[Frontiers in Bioscience 19, 687-706, January 1, 2014]

Targeting FAK in human cancer: from finding to first clinical trials

Vita M Golubovskaya[1]

[1]*Department of Surgical Oncology, Roswell Park Cancer Institute, Buffalo, NY*

TABLE OF CONTENTS

1. ABSTRACT

It is twenty years since Focal Adhesion Kinase (FAK) was found to be overexpressed in many types of human cancer. FAK plays an important role in adhesion, spreading, motility, invasion, metastasis, survival, angiogenesis, and recently has been found to play an important role as well in epithelial to mesenchymal transition (EMT), cancer stem cells and tumor microenvironment. FAK has kinase-dependent and kinase independent scaffolding, cytoplasmic and nuclear functions. Several years ago FAK was proposed as a potential therapeutic target; the first clinical trials were just reported, and they supported further studies of FAK as a promising therapeutic target. This review discusses the main functions of FAK in cancer, and specifically focuses on recent novel findings on the role of FAK in cancer stem cells, microenvironment, epithelial-to-mesenchymal transition, invasion, metastasis, and also highlight new approaches of targeting FAK and critically discuss challenges that lie ahead for its targeted therapeutics. The review provides a summary of translational approaches of FAK-targeted and combination therapies and outline perspectives and future directions of FAK research.

2. INTRODUCTION

Focal Adhesion Kinase (FAK) is a 125 kDa non-receptor tyrosine kinase (1) which plays a significant role in adhesion, survival, motility, metastasis, angiogenesis, lymphangiogenesis, cancer stem cell functions (2-4), tumor microenvironment (5) and epithelial to mesenchymal transition (EMT) (6-8). FAK is overexpressed in many types of solid and non-solid tumors (9-28) (Table 1) to mediate survival and other important functions (reviewed (29)). FAK was shown to be overexpressed in 66% of preinvasive breast ductal carcinoma *in situ*, DCIS before metastasis and tumor invasion (28) , which indicates important FAK functions in the early stages of cancer. Although most studies found overexpression of FAK gene, protein or mRNA in tumors, not all studies showed correlation of FAK overexpression with the patient outcome, tumor stage or prognosis (Table 1). This shows that future detail study needs to be performed to understand differences of FAK expression and patient outcome with comparing methods analyzing FAK expression, controls on specificity of FAK antibodies, protocols for staining, pathologist's scoring methods, type and stage of tumors, patient cohorts, study mechanisms of FAK overexpression

Table 1. Overexpression of FAK in different types of cancer

Tumor type	FAK overexpression (Number of tumor samples analyzed)	Correlation of FAK overexpression with prognostic factors	References
Hepatocellular carcinoma	+ 50% (60)	+ Significant correlation with tumor size, serum AFP level. FAK was prognostic factor for disease free, overall survival	(9)
Non small cell lung cancer	+ 100% (60)	+ Positive correlation with higher disease stages	(10)
Small-cell lung carcinoma	+ 92% (85)	No correlation with SCLC disease stage	(11)
Breast cancer	+25%; +88%; +97* (629); (25) (119)	+ significant correlation with poor prognostic factors; NA;NA	(12); (16)‡; (28)
Pancreatic cancer	+ 48% (50)	Significant correlation with tumor size	(13)
Brain tumors	+ 77%; + 40.8% (13), (331)	NA***; + Significant correlation with WHO grade of malignancy	(14); (15)
Sarcoma; Osteosarcoma	+ 100%; + (13); (16)	NA;NA	(16)‡;(17)
Ovarian cancer	+ 100% (26)	No significant correlation with grade or tumor stage	(18)
Cervical Cancer	+ 77%; +32%† (30); (31)	NA; NA	(19); (20)
Colon cancer	+ 100%; +63%; +40% (17); (56); (80)	NA ; FAK higher in liver metastases compared with primary tumors; No correlation with tumor grade, stage, Ki-67 positivity or survival	(16)‡ ; (21); (22)
Neuroblastoma	+ 73% (70)	FAK staining significantly increased with stage IV with N-myc amplification	(23)
Thyroid cancer	+ 33% (27)	+ Correlation with invasiveness and metastasis	(24)
Prostate cancer	+ (25)	Increased FAK mRNA and protein with advanced stage in metastatic carcinoma	(25)
Head and neck	+ 62% 211	FAK expression correlated with nodal metastases, no correlation of FAK expression with DNA level	(26)
Hematopoietic Cancer; AML	+ 42% (60)	FAK expression correlated with high blast cell count, early death and shorter survival and poor prognosis	(27)

+ positive expression or correlation; *FAK expression is higher in early stages preinvasive DCIS breast tumors than in atypical ductal hyperplasia or dysplasia (ADH) tumors; **marks high level of FAK expression in tumors tumors (3 or 4 intensity and ≥90% positive cells by the score of immunohistochemistry staining). ***NA, not analyzed ; N, number; ‡ sarcoma, colon and breast cancer tumors were analyzed in the same report; †, not higher than in normal samples (66%); AML, acute myeloid leukemia

and activation in different types of tumors, and perform personalized approach to the patient population. Because of high percentage of FAK overexpression in different types of tumors, FAK was recently proposed as a therapeutic target (30).

