998), p130Cas (Almeida et al., 2000) and paxillin (Zouq et al., 2009), further contribute to anoikis resistance. Furthermore, in coordination with PI3K and Src, FAK sustains pro-apoptotic Bax in a conformation that prevents its mitochondrial localization (Gilmore et al., 2000). More recently, norepinephrine- and epinephrine-induced FAK activation was determined to protect ovarian cancer cells in an orthotopic mouse model from anoikis and promoted tumor growth, which involved β_2-adrenergic receptor signalling and subsequent phosphorylation of FAK Y397 and binding of Src (Sood et al., 2010) (Fig. 2, top left hand panel).

FAK signaling to PI3K/Akt also protects cells from other types of apoptotic stimuli. Chan et al. (1999) demonstrated that the FAK activation of PI3K/Akt protected MDCK cells from apoptosis induced by UV irradiation, and Sonoda et al. (1999) reported that this pathway attenuated apoptosis induced by oxidative stress in glioblastoma cells. Further, pharmacological inhibition of FAK by the FAK-selective inhibitor VS-6063 enhanced sensitivity of taxane-resistant ovarian cancer cells to paclitaxel in vitro and in vivo (as mouse xenografts) through inhibition of an Akt/YB-1/CD44 signalling pathway (Kang et al., 2013).

One of the mechanisms by which FAK kinase activity links to the PI3K/Akt signaling axis is through the recruitment of the regulatory subunit of PI3K, p85, to phosphorylated FAK Y397 (Chen & Guan, 1994; Akagi et al., 2002). The subsequent stimulation of PI3K activity leads to Akt activation, and Akt-mediated evasion of apoptosis involves, but is not limited to, phosphorylation and inactivation of the pro-apoptotic proteins BAD (Datta et al., 1997) caspase 9 (Cardone et al., 1998), or inhibition of transcription factor FKHRL1 (Brunet et al., 1999). Phosphorylation of YAP leading to association with 14-3-3 proteins and inhibition of p73-mediated apoptosis (Basu et al., 2003) and regulation of protein synthesis and nutrient uptake via mTOR and eIF4E (Edinger & Thompson, 2002; Wendel et al., 2004; Chen et al., 2005) have also been implicated in this process.

The protective role of FAK in apoptosis also includes promotion of the anti-apoptotic effects of NF-κB and inhibitory apoptosis proteins (IAPs). Sonoda et al. (2000) observed that FAK-overexpressing HL-60 cells acquire resistance to oxidative stress- and etoposide-induced apoptosis, with a concomitant activation of the PI3K/Akt survival pathway and NF-κB, inhibition of caspase-3, and induction of IAPs (Sonoda et al., 2000). Huang et al. (2007) also demonstrated that FAK prevents cytokine-induced apoptosis through upregulation of the anti-apoptotic NF-κB response and by maintaining the expression of IRS-2 and Bcl-XL. Additionally, FAK enhances anti-apoptotic signals through a direct association with RIP, a major component of the death-inducing signaling complex (DISC). Upon apoptosis-inducing stimuli, such as staurosporine and TNF-α/actinomycin D, FAK recruits RIP from the DISC, and correspondingly suppresses apoptosis (Kurenova et al., 2004; Takahashi et al., 2007).

Sandilands et al. (2012) recently reported how FAK regulates autophagy to control cell survival. They described that components of the autophagy pathway are intimately associated with focal adhesions, and that loss of FAK triggers an apoptotic response, unless the active Src released upon FAK ablation is subject to autophagic targeting. Subsequent work from the same laboratory revealed that the Ret receptor tyrosine kinase is also degraded by autophagy in cancer cells with reduced FAK signaling (Sandilands et al., 2012).

3.2. Cell proliferation

A role for FAK in regulation of cell proliferation was first demonstrated when inhibition of FAK activity upon overexpression of the C-terminal region of FAK resulted in a decrease in proliferation of Balb/c 3T3 and HUVEC cells (Gilmore & Romer, 1996). Later, it was determined that FAK catalytic activity regulates a specific stage of the cell cycle (Fig. 2, bottom left panel). Disruption of fibronectin matrix assembly suppresses FAK tyrosine phosphorylation, and results in delayed G1 to S phase transition (Sechler & Schwarzbauer, 1998), and overexpression of FAK or expression of the FAK-related non-kinase (FRNK) mutant (encompassing the C-terminal region of FAK), accelerates or inhibits G1 to S phase transition, respectively (Zhao et al., 1998). The FRNK mutant also inhibits Erk activation and cyclin D1 induction, and increases expression of the specific cyclin-dependent kinase (CDK) inhibitor p21 (Zhao et al., 1998). SCC cells with FAK ablation grown in 3D culture exhibit a block in transition from G1 to S phase and a reduction in cyclin D1 expression (Serrels et al., 2012). Furthermore, overexpression of the Y397F FAK mutant in glioblastoma cells not only inhibits Erk activation and cyclin D1 expression but also increases expression of another CDK inhibitor, p27, and attenuates expression of cyclin E (Ding et al., 2005). Importantly, FAK enhances the transcription of cyclin D1 through an Ets-binding site in the cyclin D1 promoter in order to promote cell cycle progression upon cell adhesion (Zhao et al., 2001). The effect of FAK on Erk activation is likely mediated via direct Grb2 binding to Y925 of FAK and activation of the Ras

signaling pathway (Schlaepfer et al., 1994). In melanoma cells, expression of Y925F FAK suppressed Erk phosphorylation, VEGF expression and the association of FAK with paxillin, all of which were associated with decreased adhesion-dependent proliferative potential (Kaneda et al., 2008). Using chimeric molecules that fuse the FAT domain of FAK to a number of signaling molecules revealed that targeting of Grb2 to focal adhesions enhanced cell cycle progression, which was correlated with Erk activation (Shen & Guan, 2001).

FAK regulates the cell cycle machinery through other signaling pathways in addition to the RAS-Erk pathway. For example, Oktay et al. (1999) demonstrated that FAK promotes activation of JNK and c-Jun upon integrin engagement, leading to G1 to S phase progression. The association of FAK with Src and p130Cas and the concomitant phosphorylation of p130Cas and recruitment of Crk were required in order to initiate this pathway (Oktay et al., 1999) (Fig. 2, left hand bottom panel).

In addition, an alternative mechanism by which FAK promotes cell proliferation is via its nuclear translocation and interaction with the tumor suppressor, p53, through the FAK FERM domain (Golubovskaya et al., 2005; S. T. Lim et al., 2008a). FAK is targeted into the nucleus via the FERM nuclear localizing sequence and forms a p53 degradation complex by recruiting both p53 and the E3 ubiquitin ligase Mdm2 in a kinase-independent manner (Sulzmaier et al., 2014). This leads to Mdm2-dependent p53 ubiquitination, and degradation of p53 via the 26S proteasomal pathway (Golubovskaya et al., 2005; S. T. Lim et al., 2008a). This suppresses transcriptional activation of a number of p53 target genes, including p21 and Bax, and impairs p53-mediated cell cycle arrest under stress conditions.

A pro-proliferative role for FAK signaling has also been demonstrated through the use of genetically-modified mouse models (Fig. 2, bottom left hand panel). Mammary gland-specific deletion of FAK in mice expressing the polyoma middle T (PyMT) oncogene under control of the MMTV promoter results in delayed mammary tumor formation and reduced tumor incidence (Pylayeva et al., 2009). Deletion of FAK from MMTV-PyMT-transformed mammary epithelial cells in vitro leads to decreased proliferation and invasion, and enhanced sensitivity to anoikis. Interestingly, regulation of these endpoints by FAK requires its interaction with p130Cas (Pylayeva et al., 2009). FAK signaling also provides a proliferative advantage to ErbB2-transformed mammary epithelial cells in vitro (Lahlou et al., 2012). Here, FAK deletion leads to proliferative defects as well as impaired migration, invasion and spreading along with a marked decrease in phosphorylation of FAK binding partners such as Src, paxillin and p130Cas. The anti-proliferative effect is also recapitulated in vivo, indicated by reduced tumor growth (Lahlou et al., 2012). Furthermore, conditional ablation of FAK expression in the epidermis of mice results in suppression of chemically-induced skin tumor formation, accompanied by increased keratinocyte cell death (McLean et al., 2004) and mammary gland-specific FAK deletion impairs development of p53-null and p53R270H mammary tumors (van Miltenburg et al., 2014). In Apc heterozygous mice, FAK acts downstream of Wnt signaling to promote Akt/mTOR activation in vivo and confer enhanced intestinal proliferation and tumorigenesis (Ashton et al., 2010). Further, in a mouse skin tumor model featuring inducible ROCK activation, Samuel and colleagues (Samuel et al., 2011) demonstrated that the FAK-PI3K-GSK3β pathway stabilizes β-catenin leading to its nuclear translocation and transcriptional activation, and hyperproliferation of mouse skin cells.

Despite this work characterizing FAK as a positive regulator of cell cycle progression and pro-proliferative signals, FAK has also been reported to have a dual role in cell proliferation control that is affected by cellular context (Pirone et al., 2006). Thus, under low adhesive conditions, inactive FAK exerts an inhibitory effect on cell proliferation, and elimination of inhibition of FAK function under these conditions leads to activation of RhoA, generation of cell tension, and a proliferative signal. Thus, FAK may act as a sensor that transmits appropriate signals to the cell cycle machinery depending on the adhesive context of the cell (Pirone et al., 2006).

4. Role of FAK signaling in promoting tumor progression and metastasis

4.1. Migration

Cell migration is critical to the metastatic spread of cancer cells, and involves three fundamental steps in 2D culture environments; 1) Establishment of anterior–posterior polarity in the direction of a motility attractant, a process known as polarization; 2) Formation of cell protrusions through lamellipodia at the leading edge driven by actin polymerization, and their attachment to the substratum; 3) Cell contraction and disassembly of focal adhesions at the trailing edge of a cell, and the consequent generation of traction force and net forward movement (Lauffenburger & Horwitz, 1996; Parent & Devreotes, 1999; Friedl & Wolf, 2003).

A functional connection between FAK and cell migration was first established by early observations of keratinocytes and endothelial cells, where elevated expression or phosphorylation of FAK, respectively, was observed during wound healing (Gates et al., 1994; Romer et al., 1994). A number of studies have since reported that FAK-depleted human fibroblasts or fibroblast-like cells display a rounded morphology, impaired membrane protrusions and enhanced assembly of focal adhesions, as well as defective migratory potential (Ilic et al., 1995; Sieg et al., 1999). In the next sections we summarize the FAK-mediated signaling events controlling the key processes of cell migration (Fig. 2, top right hand panel).

4.1.1. Cell polarization

For a cell to develop anterior–posterior polarity in response to a motility cue, it must undertake specific spatial localization of organelles and structures such as the Golgi and microtubule-organizing center, as well as protein complexes (Schaller, 2010). The specific role of FAK in regulation of cell polarization was demonstrated by impaired Golgi orientation towards the leading edge and decreased lamellipodial persistence in FAK-deficient cells (Tachibana et al., 1995; Tomar et al., 2009). One of the key mechanisms for regulation of cell polarity involves coordinating the actions of the Rho family GTPases and their downstream effectors. The Rho family GTPases Rac, Rho and Cdc42, are well-recognized molecular "switches" that regulate cell contractility and polarization and these GTPases function under the tight regulation of FAK (Nobes & Hall, 1995, 1999). For example, Tomar et al. (2009) demonstrated that integrin-induced tyrosine phosphorylation of FAK leads to recruitment of p120RasGAP, which in turn bridges FAK to p190RhoGAP. Tyrosine phosphorylation of the latter protein is associated with increased GAP activity, and the resulting transient decrease in RhoA activation enables directional cell movement (Tomar et al., 2009). Furthermore, the ArfGAP PKL/GIT2 is tyrosine phosphorylated by the FAK/Src complex, leading to association with paxillin (Yu et al., 2009). Perturbation of PKL expression or function led to defects in cell polarization and directional migration, and is associated with altered temporal regulation of Rac and Cdc42, and defective polarized recruitment of the Rac GEF βPIX to the leading edge (Yu et al., 2009). Serrels et al. (2011) determined that the "direction-sensing" complex formed by FAK, RACK1 and PDE4D5 serves to regulate cell polarization by signaling to the GEF EPAC and the low molecular weight G protein Rap1. This complex serves to maintain Rap1 activity low in nascent adhesive structures. Additionally, interaction between FAK and the delayed rectifier Kv2.1 potassium channel, has been reported to regulate FAK activation and directional cell migration (Wei et al., 2008).

4.1.2. Regulation of focal adhesion dynamics

FAK-depletion in fibroblasts results in an increased number and size of focal adhesions and defective cell motility, indicating that FAK plays critical roles in regulating the formation, maturation and turnover of focal adhesions (Ilic et al., 1995; Webb et al., 2004). In the canonical model for FAK recruitment and signaling at focal adhesions, FAK initially

localizes to sites of integrin engagement with the ECM through binding to paxillin and talin. Following activation, FAK phosphorylates α-actinin and modulates its ability to crosslink actin stress fibres, impacting on focal adhesion maturation and turnover, and the FAK–Src complex phosphorylates paxillin, regulating focal adhesion dynamics (Mitra & Schlaepfer, 2006). An additional mechanism whereby FAK regulates focal adhesion turnover is through modulation of Erk- and MLCK-regulated actomyosin contractility. FAK-mediated Erk localization to adhesions promotes the phosphorylation of MLCK and increases contractility, which destabilizes then disassembles focal adhesion complexes (Webb et al., 2004). Furthermore, FAK associates with the protease calpain-2, which cleaves specific focal adhesion components, including FAK itself, resulting in adhesion complex turnover (Franco & Huttenlocher, 2005; Chan et al., 2010). Of note, assembly of a calpain 2/FAK/p42Erk complex activates calpain-2 in an Erk-dependent manner, leading to FAK cleavage, focal adhesion turnover and cell migration (Carragher et al., 2003). Caspase-8 also forms a complex with FAK and calpain 2 to destabilize focal adhesions and promote efficient cell motility (Barbero et al., 2009).

However, ongoing research continues to challenge the canonical model of FAK signaling at focal adhesions. For example, a recent study identified that FAK can recruit talin to nascent adhesions independently of integrins. This involves direct binding of talin to FAK, with a critical role for the FAK Glu1015 residue (Lawson et al., 2012). Mutations that disrupt FAK-talin binding inhibit proteolytic talin cleavage, thereby preventing efficient focal adhesion turnover (Lawson et al., 2012). In addition, while the canonical model originally placed p190RhoGEF downstream of the integrin/FAK complex (see below), it now appears that p190RhoGEF plays an important scaffolding role, promoting FAK localization to early peripheral adhesions and FAK activation in a manner dependent on the p190RhoGEF PH domain (Miller et al., 2013).

4.1.3. Reorganization of actin-cytoskeletal structures

4.1.3.1. p130Cas and paxillin. The best-characterized FAK/Src downstream targets are p130Cas and paxillin, both of which promote migration by signaling to Rho family GTPases and influencing the dynamics of cell adhesion sites (Timpson et al., 2001; Hanks et al., 2003; Chodniewicz & Klemke, 2004; Mitra et al., 2005). SH3-domain-mediated binding of p130Cas to FAK, which is enhanced by FAK/Src-induced phosphorylation of Y861 FAK, leads to enhanced phosphorylation of p130Cas at multiple sites (Polte & Hanks, 1995; Schlaepfer et al., 1997; Mitra et al., 2005). This mediates SH2-mediated binding of the Crk adaptor protein, which in turn recruits the Crk binding partners ELMO and Dock180 that provide GEF activity towards Rac and hence promote lamellipodia formation and stabilization of focal complexes (Brugnera et al., 2002; Parsons, 2003; Playford & Schaller, 2004; McLean et al., 2005). An alternative signaling route of the FAK/Src complex affecting cell migration is through the paxillin/Crk interaction. Phosphorylation of paxillin on Y31 and Y118 by the FAK/Src complex promotes SH2-mediated coupling of Crk to paxillin (Burridge et al., 1992; Birge et al., 1993). Overexpression of paxillin harboring mutations at these sites blocks the turnover of focal adhesions (Webb et al., 2004) and cell motility (Subauste et al., 2004).