The Timeline (Figure 1) shows the main twenty-year findings starting from the discovery of FAK and to the idea of using FAK as a potential therapeutic target (30) to the results of the first clinical trials (31). FAK was first discovered in 1992 by several different groups (1), (32), the next year human FAK was isolated (33-34) and later it was found in Drosophila (35-37) and other species. Once FAK was isolated in humans it was directly linked to cancer due to its high expression in primary tumors (Table 1) and overexpression in nearly all metastatic tumors, with no detectable FAK mRNA or protein in normal tissues (34).

In this review we discuss important functions of FAK in survival, metastasis, angiogenesis (5), and highlight its kinase-dependent and kinase-independent scaffolding functions (7),(38) . We will especially focus on recent studies revealing novel functions of FAK in cancer stem cell signaling and provide a critical overview of different approaches to FAK inhibition with pharmacological inhibitors, discuss the first clinical trial results, and finally propose translational perspectives for future research on FAK biology and therapeutics development in cancer.

3. FAK STRUCTURE

The *FAK (PTK2)* gene coding sequence contains 34 exons (39) (Figure 2). There are several mechanisms of regulation of FAK expression and activation: on the genetic level through gene amplification(40, 41) ; on the level of RNA by alternative splicing(39), or by FAK mRNA up-regulation (34), (21), (42), (4); and on the translational and post-translational levels (phosphorylation (43), dephosphorylation (44), sumoylation (45), regulation by MicroRNA (46-48).

The FAK protein domains are shown in Figure 2. The FERM domain of FAK contains one nuclear export sequence (NES) and one nuclear localization sequence (NLS), and the FAK kinase domain contains another NES sequence close to the major FAK phosphorylation sites Y576/Y577(49), suggesting the regulation of FAK through nucleo-cytoplasmic shuttling and supporting nuclear functions of FAK (binding with p53 (50-51), and Mdm-2 (51) and other partners) (reviewed (52-53).

The crystal structures of the C-terminal FAT (54-55); N-terminal FERM (56) and kinase domain (57) of