4.1.3.2. Rho GTPases; GAPs and GEFs. Multiple signaling mechanisms are employed by FAK to maintain the activation of Rho GTPases in balance by regulating the opposing actions of GAPs and GEFs, and thereby coordinating cell migration (Tomar & Schlaepfer, 2009; Schaller, 2010). For example, upon fibronectin-stimulated cell spreading, FAK mediates cycles of RhoA inactivation/activation through the selective interaction with p190RhoGAP (Tomar et al., 2009) and p190RhoGEF (Y. Lim et al., 2008), respectively. Tomar and Schlaepfer (2009) proposed a model, in which, at early stages of fibronectin-mediated cell spreading, FAK recruits p190RhoGAP and triggers "push" migratory signals through RhoA inhibition, while at a later stage, FAK-mediated activation of p190RhoGEF and Rho exerts subsequent "pull" signals by promoting cell contractility (Y. Lim et al., 2008; Tomar & Schlaepfer, 2009).

Importantly, Rho is also a key regulator of focal adhesion disassembly at the rear of migrating cells (Gupton & Waterman-Storer, 2006). In association with RhoGEFs, FAK functions to promote RhoA and ROCK activation, which in turn results in increased contractility and disassembly of focal adhesions at the trailing edge (Iwanicki et al., 2008).

4.1.3.3. N-WASP and Arp2/3 complex. The major effector of Cdc42 is a member of WASP/WAVE family, N-WASP, which functions to activate the Arp2/3 complex, a major mediator of the formation of branched actin networks (Machesky & Insall, 1998; Ridley et al., 2003). Importantly, FAK can regulate cell migration by binding to and phosphorylating the Cdc42 target, N-WASP on Y256 residue (Wu et al., 2004). FAK can also bypass the association with N-WASP, and directly bind to the Arp2/3 complex via the FERM domain (Serrels et al., 2007). Recruitment of Arp2/3 does not require FAK catalytic activity and indeed, assembly of the Arp2/3-FAK complex is prevented upon FAK Y397 phosphorylation (Serrels et al., 2007). These findings suggest a possible model in which FAK serves to recruit Arp2/3 to nascent adhesions, and upon phosphorylation FAK releases the Arp2/3 complex, which then promotes the formation of lamellipodia (Serrels et al., 2007).

4.2. Invasion

Tumor invasion is characterized by the penetration of cancer cells through the ECM and into neighboring tissue, which requires combined effects of enhanced cell motility and alterations in dynamics of focal adhesions, together with proteolytic degradation of the matrix (Mitra et al., 2005; Mitra & Schlaepfer, 2006). A potential role of FAK in tumor invasion was revealed by early in vitro findings, in which a gain-of-function mutation of the SH3 domain in v-Src conferred enhanced binding to and phosphorylation of FAK, and this was associated with an elevated invasive phenotype in matrigel and co-localization of FAK, v-Src and β1 integrin at invadopodia (Hauck et al., 2002a) (Fig. 2, bottom right hand panel). Consistent with this finding, several studies have demonstrated that FAK expression and phosphorylation are elevated in invasive human cancers (Gabarra-Niecko et al., 2003; Oktay et al., 2003; Lark et al., 2005; Madan et al., 2006; Theocharis et al., 2009; Alexopoulou et al., 2014) and cancer cell lines (Hauck et al., 2001; Schneider et al., 2002).

Importantly, over the last decade, increasing fidelity of 3D models to recapitulate in vivo settings have enhanced our understanding of the differential mechanism by which FAK functions in 2D migration versus 3D invasion of cancer cells. While v-Src transformation of FAK-null fibroblasts restores migratory defects, invasion through 3D matrices is not rescued (Hsia et al., 2003). To regain the invasive phenotype, the integrity of the C-terminal proline-rich SH3 binding sites on FAK, Y397 phosphorylation and FAK kinase activity were all required (Hsia et al., 2003). Furthermore, while the Scar/WAVE regulatory complex (WRC) normally drives lamellipodia assembly via the Arp2/3 complex and is required for 2D migration, loss of WRC promotes FAK-dependent invasion of SCC cells in a 3D organotypic assay, as well as tumor growth in vivo (Tang et al., 2013). Loss of WRC leads to increased FAK expression and activation, recruitment of activated N-WASP/Arp2/3 complex to leading invasive edges by FAK-containing focal complex structures and subsequent Arp2/3-mediated actin assembly (Tang et al., 2013).

Additional mechanisms employed by FAK to drive 3D invasion have been demonstrated. Fibroblasts transformed by v-Src exhibit accumulation of v-Src/FAK complexes within invadopodia and the concomitant formation of a FAK-Src-p130Cas-Dock180 signaling complex that leads to increased Rac and JNK activation as well as enhanced matrix metalloproteinase-2 (MMP2) activity and MMP9 expression (Hsia et al., 2003) (Fig. 2, right bottom panel). Further, inhibition of v-Src-induced invasion of fibroblasts through matrigel in vitro by expression of dominant-negative FAK (FRNK) involves attenuation of p130Cas

phosphorylation and Erk2 and JNK activation, as well as reduced gene transcription and secretion of MMP2 (Hauck et al., 2002b), and both FRNK expression and FAK antisense treatment inhibited MMP9 secretion and blocked serum-stimulated invasion of lung adenocarcinoma cells through matrigel (Hauck et al., 2001). Other groups have also reported a role for FAK-induced MMP9 production in cell invasion (Shibata et al., 1998; Meng et al., 2009). FAK can also alter MMP expression and localization via its scaffolding function. FAK binds to endophilin A2 via the latter's SH3 domain, which in turn promotes tyrosine phosphorylation of endophilin A2 by Src. This modification reduces endocytosis of MT1-MMP, thereby increasing its cell surface expression (Wu et al., 2005). In addition, in association with p130Cas, FAK regulates the targeted action of MT1-MMP facilitating degradation of ECM at focal adhesion sites in a Src-dependent manner (Wang & McNiven, 2012). Impairment of the FAK–p130Cas–MT1-MMP complex suppresses ECM degradation and invasion of fibrosarcoma cells through matrigel.

Interaction with the hepatocyte growth factor (HGF) receptor c-Met regulates FAK to promote cell invasion (Chen & Chen, 2006). HGF stimulation leads to recruitment of tyrosine phosphorylated c-Met to the FERM domain of FAK (Chen & Chen, 2006). While the FAK K222A mutant defective in c-Met binding can still promote HGF-induced motility in MDCK cells, acquisition of an invasive phenotype was prevented. In lung cancer cells, formation of a c-Met/FAK complex was associated with an invasive phenotype, and expression of the N-terminal region of FAK suppressed cellular invasion in vitro (Chen & Chen, 2006).

Furthermore, a recent study by Jean and colleagues identified that endothelial FAK kinase activity exerts an important role in regulating trans-endothelial migration of tumor cells and hence metastasis (Jean et al., 2014). Endothelial cell (EC)-specific FAK catalytic activity was required for VE-cadherin Y658 phosphorylation in response to tumor-associated VEGF in melanoma and ovarian cancer mouse models. Both EC-specific FAK inhibition and VE-cadherin Y658F mutation blocked VEGF-stimulated tumor cell migration across endothelial cell barriers, and EC-FAK inhibition prevented melanoma dermal to lung metastasis without affecting primary tumor growth.

4.3. Epithelial–mesenchymal transition

Epithelial–mesenchymal transition (EMT), characterized by acquisition of a mesenchymal phenotype, increased migratory and invasive potential, enhanced resistance to apoptosis, and increased production of ECM components, is thought to represent an important step in cancer progression (Thiery & Sleeman, 2006; Kalluri & Weinberg, 2009). This also involves downregulation of intercellular contacts and increased formation of cell-matrix adhesions (Avizienyte & Frame, 2005). Importantly, FAK cooperates with Src to exert an important role in these events (Fig. 2, middle bottom hand panel). Specifically, Src-induced FAK phosphorylation promotes the dissolution of E-cadherin-containing intercellular junctions in colon cancer cells (Avizienyte et al., 2002) and in a TGF-β-induced model of EMT in hepatocytes, the FAK/Src signaling complex is required for establishment of EMT features, including upregulated transcription of mesenchymal and invasiveness markers, such as matrix metalloproteinase-9 (MMP9) and fibronectin, and downregulation of membrane-bound E-cadherin (Cicchini et al., 2008). In addition, type II transmembrane serine protease, TMPRSS4, induces EMT in colon cancer cells through activation of the FAK and Erk signaling axes via integrin α5 upregulation (Kim et al., 2010), and FAK, via its scaffolding capability, directs Src-mediated phosphorylation of endophilin A2 at Y315 and thereby promotes EMT characteristics such as surface expression of MT1-MMP, associated with tumor development and progression in the MMTV-PyMT mouse model of breast cancer (Fan et al., 2013). Consistent with these data from in vitro and in vivo models, immunohistochemical examination of human laryngeal tumors revealed that elevated FAK expression was associated with the loss of E-cadherin in nodal metastases (Rodrigo et al., 2007).

Interestingly, using FRAP technology (Serrels et al., 2009), Canel et al. demonstrated that FAK plays an important role in E-cadherin-dependent collective movement of SCC cells in 3D culture and in vivo (Canel et al., 2010). Here, the authors reported that E-cadherin internalization and the integrity and strength of cell–cell junctions are under FAK regulation (Canel et al., 2010), and FAK/Src inhibition stabilized cell–cell contacts to impair collective cell movement (Fig. 2, middle bottom panel). Overall, these findings highlight the role of FAK, in association with Src, in regulating the dissolution of E-cadherin-based intercellular adhesions in vitro and in vivo.

4.4. Angiogenesis

Angiogenesis is critical to malignant progression and involves the local formation of nascent blood vessels from pre-existing vasculature through stimulation of ECs and subsequent mobilization, proliferation and sprout formation (Sulzmaier et al., 2014). FAK integrates angiogenic signals from vascular endothelial growth factor receptors (VEGFRs) and integrin receptors, and directs the migration and growth of endothelial cells to promote angiogenesis (Veikkola et al., 2000) (Fig. 2, top middle panel). The requirement of FAK in angiogenesis was initially suggested by early observations of restricted patterns of enriched FAK expression in the embryonic vasculature (Polte et al., 1994) and the embryonic lethality conferred upon FAK gene ablation in mice, which is due to cardiovasculature defects (Ilic et al., 2003).

A direct role of endothelial FAK in regulating vascular permeability, a critical pro-angiogenic feature, has been reported (X. L. Chen et al., 2012). VEGF-induced FAK activation promotes rapid localization of FAK to endothelial adherens junctions and binding of FAK to vascular endothelial (VE)-cadherin via its FERM domain and FAK-mediated phosphorylation of β-catenin. This subsequently induces β-catenin/VE-cadherin dissociation and increased junctional breakdown. Pharmacologic or genetic blockade of FAK signaling in endothelial cells suppresses VEGF-stimulated vascular permeability and β-catenin Y142 phosphorylation (X. L. Chen et al., 2012).

In the context of cancer, the angiogenic function of FAK was demonstrated using EC-specific FAK-null melanoma- or lung-carcinoma-bearing mice, which exhibit suppressed VEGF-mediated tumor angiogenesis and growth (Tavora et al., 2010). Treatment of mice bearing glioblastoma cell line xenografts with the FAK inhibitor PF-00562271 reduced tumor microvasculature density (Roberts et al., 2008). Additionally, elevated FAK expression was observed in the vascular and tumor cell compartments of invasive breast cancer specimens (Alexopoulou et al., 2014). High microvessel density was also observed in epithelial ovarian cancers with elevated endothelial cell FAK protein expression and FAK phosphorylation (Stone et al., 2014).

A key mechanism underpinning the pro-angiogenic role of tumoral FAK is induction of VEGF expression. Inhibition of FAK catalytic activity in breast carcinoma cells by stable expression of FRNK reduces FAK Y925 phosphorylation, the ability of the Grb2 adaptor protein to bind to FAK, as well as Erk2 activation (Mitra et al., 2006). The concomitant impairment of FAK–Grb2–Erk2 signaling results in decreased VEGF expression in vitro and in vivo together with small avascular tumors in mice without affecting cell survival or proliferation in vitro (Mitra et al., 2006). Reconstitution experiments with a FAK Y925F or impaired kinase activity mutants in Src-transformed FAK-null fibroblasts confirmed the role of this FAK phosphorylation site and catalytic activity in regulating VEGF-associated angiogenesis. Suppression of FAK expression in neuroblastoma, breast and prostate carcinoma cells also results in reduced VEGF expression (Mitra et al., 2006).

In contrast to these findings, however, a recent study by Kostourou et al. (2013) highlighted a counter-intuitive role of FAK in tumor angiogenesis, where FAK-heterozygous mice exhibited enhanced growth and angiogenesis of melanoma and lung carcinoma xenografts. Furthermore, FAK heterozygous endothelial cells were characterized by increased survival and microvessel sprouting ability and elevated

serum-induced Akt phosphorylation. The mechanism for these effects is not clear but may reflect an impact of altered expression levels on the scaffolding function of FAK.

Overall, these findings indicate that FAK can play contrasting roles within cancer cells and the surrounding tumor microenvironment, and highlight novel rationales for therapeutic targeting of FAK.

5. FAK and cancer stem cells

Cancer stem cells refer to a subset of tumor cells that exhibit "stem-like" properties, such that they exhibit the potential to self-renew and also generate the different cell types that comprise the tumor (Visvader & Lindeman, 2008). Consequently, they contribute to intratumoral heterogeneity and sustained tumorigenesis. Cancer stem cells infrequently enter the cell cycle, and thereby constitute a subpopulation refractory to conventional cancer therapies that target rapidly dividing cells (Al-Hajj et al., 2003; Eramo et al., 2008; Hurt et al., 2010).

Luo and colleagues (Luo et al., 2009) identified that the conditional targeting of FAK expression specifically in mammary epithelial cells of MMTV-PyMT mice reduced the pool of mammary cancer stem/progenitor cells in primary tumors as well as their self-renewal and migratory potential in vitro (Fig. 2, center panel). Furthermore, cancer stem/progenitor cells from these mice exhibited not only decreased tumorigenicity but also maintenance in tumors, factors that likely underpin reduced tumor growth and metastasis in the FAK-deficient mice (Luo et al., 2009). Interestingly, the Guan group recently extended their findings by probing the requirement for FAK kinase activity in mammary stem/progenitor cells via knock-in of a kinase-dead mutant. This demonstrated that FAK kinase activity is required for luminal progenitor proliferation, whereas kinase-independent functions support basal mammary stem cell activities (Fig. 2 center). Consistent with these data, FAK kinase inhibitors inhibited proliferation and spheroid formation by luminal progenitor-, but not mammary stem cell-like, breast cancer cells (Luo et al., 2013). In addition, the scaffolding function of FAK contributes to tumor development and progression by coordinating EMT and mammary cancer stem cell activities in vivo (Fan et al., 2013) (Fig. 2, center). In the MMTV-PyMT model, disruption of the scaffold function of FAK through a P878A/P881A mutation reduced mammary tumor development and metastasis and decreased the expression of markers for EMT and mammary cancer stem cell activities, as well as surface expression of MT1-MMP. The underlining mechanism for these effects was disruption of FAK-endophilin A2 binding, and hence a reduction in the ability of Src to phosphorylate endophilin A2 (Fan et al., 2013). Further, although the effect on cancer stem cells was not specifically documented, studies by other groups reported that deletion of FAK attenuates tumorigenesis and progression in mouse models of breast cancer (Lahlou et al., 2007; Provenzano et al., 2008; Pylayeva et al., 2009).

FAK regulation of cancer stem cell activities has also been reported in other cancer types. Crosstalk between FAK and the Wnt/β-catenin signaling pathway regulates an early stage of tumorigenesis involving proliferation of epidermal stem cells in vivo (Ridgway et al., 2012). Here, FAK promotes TPA-induced proliferation of epidermal stem cells within the mouse skin by regulating nuclear localization of β-catenin and transcriptional activation of key Wnt targets such as c-Myc (Ridgway et al., 2012) (Fig. 2, center). In addition, loss of the transcription factor Ikaros arrests precursor B cells at a highly-adherent and proliferative stage with augmented self-renewal and impaired differentiation properties. This occurs in a manner dependent on FAK activation and predisposes the cells towards transformation to a leukemic state (Joshi et al., 2014). A further link between FAK and cancer stem cells was recently identified in mesothelioma. In a patient-derived xenograft model of this malignancy, the FAK tyrosine kinase inhibitor VS-4718 preferentially eliminated the cancer stem cells that were enriched following treatment with the standard-of-care pemetrexed and cisplatin chemotherapy agents (Shapiro et al., 2014).