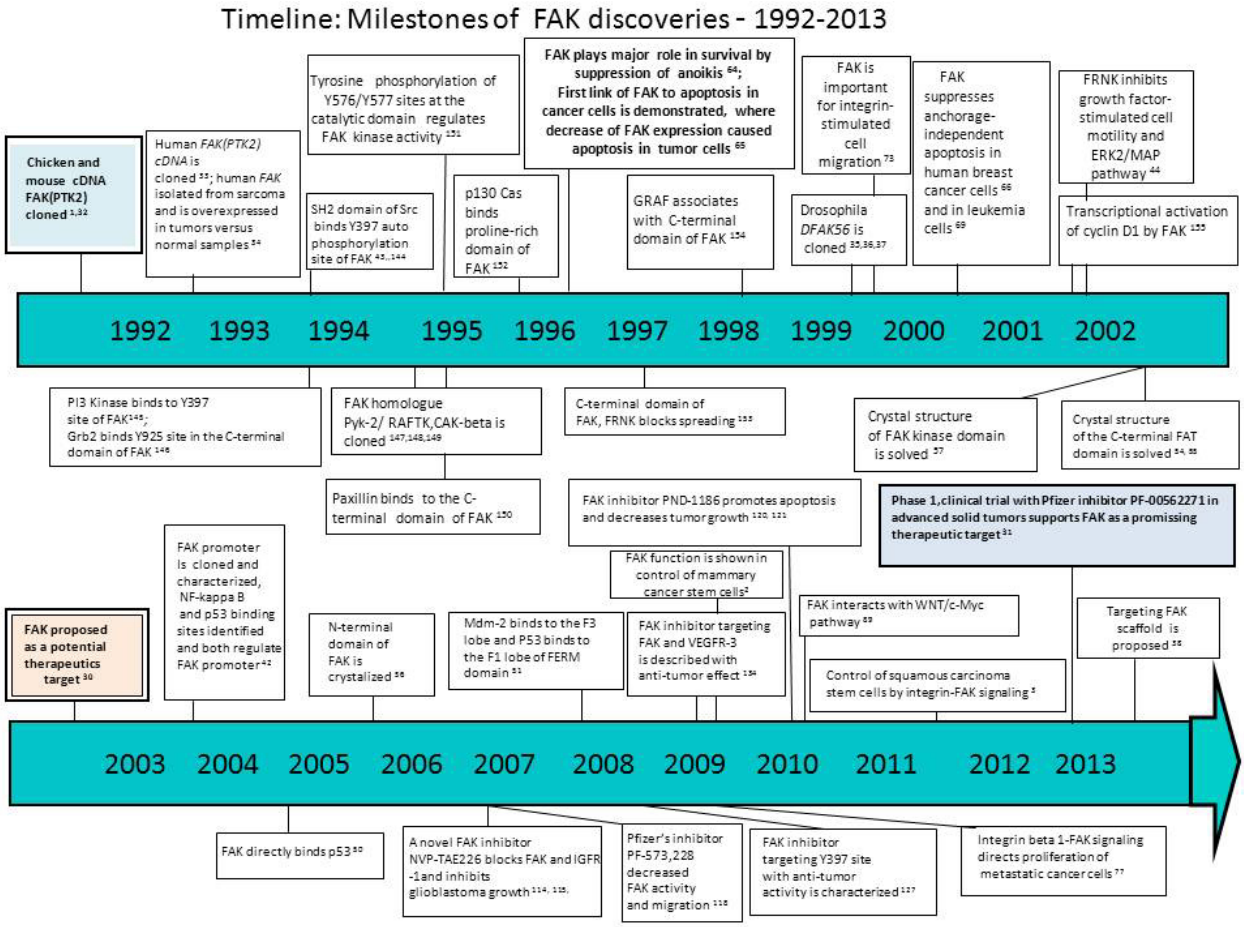


Figure 1. Timeline. Milestones of FAK discoveries: 1992-2013. 1992- Chicken and mouse FAK(PTK2) cDNA is cloned (1),(32). 1993 -Human FAK cDNA is cloned (33); Human FAK is isolated from sarcoma and is overexpressed in tumors versus normal tissues (34). 1994-SH2 domain of Src binds to Y397 autophosphorylation site of FAK (43, 144); PI3Kinase binds Y397 site of FAK (145); Grb-2 binds C-terminal domain of FAK (146). 1995-FAK homologue Pyk-2/RAFTK, CAK-beta is cloned (147-149); Paxilin binds to the C-terminal domain of FAK (150); Tyrosine phosphorylation of Y576/Y577 sites at the catalytic domain of FAK regulates FAK kinase activity (151). 1996- p130 Cas binds proline-rich domain of FAK (152); FAK plays major role in survival by suppression of anoikis (64); The first link of FAK to apoptosis is demonstrated in cancer cells: Decrease of FAK expression caused apoptosis in tumor cells (65). 1997-C-terminal domain of FAK, FRNK blocks spreading (153). 1998 - GRAF associates with C-terminal domain of FAK (154). 1999-Drosophila DFAK56 is cloned (35-37); FAK is important for integrin-stimulated cell migration (73). 2000- FAK suppresses anchorage-independent apoptosis in human breast cancer cells (66) and in human leukemia cells (69). 2001- Transcriptional activation of cyclin D1 by FAK (155); FRNK inhibits growth-factor stimulated motility and ERK2/MAP pathway (44). 2002-Crystal structure of FAK kinase domain is solved (57); Crystal structure of the C-terminal FAT domain is solved (54-55). 2003 -FAK proposed as a potential therapeutics target (30). 2004-FAK promoter is cloned and characterized, containing NF-kappa B and p53 binding sites; both transcription factors regulate FAK (42). 2005-FAK directly binds p53(50). 2006-The N terminal domain of FAK is crystalized (56). 2007- FAK inhibitor NVP-TAE226 blocks FAK and IGFR-1 and inhibits glioblastoma growth (114-115); Pfizer's inhibitor PF-573228 decreases FAK activity and inhibited migration (118). 2008-Mdm-2 binds to F3 lobe and p53 binds to F1 lobe of FAK FERM domain (51); FAK inhibitor targeting Y397 site of FAK with anti-tumor activity of FAK is characterized (127). 2009-FAK inhibitor targeting FAK and VEGFR-3 is described with anti-tumor effect(134); FAK function is shown in the control of mammary cancer stem cells(2); Integrin beta 1-FAK signaling directs proliferation of metastatic cancer cells (77). 2010- FAK inhibitor PND-1186 promotes apoptosis and decreases tumor growth (120-121). FAK interacts with WNT/c-Myc pathway (89). 2011- Control of squamous carcinoma stem cells by integrin-FAK signaling (3). 2012-Phase-1 clinical trial with Pfizer inhibitor PF-00562271 in advanced solid tumors supports FAK as a promising therapeutic target (31). 2013- Targeting scaffolding function of FAK is proposed (38). Bold font shows main findings in FAK therapeutics

FAK were solved (Timeline), which allowed to develop small molecules targeting FAK. Interaction of the F2 lobe of the FERM domain and C-lobe of FAK kinase domain caused locked inactive conformation resulting in