Taken together, these findings highlight important roles for FAK in regulating cancer stem cells (Fig. 2, center) and highlight novel opportunities for therapeutic intervention, discussed later in the review.

6. FAK expression in human cancers

It is now well-established that FAK expression is elevated in certain human cancers. A potential link between FAK and cancer was first reported over twenty years ago in a study that identified elevated levels of FAK transcripts in various cancer types (Weiner et al., 1993). One of 8 adenomatous tissues, 17 of 20 invasive tumors, and all 15 metastatic cancers showed increased FAK mRNA levels, whereas 6 normal tissue samples displayed no detectable FAK mRNA, suggesting that FAK overexpression may be an early event in cancer development and FAK may play a role in tumor progression and metastasis. Similarly, a number of subsequent studies reported upregulation of FAK expression in a broad range of tumors including astrocytic (Jones et al., 2001), breast (Weiner et al., 1993; Cance et al., 2000; Garcia et al., 2007), cervical (Gabriel et al., 2006), colorectal (Owens et al., 1995; Cance et al., 2000; Yu et al., 2006), endometrial (Livasy et al., 2004), esophageal (Miyazaki et al., 2003), gastric (Su et al., 2002), head and neck (Canel et al., 2006), hepatocellular (Fujii et al., 2004), laryngeal (Rodrigo et al., 2011), lung (Carelli et al., 2006; Hsu et al., 2007), ovarian (Judson et al., 1999), pancreatic (Furuyama et al., 2006), prostate (Tremblay et al., 1996) and thyroid (Kim et al., 2004) cancers.

Despite these lines of evidence, the precise molecular mechanisms responsible for the increased FAK expression in human cancers remain largely uncharacterized. One of the proposed mechanisms underlying FAK overexpression in cancer is via FAK amplification. Using in situ hybridization, copy number gains at 8q24.3, the cytogenetic locus of FAK, were first reported in cell lines derived from human cancers of lung, breast and colon (Agochiya et al., 1999). Elevation of FAK protein expression in cell lines derived from invasive squamous cell carcinomas as well as in frozen sections of these cancers is associated with gains in copy number of the human FAK gene (Agochiya et al., 1999), and additional studies have reported a correlation between FAK amplification and FAK expression in breast (Yom et al., 2011) and other tumor types, including ovarian (Stone et al., 2014), gastric (Park et al., 2010), hepatocellular (Okamoto et al., 2003) and prostate (Menon et al., 2013) carcinoma. However, increased FAK expression can also occur independently of FAK gene amplification, as shown in a study on head and neck carcinoma (Canel et al., 2006), indicating that transcriptional and/or post-transcriptional mechanisms may contribute.

Analysis of the FAK promoter region revealed putative binding sites for the transcription factor NF-κB and the tumor suppressor p53 (Golubovskaya et al., 2004; Golubovskaya & Cance, 2007). FAK promoter activity is stimulated by NF-κB and suppressed by p53 through their binding to this promoter (Golubovskaya et al., 2004, 2008) and primary breast and colon cancers harboring p53 mutations exhibit increased FAK expression (Golubovskaya et al., 2008). In addition, immunohistochemical, single strand conformational polymorphism and sequencing analyses of 622 breast cancers revealed that expression of FAK was associated with p53 mutation, and that FAK-positive tumors were more likely to harbor p53 mutation by 2.5-fold in comparison to FAK-negative tumors (Golubovskaya et al., 2009). These findings highlight how FAK expression can be perturbed by transcriptional regulators implicated in cancer. Moreover, they indicate that aberrant regulation of FAK may contribute to the gain-of-function role of mutant p53 in driving metastasis of many cancer types in vivo (Morton et al., 2010).

Elevated FAK expression is correlated with grade, stage and nodal disease in most malignancies examined (Chatzizacharias et al., 2008). High FAK expression is associated with an aggressive phenotype in breast cancer specimens characterized by high mitotic index, HER2/neu overexpression (Lark et al., 2005) and estrogen and progesterone receptor negativity (Lark et al., 2005; Yom et al., 2011). FAK amplification is also positively correlated with tumor size, nodal metastasis,

distant metastasis, lymphatic invasion, venous invasion and perineural invasion in gastric cancer (Park et al., 2010). Further, phosphorylation on FAK Y397 and Y576, key activation sites of the kinase, correlated with enhanced invasion, migration and vasculogenic mimicry plasticity in a panel of uveal and cutaneous melanoma cell lines (Hess et al., 2005). Interestingly, a number of studies have shown that increased FAK expression and activity are associated with not only malignant and/or metastatic disease (Oktay et al., 2003; Madan et al., 2006), but also with poor prognosis (Park et al., 2010). In particular, elevated expression of FAK mRNA was inversely correlated with metastasis-free survival in the large cohort of breast cancer patients from the NKI dataset (Pylayeva et al., 2009). In this study, multivariable analysis indicated that elevated FAK mRNA expression was an independent predictor of poor outcome and that it outperformed many commonly used clinical parameters, such as lymph node involvement, ER negativity and poor differentiation (Pylayeva et al., 2009). Furthermore, FAK overexpression was positively correlated with lymph node and distal metastasis in ovarian cancer patients, as well as with a significant reduction in patient overall survival (Sood et al., 2004). Elevated FAK mRNA levels in serious ovarian carcinoma are also associated with reduced patient overall survival (Ward et al., 2013). These studies indicate that FAK could potentially be used as a prognostic marker.

Overexpression of wild type FAK likely enhances certain cancer hallmarks, such as increased cell survival under anchorage-independent conditions and migratory capability (Fig. 2). It is also likely to cooperate with certain signals provided within the cancer cell (e.g. increased activation of certain receptor tyrosine kinases, such as Met) or by the tumor microenvironment (e.g. tissue stiffness). However, FAK is also subject to alternative splicing in human cancer, leading to the generation of distinct isoforms, which contribute to tumor progression. In this regard, a novel gain-of-function somatic mutation leading to exon 33 deletion (FAK-Del33) has recently been detected in breast and thyroid cancers (X. Q. Fang et al., 2014). Overexpression of FAK-Del33 in vitro resulted in elevated FAK Y397 phosphorylation and enhanced cell migration. This deletion occurs in the FAT domain, hence, the binding of paxillin and/or talin may be compromised (X. Fang et al., 2014; X. Q. Fang et al., 2014). In addition, exon 26 deletion was detected in 6 of 102 breast cancer specimens. This splice variant exhibits resistance to caspase-mediated cleavage in vitro and protects cells from apoptosis (Yao et al., 2014).

7. Pharmacologic strategies targeting FAK

FAK has long been considered as a potential target for cancer therapeutics, reflecting its pivotal role in governing malignant characteristics and the evidence of high expression and activity in both preclinical cancer models and human cancers. A number of inhibitory approaches were initially employed to functionally interrogate the oncogenic role of FAK. These included antisense oligonucleotide (Sonoda et al., 1997; Judson et al., 1999), siRNA- (Ding et al., 2005; Huang et al., 2005; Tilghman et al., 2005) and shRNA-based (Mitra et al., 2006; S. T. Lim et al., 2008a; Chen et al., 2010) abrogation of FAK expression, and overexpression of FRNK (Richardson et al., 1997; Hauck et al., 2001, 2002b; Taylor et al., 2001). Attenuation of FAK signaling through these approaches led to decreased cell viability through induction of apoptosis, as well as impaired migratory and angiogenic capacity of cancer cells in vitro and in vivo, and provided proof-of-principle for the development of more clinically relevant pharmacologic approaches such as small molecule inhibitors.

Over the past decade, a number of preclinical and clinical studies have employed a variety of pharmacologic agents that utilize different mechanisms for the blockade of FAK signaling in cancer. Of these, several orally bioavailable ATP-competitive FAK inhibitors have entered early clinical testing. In the following section, we summarize recent advances in the development of small molecule FAK inhibitors (Table 1).