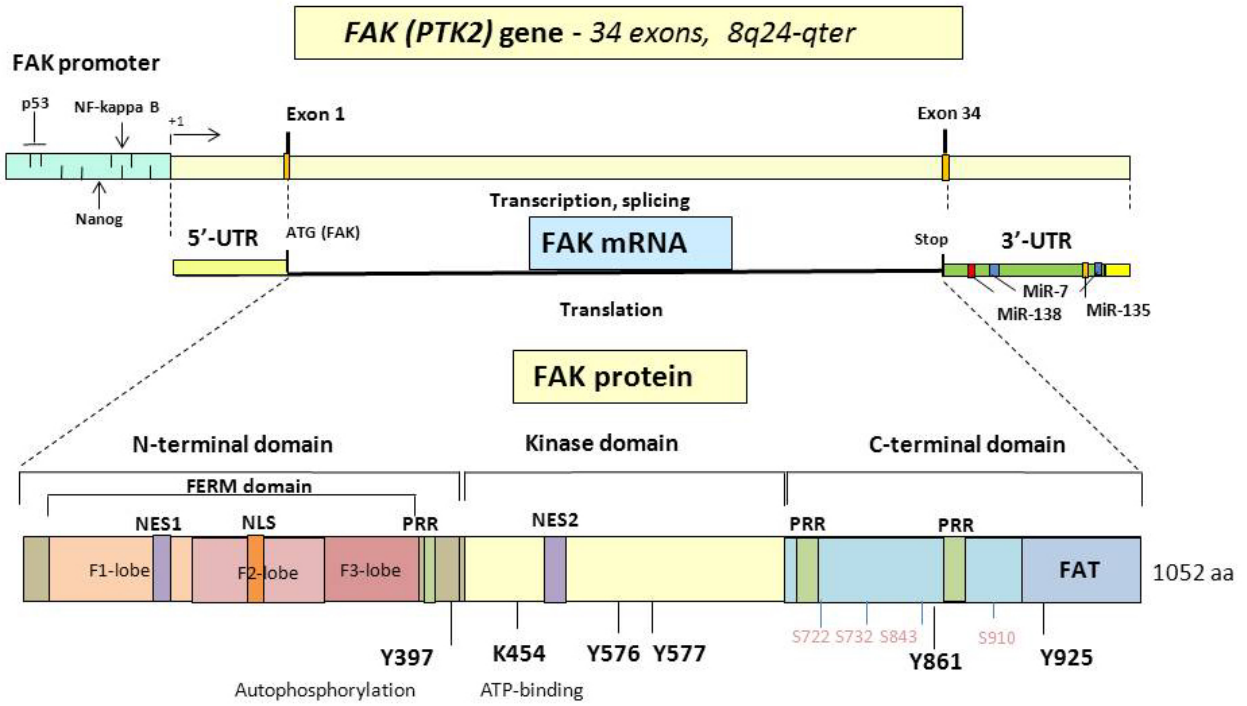


Figure 2. Structure of Focal Adhesion Kinase genomic, RNA and protein structure. FAK gene has 34 exons. FAK promoter contains two p53, two NF-kappa B and four Nanog transcription factor binding sites. P53 inhibits and NF-kappa B and Nanog induce FAK promoter activity. 5-UTR, mRNA and 3-UTR (untranslated region) are shown. MiR-138, MiR-135 and MiR-7 bind to FAK 3-UTR and repress its expression. The N-terminal domain includes FERM domain (F1-F3 lobes). The N-terminal domain includes Y397- autophosphorylation tyrosine site; the Kinase domain includes Y576/577 tyrosines important for catalytic activity of FAK and the C-terminal domain of FAK has Y861 and Y925 tyrosine sites. Tyrosine phosphorylation sites are shown in black. The serine phosphorylation sites (S722, S732, S843, and S910) are shown in green. PRR, proline-rich domains are marked by green color. NES an NLS mark nuclear export and nuclear localization signals, respectively.

autoinhibition of FAK. Integrin or growth factor interactions cause the release of the linker containing autophosphorylation Y397 site, providing its activation and induction of downstream signaling (58). These structural studies provided a basis for developing of small molecule inhibitors targeting FAK.

The main FAK binding partners form FAK scaffold and have been discussed in many reviews (59-63), (Figure 3). FAK integrates signals from growth factor, integrin, vascular endothelial growth factor receptors (VEGFR) and activates PI-3Kinase, AKT, MAPK and other down-stream signaling, regulating intracellular functions.

4. FAK FUNCTIONS IN CANCER

4.1. Survival signaling

The first demonstration of a survival function for FAK was performed by Frisch *et al* (64), where expression of active FAK was shown to suppress anoikis in Madin Darbin canine kidney (MDCK) and immortalized keratinocyte HaCat epithelial cells. Then FAK was linked to apoptosis in cancer cells, where inhibition of FAK with anti-sense oligonucleotides or with dominant negative FAK (FAK-CD (FAK C-terminal domain)) caused loss of adhesion and apoptosis in tumor cells (65-66). Down-regulation of FAK with FAK siRNA decreased MCF-7 breast cancer viability and inhibited tumor growth (67). FAK binding to the death domain kinase receptor interacting protein RIP was demonstrated, where FAK sequestered and inhibited the tumor-suppressing apoptotic function of RIP. (68) In addition, FAK was shown to interact with p53 and inhibit its apoptotic activity (50). Nuclear FAK regulated survival through its direct binding to Mdm-2, which promoted p53 ubiquitination and degradation(51). The anti-apoptotic function of FAK was demonstrated in HL-60 leukemia cells, where FAK activated the PI-3-Kinase/AKT pathway and induced functions of NF-kappa B and inhibitor of apoptosis proteins (IAPs) (69). In addition, recently inhibition of heat shock protein HSP90 was shown to decrease FAK phosphorylation and protein level, demonstrating novel heat shock-FAK-regulated survival pathway (70). Recently, connection of FAK signaling to autophagy was demonstrated, where absence of FAK accelerated autophagy and caused sequestration of active Src from focal adhesions in squamous cancer cells, which provided a new therapeutic opportunity for developing FAK and autophagy inhibitors (71).

4.2. Motility, EMT, invasion and metastasis

FAK has been shown to be important for motility (44, 72-73). FAK-null embryos exhibited decreased cell

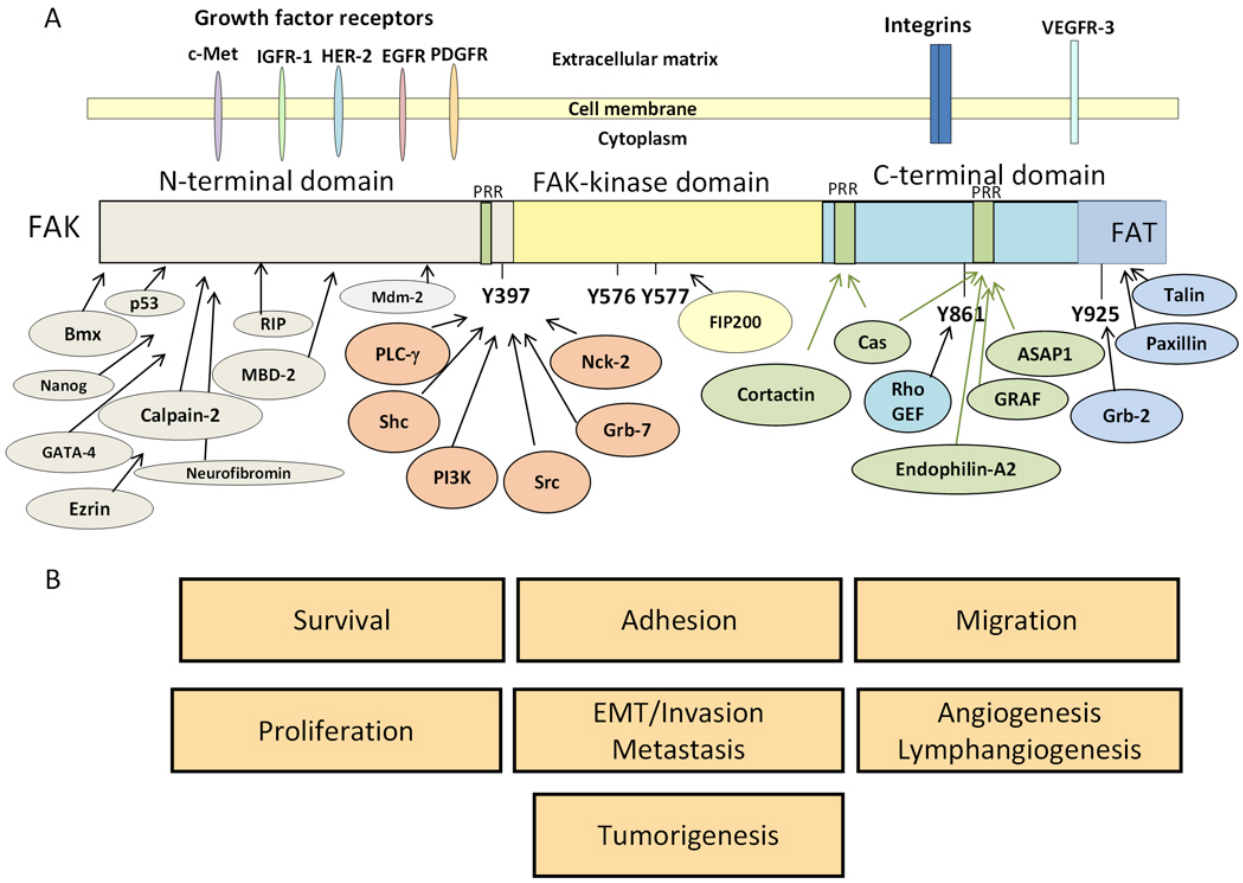


Figure 3. Multiple FAK binding proteins and FAK functions in cancer. A. Many proteins bind to FAK and form its scaffold. Several proteins with SH2 domains are shown that bind to the Y397 site of FAK by orange color. Different proteins bound to FAK domains are involved in motility, survival, angiogenesis signaling and other FAK-regulated signaling (B). FAK integrates signals from growth factor receptors, integrins and angiogenesis receptors to regulate adhesion, survival, migration, metastasis, invasion, lymphangiogenesis and angiogenesis. FAK pathways are linked with Src, PI3-Kinase and other multiple signaling pathways.

motility (74), and overexpression of FAK induced cell motility (73). The Src (75) and PI-3 Kinase (76) downstream signaling pathways have been shown to be important for FAK-mediated cell motility (reviewed (29, 59)).

FAK was shown to mediate cell invasion and metastasis through promotion of epithelial-to-mesenchymal transition (EMT). Recent study of mechanism of mouse mammary carcinoma cells showed the importance of FAK activation to enable proliferation of micrometastatic cancer cells disseminated in the lungs (77). Recently the novel role and mechanisms of FAK role in EMT were reviewed (78-79). The phosphorylation of FAK was required for Src-induced down-regulation of E-cadherin in colon cancer cells (80), and inhibition of FAK activity reduced Src-mediated cell invasion and blocked metastasis (81), providing basis for targeting invasion and metastasis with FAK pharmacological inhibitors. TGF-beta 1-induced Slug regulated EMT and promoted cell migration of human squamous cell carcinoma, which was repressed by FAK inhibitor (82). FAK was shown to affect E-cadherin expression by different mechanisms (reviewed (79)). Thus, FAK plays an important role in EMT, invasion and metastasis and the details of the down-stream molecular mechanisms of FAK-regulated EMT and E-cadherin mediated cell-cell adhesions or integrin-ECM mediated adhesions and their cross-talk and role in metastasis remain to be discovered (79).