7.1. PF-573,228

Initial drug discovery by Pfizer identified PF-573,228 as a prototype ATP-competitive inhibitor of FAK (Slack-Davis et al., 2007). This is the mother compound for the derivative FAK-directed drugs (VS-6062 and VS6063) that are currently being evaluated by Verastem. PF-573,228 exhibits an IC_{50} value of 4 nM, and inhibits cell migration by blocking focal adhesion turnover, but has no effect on cell growth or survival in fibroblast or prostate cancer cell lines (Slack-Davis et al., 2007). Despite its potent efficacy in FAK inhibition, PF-573,228 showed limited anticancer effects possibly due to the compensatory role of the FAK homologue, Pyk2 (Schultze & Fiedler, 2010). There is no report that further evaluates this compound in the pre-clinical or clinical settings.

7.2. TAE-226

This pre-clinical compound is an orally bioavailable, ATP-competitive inhibitor of FAK, Pyk2 and IGF-1R, exhibiting highly potent inhibitory activity towards FAK as indicated by an IC_{50} value of 5 nM (Liu et al., 2007). TAE-226 demonstrated potent antitumor activities in a panel of in vitro and in vivo cancer models including glioma (Liu et al., 2007; Shi et al., 2007), ovarian cancer (Halder et al., 2007), neuroblastoma (Beierle et al., 2008), esophageal cancer (Watanabe et al., 2008), imatinib-resistant GIST (Sakurama et al., 2009), pancreatic cancer (Liu et al., 2008), and oral squamous cell carcinoma (Kurio et al., 2012). TAE-226-treated tongue squamous cell carcinoma and esophageal cancer cell lines exhibited impaired cell attachment and time- and dose-dependent growth inhibition as well as concomitant inhibition of Akt S473 phosphorylation and induction of caspase-mediated apoptosis (Watanabe et al., 2008; Kurio et al., 2012). Oral administration of TAE-226 in xenograft mouse models of these cancers markedly suppressed tumor growth (Watanabe et al., 2008; Kurio et al, 2012). Furthermore, administration of TAE-226 at concentrations of 50–75 mg/kg significantly increased median survival in an in vivo intracranial glioma xenograft model (Liu et al., 2007). In a mouse xenograft model of MDA-MB-231 human breast cancer cells, oral administration of TAE-226 not only conferred tumor regression, but also decreased bone metastasis and prolonged survival (Kurio et al., 2011). Despite these encouraging pre-clinical findings, at this point development stalled due to the drug failing clinical trials for undisclosed reasons.

7.3. PF-00562271

PF-00562271, also known as VS-6062, is an orally bioavailable, potent ATP-competitive dual inhibitor of both FAK and Pyk2, developed by Pfizer and now acquired by Verastem. This compound exhibits greater selectivity for FAK and Pyk2 than its predecessor PF-573,228 compound, > 100-fold selectivity for FAK and Pyk2 in comparison to a panel of non-target kinases with an IC_{50} of 1.5 and 14 nM, respectively (Roberts et al., 2008). PF-00562271 robustly inhibits Y397 FAK phosphorylation in a dose-dependent manner both in vitro and in vivo, and shows a broad pre-clinical activity against cancer types (Roberts et al., 2008). Roberts et al. first reported the antitumor efficacy of PF-00562271, with tumor regression observed in multiple xenograft models following PF-00562271 treatment without weight loss, morbidity or mortality in mice (Roberts et al., 2008). Although this compound, like its predecessor PF-573,228, does not affect proliferation (Stokes et al., 2011) or apoptosis (Roberts et al., 2008) of cells grown in monolayer, it strongly inhibits anchorage-independent growth of basal breast cancer cells in soft agar (Hochgrafe et al., 2010) and decreases xenograft growth of several human cancer cell lines including those derived from prostate, pancreatic, breast, lung and colon cancer, as well as glioblastoma (Roberts et al., 2008; Sun et al., 2010). In line with these studies, Serrels et al. (2012) showed that PF-00562271 decreased growth of SCC in 3D culture and as xenografts, but an additional finding was that this tyrosine kinase inhibitor also reduced FAK-mediated Src activation.

Furthermore, Crompton et al. (2013) demonstrated induction of apoptosis upon PF-00562271 treatment concomitant with downregulation of Akt/mTOR and p130Cas activity in Ewing sarcoma cell lines. PF-00562271 also impacts biological processes related to cancer cell migration, invasion and metastasis. In a pancreatic cancer model, PF-00562271-induced FAK inhibition attenuated migration of tumor-associated macrophages and fibroblasts in vitro, and reduced tumor growth, invasion and metastasis in vivo (Stokes et al., 2011). Canel et al. (2010) reported that FAK inhibition by PF-00562271 treatment suppressed collective motility of A431 SCC cells in vivo through alteration of E-cadherin dynamics. Interestingly, Bagi et al. (2008) demonstrated that PF-00562271 suppressed local invasion of intratibial tumors, and restored tumor-induced bone loss in MDA-MD-231-bearing nude rats. This indicates that PF-00562271 may represent a potential therapeutic option for bone metastases. PF-00562271 also blocks migration of epithelial ovarian cancer and endothelial cells, inhibits endothelial cell tube formation in vitro, and reduces tumor microvessel density in vivo (Stone et al., 2014).

PF-00562271 was the first FAK-directed agent to enter clinical testing. A phase I clinical trial with PF-00562271 (NCT00666926) recruited 99 patients with solid cancers including glioblastomas, pancreatic, breast, lung, colon cancers, and SCC (Table 1). Results showed a manageable safety profile with the maximum tolerated dosage (MTD) of 125 mg PF-00562271 orally administered twice daily with food. Grade 3 dose limiting toxicities included headache, nausea/vomiting, dehydration and edema (Infante et al., 2012). Of the total cohort of 99 patients, the tumor responses of 14 patients were evaluated by positron emission tomography in the expansion cohorts, and seven had metabolic responses. With conventional imaging, 31 patients had stable disease at first restaging scans, and 15 of these remained stable for six or more cycles, supporting FAK as a promising therapeutic target for further evaluation in patients with solid tumors.

7.4. Defactinib VS-6063 (PF-04554878)

Defactinib, also known as VS-6063, is an ATP-competitive FAK inhibitor, which was developed by Pfizer and has now been acquired by Verastem. This compound exhibits a superior pharmacodynamic profile compared to its predecessor PF-00562271 (Infante et al., 2012). As of June, 2014, there are three phase II clinical trials that are active or recruiting to evaluate the clinical benefits of VS-6063 in patients with advanced ovarian cancer, malignant pleural mesothelioma and non-small cell lung cancer (Table 1).

The initial phase I study showed that VS-6063 was well-tolerated in patients with advanced non-hematologic malignancies (Jones et al., 2011) (NCT00787033). The most common adverse events associated with VS-6063 were nausea, vomiting, unconjugated hyperbilirubinemia, fatigue, headache, diarrhea and decreased appetite with dose-limiting toxicity of headache and hyperbilirubinemia. Twelve patients administered with ≥100 mg BID VS-6063 showed stable disease, and the recommended phase II dose (RP2D) was determined to be 425 mg BID.

A phase II randomized, double-blind, placebo-controlled, multicenter study of VS-6063 in malignant pleural mesothelioma (MPM) known as COMMAND, (NCT01870609) is currently recruiting at clinical sites in 12 countries. Eligible patients have MPM and have not progressed following ≥4 cycles of treatment with standard-of-care pemetrexed/cisplatin or pemetrexed/carboplatin. Based on preclinical data indicating that low merlin levels may be predictive of increased responsiveness to FAK inhibitors (Shapiro et al., 2014), patients will be stratified according to tumor merlin status of high or low, established by immunohistochemistry. Patients will be randomized to receive oral VS-6063 at 400 mg twice per day, or matched placebo. Primary endpoints include overall survival (OS) and progression free survival (PFS) together with secondary endpoints of time to new lesion, pharmacokinetics, safety and tolerability of defactinib. The study started in September, 2013, and is scheduled to complete in December, 2016.

Another phase II, open-label, multicenter, multi-cohort trial of VS-6063 (NCT01951690) is estimated to recruit 150 participants with KRAS mutant non-small cell lung cancer (NSCLC) to test whether VS-6063 improves PFS. The requirement for entry into the study is NSCLC patients with a KRAS mutation, and the subjects will be subsequently enrolled into 1 of 4 cohorts based on INK4a/Arf and p53 mutation status. The safety and tolerability of VS-6063, tumor response rate, PFS and OS will be assessed as primary endpoints. Pharmacodynamics of VS-6063 will be assessed using tumor biopsy and blood samples. This study commenced in September, 2013, and is expected to complete in November, 2015.