4.3. Angiogenesis and Lymphangiogenesis

FAK was shown to play an important role in tumor angiogenesis, which was demonstrated in several *in vivo* mouse models (reviewed (83)). FAK-knock-out mice displayed embryonic lethal phenotype with defects of vasculogenesis, but homologous Pyk-2 knock-out mice developed normally (84), which is important fact for designing FAK and Pyk-2 targeted therapy. Down-regulation of FAK in FRNK-expressing 4T1 breast carcinoma cells expressed less VEGF and formed decreased avascular tumors (85). The production of VEGF was controlled by Y925 FAK phosphorylation, facilitating Grb-2 binding and Ras-ERK pathway activation (85). The interaction of FAK and VEGFR-3 linked FAK to

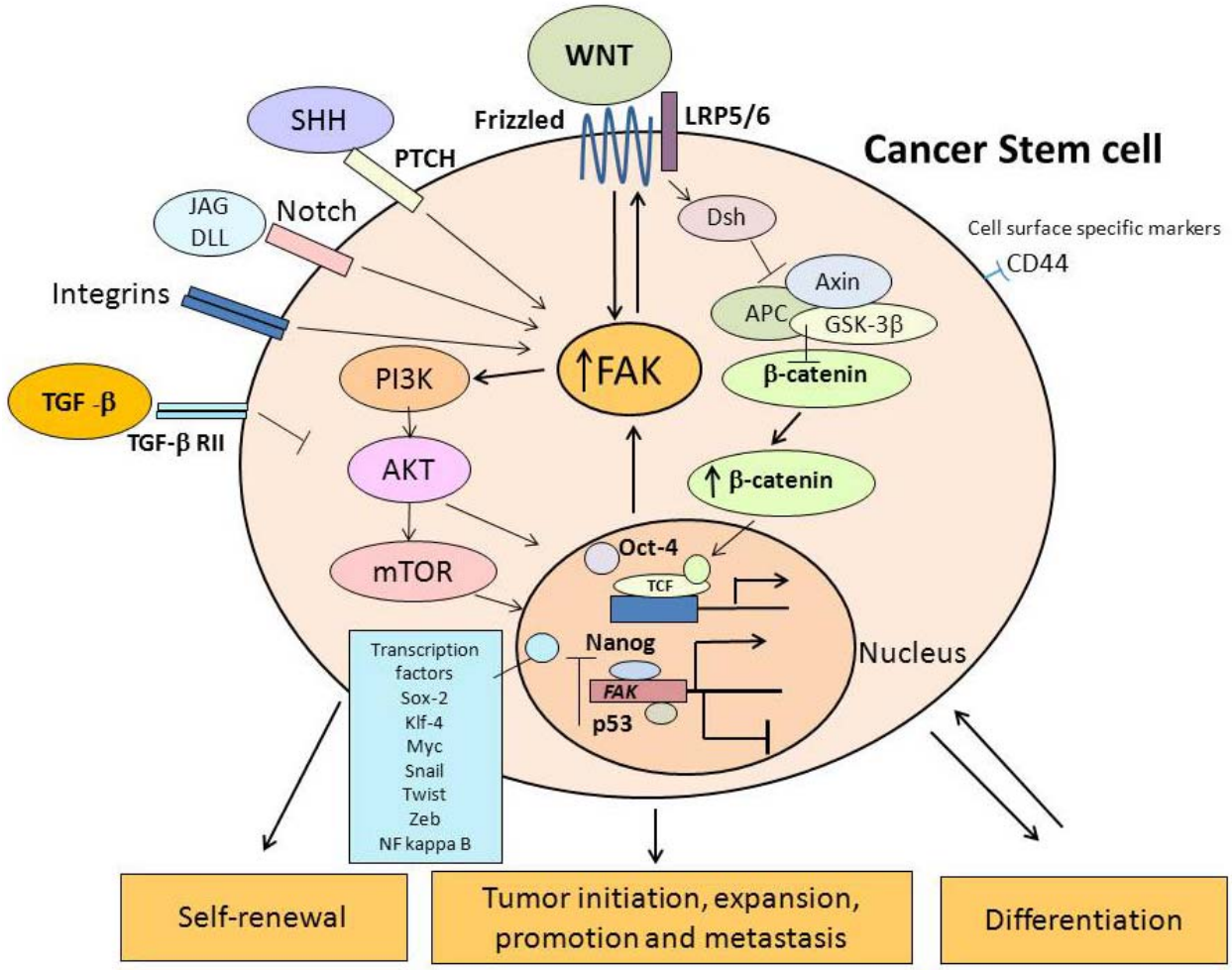


Figure 4. FAK functions in cancer stem cells. Cancer stem cells are able to self-renew, differentiate/ redifferentiate, and promote tumor growth. FAK signaling is linked with WNT, TGF-beta, Integrin and Hedgehog pathways. Activation of WNT pathway causes activation of FAK and downstream PI3K, AKT and mTOR pathways. In reverse, FAK regulates WNT pathway. WNT binding to Frizzled and LRP5/6, activation of Dsh, blocking of the beta-catenin destruction APC-Axin-GSK-3β complex, nuclear translocation of beta-catenin, binding to TCF factors activates of transcription of target genes. Sonic Hedgehog and Notch pathways activate FAK signaling. In the absence of inhibiting signals from TGF beta RII integrin-FAK-AKT pathways activate cancer stem cell functions. Cancer stem cells are regulated by many transcription factors (shown by blue color) and contain different cell surface specific markers (CD44 is shown). FAK transcription is up-regulated by transcription factor Nanog, which plays a significant role in cancer stem cell maintenance. The p53 transcription factor blocks FAK transcription, and blocks Nanog and inhibits cancer stem cell functions. Transcription factor Oct-4 up-regulates FAK.

lymphangiogenesis and also demonstrated its important role in tumorigenesis (86). These functions of FAK are connected to FAK functions in endothelial cells, and important for development of FAK-targeted therapeutics in tumors and tumor microenvironment.

5. NOVEL FUNCTIONS OF FAK IN CANCER STEM CELLS

Cancer stem (or tumor-initiating) cells have the ability to self-renew, differentiate into lineage-specific cell types and promote tumorigenesis (Figure 4). Cancer stem cells can be resistant to many chemotherapy treatments (87), making these cells crucial for studies of the mechanism of their functions and the development of therapies. Several key signal transduction pathways are involved in regulation and activation of cancer stem cell functions such as WNT, Notch, Hedgehog, TGF-beta, growth factor and integrins. Cancer stem cells contain different cell surface specific markers (CD44, CD133, CD34, etc) and are regulated by the transcription factors Sox-2, c-Myc, Oct-4, Klf-4, Twist, Snail, Nanog, etc. (88) (Figure 4).

5.1. FAK and cancer stem cell signaling pathways

Recently an important novel role of FAK in cancer stem cell functions was demonstrated by several groups (2-3, 77). FAK was shown to be linked with WNT