Verastem has reported their interim data from an ongoing phase I/Ib clinical trial of VS-6063 in combination with paclitaxel in patients with ovarian cancer (NCT01778803). The study has now completed enrollment, and is evaluating 22 patients at three sites in the U.S. The RP2D of 400 mg BID of VS-6063 was well tolerated in combination with weekly paclitaxel with no toxicity. Early clinical activity was observed where 14 of 22 (64%) patients showed stable disease including two partial responses and two complete responses to date with nine patients remaining on the study (www.verastem.com). These findings support further testing of this combination in malignancies where paclitaxel is the standard-of-care therapeutic.

7.5. VS-4718

The substituted pyridine VS-4718, formerly known as PND-1186 (Shapiro et al., 2014), is the newest FAK inhibitor acquired by Verastem. VS-4718 is a potent reversible inhibitor of FAK, exhibiting an IC_{50} of 1.5 nM, and is capable of inducing a robust FAK inhibition in cultured breast carcinoma cells at a concentration of 0.1 μM (Tanjoni et al., 2010).

An initial preclinical study by Tanjoni et al. indicated that VS-4718 showed limited effects on cell proliferation in adherent cancer cells, whereas it induced marked inhibition of FAK and p130Cas phosphorylation in cells grown in suspension or as spheroids, resulting in caspase-3 activation and apoptosis (Tanjoni et al., 2010). This finding is consistent with other potent FAK inhibitors, PF-00562271 and PF-573,228, which do not affect proliferation and apoptosis in monolayers, while PF-00562271 inhibits anchorage independent growth of basal breast cancer cells in soft agar (Hochgrafe et al., 2010). Additionally, VS-4718 also exerted anti-tumor and anti-metastatic effects in orthotopic breast and ovarian carcinoma mouse tumor models (4T1 and MDA-MB-231) without conferring animal morbidity, death or weight loss (Tanjoni et al., 2010; Walsh et al., 2010). The efficacy of VS-4718 was demonstrated by a marked reduction in both subcutaneous tumor growth of breast carcinoma cells (Tanjoni et al., 2010; Walsh et al., 2010) and their metastasis to lungs that was accompanied by inhibition of FAK Y397 and p130Cas phosphorylation and elevated caspase-mediated apoptosis (Walsh et al., 2010).

A more recent study (Shapiro et al., 2014) reported that low expression of the tumor suppressor merlin predicts for enhanced responsiveness of MPM cells to VS-4718 in vitro and in xenograft models. The proposed mechanism of increased sensitivity to VS-4718 is through enhanced dependence of merlin-negative MPM cells on ECM-induced FAK signaling. Furthermore, the authors reported that standard-of-care agents for MPM such as pemetrexed and cisplatin enrich for CSC populations, and VS-4718 effectively reduces these cells in MPM models (Shapiro et al., 2014). These data provide the rationale for a clinical trial in MPM patients using VS-4718 as a single agent after first-line chemotherapy, where merlin-negative patients may especially benefit from this regimen.

VS-4718 is currently undergoing open-label, multicenter, dose-escalation phase I clinical testing in patients with metastatic non-hematologic malignancies (NCT0184944). This phase I study has an

estimated enrollment of 40 participants, and commenced in June, 2013, with an expected completion date of December, 2014.

7.6. GSK2256098

GSK2256098 is a FAK-directed small molecule inhibitor developed by GlaxoSmithKline that is currently in clinical development. Two preliminary preclinical studies have been reported thus far. Pazopanib, a pan VEGFR and PDGFR inhibitor, enhanced the anti-tumor activity of GSK2256098 in ovarian cancer cells in vitro and in vivo (Bottsford-Miller et al., 2011). In addition, GSK2256098 inhibited migration and invasion through matrigel in eight of 26 glioblastoma cell lines tested in vitro, with minimal effect on 2D cell proliferation (S. Chen et al., 2012).

There are three phase I dose-escalation studies of GSK2256098, one of which is now completed. GSK2256098 was initially tested in a randomized, single-blind, placebo-controlled, dose-escalation phase I study (NCT00996671) in 38 healthy subjects. Its goal was to evaluate the safety, pharmacokinetics, pharmacodynamics and preliminary food effect of the drug following single oral doses as a prelude to studies in cancer patients where the drug will be given at higher doses over an extended period of time. No results have yet been reported to the public. Another phase I study (NCT01138033) is currently recruiting subjects with solid tumors. This study commenced in July, 2010, with an estimated enrollment of 138 subjects, and is estimated to complete in December, 2014. Preliminary results show that GSK2256098 was well tolerated with early evidence of clinical activity. MTD was determined to be 1000 mg BID with nausea, diarrhea, vomiting, decreased appetite and asthenia as the most frequent toxicities. Minor responses were observed in patients with mesothelioma, melanoma and nasopharyngeal cancer and stable disease in renal cell carcinoma (Soria et al., 2012). In addition, a further phase I, open-label and dose-escalation study (NCT01938443) is currently recruiting 35 estimated participants to assess the safety of a combination treatment of GSK2256098 with a MEK inhibitor, trametinib in subjects with mesothelioma or other selected tumor types. The study is designed to determine the MTD and RP2D of GSK2256098 in combination with trametinib, and to undertake safety assessment of these selected doses. This study commenced in November, 2013, and is expected to complete in December, 2014.

7.7. Y15

Y15, developed by a group at Roswell Park Cancer Institute, is an allosteric FAK inhibitor with a robust inhibition of Y397 autophosphorylation at a concentration range of 25 nM–1 μM. Y15 differs from ATP-competitive FAK inhibitors that bind the ATP-binding domain in that it targets the Y397 site of FAK, and reflecting this, it does not inhibit other tyrosine kinases such as the FAK homologue Pyk2, EGFR, Src and IGF1R.

FAK inhibition with Y15 induced cell detachment and inhibited cell adhesion in a dose-dependent manner (Hochwald et al., 2009), and decreased the survival, via increased apoptosis, of pancreatic cancer cells in vitro (Zheng et al., 2010). Administration of Y15 decreased tumor growth in breast, pancreatic and colon cancer, as well as neuroblastoma and glioblastoma, xenograft mouse models (Golubovskaya, 2014), and impaired liver metastasis of neuroblastoma cells (Lee et al., 2012). However, the drug is yet to be clinically tested.

7.8. BI 853529

This compound inhibits Y397 FAK phosphorylation with an EC50 of 1nM in PC3 prostate cancer cells and exhibits 1000-fold selectivity for FAK over Pyk2 (Hirt et al., 2011). A daily oral dosage of 50 mg/kg of this compound led to a marked tumor suppression in several tumor xenograft models including PC3 prostate carcinoma.

BI 853520 is currently being tested in two clinical trials. Both studies are phase I studies to determine the safety and tolerability and MTD of the compound for patients with advanced or metastatic solid tumors (NCT0190511 and NCT01335269).

7.9. Pharmacologic targeting of the FAK scaffold function

FAK also signals via non-kinase scaffold functions that cannot be affected using conventional small molecule FAK inhibitors. Development of pharmacologic interventions disrupting the protein–protein interactions of FAK has recently begun and is at an early stage. For example, a small number of compounds developed by CureFAKtor Pharmaceutical are currently undergoing pre-clinical testing. In particular, a compound known as C4 disrupts FAK and VEGFR4 interactions (Kurenova et al., 2009), whereas the M13 compound blocks FAK and Mdm-2 interaction (Golubovskaya et al., 2013c). Other inhibitors of FAK-scaffold functions include INT2-31 that blocks FAK and c-Met/IGFR1 interactions (Ucar et al., 2012, 2013) and R2 which targets the FAK–p53 interaction (Golubovskaya et al., 2013a). All of these inhibitors effectively reduce cell viability and tumor growth through inhibiting angiogenesis and Akt signaling, or by activating p53 signaling with a resulting enhanced expression of downstream targets of p53 such as p21 and Bax. Importantly, as opposed to loss of p53, mutant p53 drives a metastatic program in many cancers and while re-expression of wild type p53 is a feasible mechanism for induction of cell death, caution should be taken on enhancing mutant p53 levels, which could lead to increased metastasis. The assessment of p53 mutation status should therefore be considered when rationally designing therapeutic targeting of the FAK–p53 interaction.

7.10. Combinatorial use of FAK-directed agents with anticancer therapies for enhanced efficacy

Over the past couple of decades, the treatment modality directed against metastasized cancer cells has evolved from heavy dependence on “one-size-fits-all” cytotoxic chemotherapy to inclusion of more molecularly targeted anticancer therapeutics, such as TKIs (Druker et al., 2001; Al-Lazikani et al., 2012). Therapeutic intervention with a combination of multiple molecularly targeted anticancer agents aimed at different, yet interrelated, tumorigenic mechanisms is more likely to exert enhanced impact on the cancer cell and reduce the likelihood of drug-resistance arising. In particular, accumulating empirical clinical experience, supported by experimental studies in vitro and in vivo, indicate that cytotoxic drugs can be more effective when given in combination with particular targeted therapies.

With regard to FAK, an initial attempt to target FAK signaling in order to overcome chemoresistance was based on FAK silencing via RNA interference (Duxbury et al., 2003). This enhanced chemosensitivity of pancreatic cancer cells towards gemcitabine in vitro and in a xenograft model (Duxbury et al., 2003). Similarly, ablation of FAK expression using FAK siRNA sensitized ovarian cancer cells to docetaxel chemotherapy through promoting docetaxel-mediated growth inhibition and apoptosis (Halder et al., 2005), and shRNA-based targeting of FAK significantly decreased the IC_{50} value of 5-fluorouracil (5FU) against 3D multicellular spheroids of colon carcinoma cells (Chen et al., 2009). This effect appears to be partially mediated by inhibition of antiapoptotic Akt and NF-κB signaling upon FAK targeting (Chen et al., 2010). Further, the individual antitumoral effects of antisense FAK oligonucleotides and chemotherapeutics such as cisplatin, etoposide and nimustine hydrochloride were enhanced when administered in combination in human glioblastoma cells in vitro (Wu et al., 2006). These findings suggest that targeting FAK using small molecule FAK inhibitors represents a potential strategy for enhancing chemosensitivity in the clinic.

A number of subsequent studies showed that pharmacologic targeting of FAK activity enhanced chemosensitivity in cancers

exhibiting elevated FAK activity or expression. FAK inhibition with TAE-226 sensitized docetaxel-resistant ovarian cancer cells to docetaxel, promoting tumor regression with suppressed levels of angiogenesis, invasion and apoptosis, and prolonged survival of tumor-bearing mice (Halder et al., 2007). Additionally, in brain tumor models with elevated FAK expression and activity, the combination treatment of Y15 with a chemotherapeutic such as temozolomide more effectively decreased viability and induced apoptosis in vitro, and blocked tumor growth in vivo, in comparison to temozolomide monotherapy (Golubovskaya et al., 2013b). Y15 also chemosensitized colon cancer cells to 5FU and/or oxaliplatin in vitro and to 5FU or oxaliplatin in vivo (Heffler et al., 2013). Further, combining Y15 with gemcitabine resulted in more effective inhibition of growth of xenografted pancreatic cancer tumors (Hochwald et al., 2009). Furthermore, our group have reported that PF-00562271 significantly sensitized docetaxel-resistant prostate cancer cells with highly elevated FAK activity to docetaxel in vitro (Lee et al., 2014). The enhanced sensitivity of the resistant cells to the combination treatment was associated with more effective targeting of FAK phosphorylation when compared to individual treatments. Notably, the enhanced chemosensitivity of docetaxel-resistant prostate cancer cells upon FAK inhibition was not via enhanced apoptosis, rather through enhanced autophagic cell death. These findings revealed an effective strategy to enhance cellular sensitivity towards the standard-of-care chemotherapy in prostate cancer. Interestingly, PF-00562271 has recently been confirmed as a potent inhibitor of CYP3A, one of the major metabolizing enzymes of cytotoxics, including docetaxel, hence it may cause a toxicity issue in patients when used in combination with docetaxel (Infante et al., 2012). In contrast, VS-6063 is a weak CYP3A inhibitor, making this compound more appropriate for combination treatment with cytotoxics. In addition, Kang et al. recently identified a novel pathway whereby FAK inhibition with VS-6063 overcomes paclitaxel resistance in ovarian cancer models that exhibit elevated FAK Y397 phosphorylation (Kang et al., 2013). VS-6063-induced FAK inhibition increased chemosensitivity in these cells through decreasing YB-1 phosphorylation and suppressing expression of its downstream target CD44 in an Akt-dependent manner in vitro and in vivo. Examination of human ovarian cancer samples revealed that co-expression of nuclear FAK and YB-1 was associated with significantly worse median overall survival (Kang et al., 2013). This pre-clinical study provides a proof-of-principle for use of VS-6063 in patients with advanced ovarian cancer and supports the currently undergoing phase I/Ib study of VS-6063.

C4 and R2, which target the scaffolding function of FAK, also sensitized tumors to cytotoxic agents. Combined treatment of C4 with doxorubicin resulted in more effective regression of tumors in a breast cancer xenograft model (Kurenova et al., 2009), and R2 sensitized colon cancer cells to doxorubicin and 5FU (Golubovskaya et al., 2013a).

In addition, co-administration of PF-00562271 with a multi-targeted TKI, sunitinib, in a hepatocellular carcinoma xenograft model exhibited a significantly greater effect than monotherapy, blocking tumor growth and recovery after treatment (Bagi et al., 2009), and the combination of Y15 with Src inhibitor PP2 effectively suppressed colon cancer viability via robust inhibition of Y397 FAK and Y418 Src phosphorylation (Heffler et al., 2013).

While FAK-knockdown has previously been demonstrated to sensitize pancreatic cancer cells to ionizing radiation (Cordes et al., 2007), Graham and colleagues (Graham, 2011) demonstrated that FAK kinase inhibition by PF-00562271, as well as FAK deletion, promotes radioresistance in SCC cells through p53-mediated induction of p21. These results indicate that the role of FAK in radioresistance is context-dependent.

A recent study by Tavora and colleagues reported that cancer cells in mouse models of melanoma and lymphoma could be chemosensitized through EC-specific FAK targeting (Tavora et al., 2014). EC-specific deletion of FAK induced apoptosis and decreased proliferation within perivascular tumor-cell compartments of doxorubicin- and radiotherapy-treated mice through suppression of NF-κB activation in EC cells and hence reduced production of EC-derived cytokines in vitro and in vivo (Tavora et al., 2014). These results provide the rationale for a novel strategy of sensitizing tumor cells to DNA-damaging chemotherapy through targeting of FAK signaling in tumor-associated ECs.

8. Conclusions and future perspectives

In this review, we have highlighted current knowledge and emerging findings regarding the effects of FAK signaling on cancer development and progression, and its potential as a target for cancer therapeutics. Although FAK was first identified over twenty years ago, research on this multifunctional kinase and scaffold continues apace, and is still providing significant surprises. For example, it is now apparent that FAK signals in several cellular subcompartments, including the nucleus (Schaller, 2010), and further characterization of such localization-specific roles is likely to provide new insights into cell biology and signaling, and identify novel opportunities for therapeutic intervention. The latter possibility is highlighted by the progress made in developing small molecule drugs that target the scaffolding function of FAK (Cance et al., 2013). Further development and application of FAK biosensors (Seong et al., 2011) and use of intravital imaging approaches (Conway et al., 2014), will provide important novel information regarding spatiotemporal regulation of FAK signaling not only in cancer cells, but also the other cell types that constitute the complex tumor microenvironment. From a translational standpoint, while FAK tyrosine kinase inhibitors appear to be well-tolerated (Jones et al., 2011; Infante et al., 2012), much remains to be learned regarding the most appropriate cancer types and stages for their clinical use, and whether patients can be stratified for treatment using predictive biomarkers. With regard to the latter possibility, the recent finding that low levels of merlin in mesothelioma predict responsiveness to specific FAK TKIs represents an important step forward (Shah et al., 2014; Shapiro et al., 2014). Given the complexity of FAK signaling and function, it appears likely that further progress in the development and application of FAK-directed therapeutics will require a multidisciplinary approach that integrates cancer cell biology, animal and 3D models of cancer, and drug and biomarker development.

Conflict of interest

Lisa G. Horvath received an honorarium for being on the organizing committee of the Australian Pfizer Oncology Forum and attended a research forum with Pfizer in La Jolla, California paid for by Pfizer. No potential conflicts of interest were disclosed by the other authors.

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