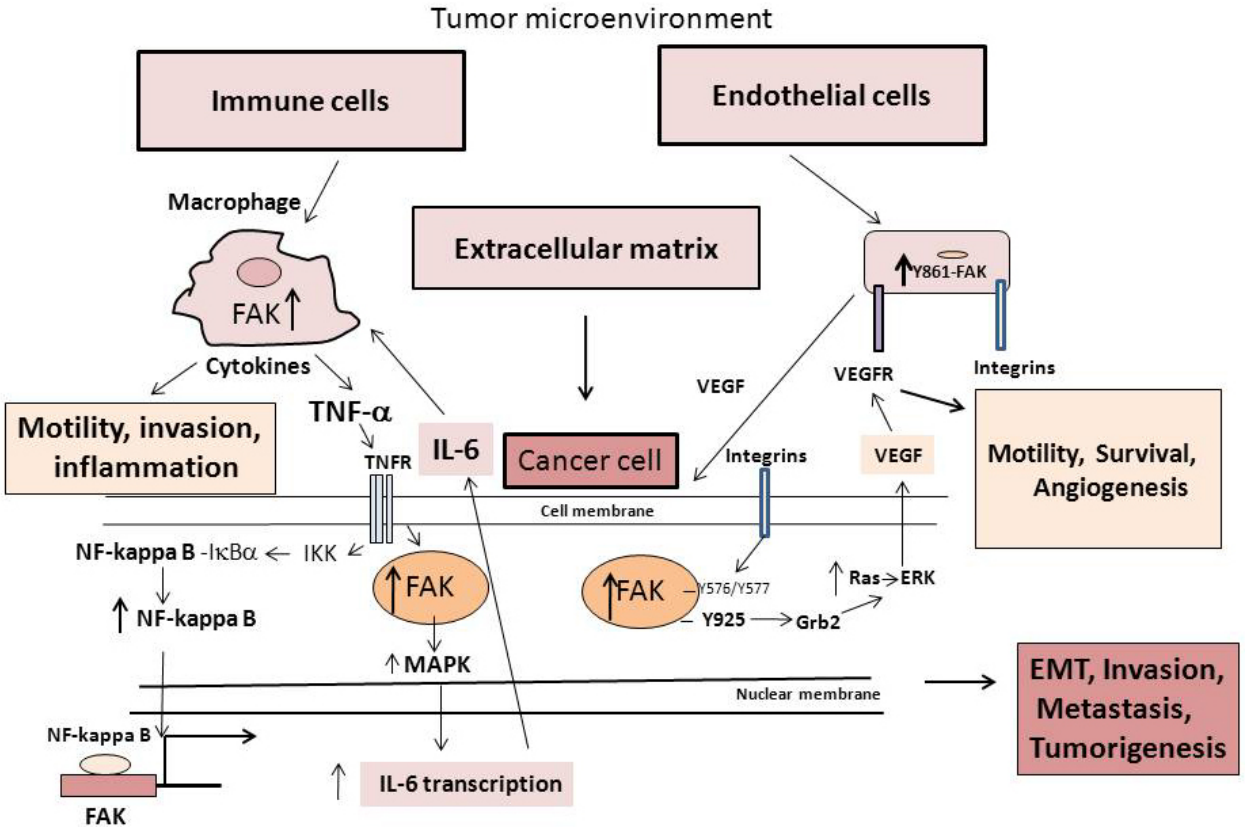


Figure 5. FAK and tumor microenvironment Cancer cells are surrounded by cancer cell niche or tumor microenvironment components, such as extracellular matrix, endothelial cells, blood vessels; and immune cells, playing a significant role in inflammation. FAK plays important role in motility and invasion of macrophages. Macrophages release cytokines that affect NF-kappa B and FAK signaling, inflammation and tumor growth. TNF alpha binds TNFR receptor, activates I kappa B kinase (IKK), which phosphorylates IkBα, causing its dissociation from NF-kappa B and activating NF-kappa B. Activated NF-kappa B translocates to the nucleus, binds FAK promoter and induces FAK transcription. FAK is required for TNF-alpha induced interleukin-6 transcription, MAPK activation and IL-6 increased transcription and production. Endothelial cells: VEGF production stimulates FAK activation and induces Src-dependent phosphorylation of Y861 FAK in endothelial cells, playing important role in motility, angiogenesis and survival of endothelial cells. In tumors integrin signals activate FAK Y397, Y576/Y577 and Y925 phosphorylation, which leads to binding of Grb-2, Ras/ERK pathway activation and secretion of VEGF, which induce endothelial cells function. Tumor microenvironment regulate cancer cell EMT, metastasis, invasion and tumorigenesis.

pathway (one of the main signaling pathways of cancer stem cells) and regulated intestinal regeneration and tumorigenesis (89). FAK was required down-stream of WNT/c-Myc signaling to induce AKT-mTOR signaling pathways and promote intestinal tumorigenesis in mice following Apc tumor-suppressor loss (89). Also, FAK regulated expression of WNT3a in human breast cancer cells, where down-regulation of FAK with siRNA caused decreased WNT3a transcription and increased WNT3a protein levels (90) (Figure 4). In addition, down-regulation of FAK with autophosphorylation inhibitor in colon cancer cells decreased transcription of *Frizzled* and *LRP5* and increased transcription of WNT pathway inhibitor, *Dickkopf-1 (DKK1)*, demonstrating that FAK also acts up-stream of WNT pathway (91). The detail molecular mechanisms of FAK and WNT interaction and cross-signaling pathways in cancer stem cells need to be discovered in future.

Recently targeted deletion of FAK in mammary epithelium suppressed mammary tumorigenesis and demonstrated that FAK plays a significant role in the maintenance of mammary cancer stem cells (2). It was shown using mammary cancer stem cell markers: ALDH, aldehyde dehydrogenase; CD24, CD29 and CD61 that down-regulation of FAK reduced pool, self-renewal, stem sphere formation and migration of mammary cancer stem cells *in vitro* (2). Down-stream PI3Kinase/AKT signaling was found to be a major mediator of FAK signaling, regulating mammary cancer stem cells (92). In case of FAK deletion in mammary cancer stem cells Pyk-2 had compensatory function mediated by AKT (92). Recently, novel kinase-dependent and kinase-independent functions of FAK were defined in regulation of mammary stem and progenitor cells, where FAK kinase activity preferentially regulated proliferation and tumor sphere formation of luminal progenitors, while scaffolding function of FAK

was required for regulation of the basal mammary stem cells (93). The mammary cancer stem cells required PI3K for maintenance, while normal mammary stem cells did not, which supports that FAK/PI3 kinase pathway can be used for specific targeting of cancer stem cells. Another report using FAK knock-in mice with mutation of prolines P878A/P881 (a binding site with endophilin A) demonstrated that the kinase-independent scaffolding function of FAK and its interaction with endophilin A2 were important for mammary cancer stem cell activities (self-renewal and tumorigenesis) (7). Disruption of FAK scaffolding function to mediate endophilin phosphorylation inhibited tumor growth and metastasis, and decreased markers of epithelial to mesenchymal transition.

Recently it was shown that the receptor tyrosine kinase Ephrin B2 (EphB2) stimulated glioblastoma-derived stem-like neurosphere invasion through interaction with FAK(94). FAK and PI3K-AKT pathways were required for migration of GBM stem-like neurospheres (94).

The proliferation of tumor-initiating stem cells of squamous carcinoma was controlled by interaction of integrin, FAK and TGF-beta receptor II signaling (3). TGF beta receptor and integrin, FAK signaling had an opposite effect on the self-renewal and tumor initiation functions of cancer stem cells, which explains the increased aggressiveness of TGF beta receptor II-deleted mice with activated FAK signaling (3).

Activated Sonic hedgehog (SHH) induced hepatoma cell migration and invasion and induced phosphorylation of FAK, activation of AKT signaling and caused activation of matrix metalloproteinases MMP-9 and MMP-2 (95).

Down-regulation of Notch pathway by silencing of Notch 1 expression caused decrease of FAK and down-stream AKT phosphorylation in MDA-231 breast cancer cells, which decreased cell migration and invasion (96), demonstrating cross-talk of Notch and FAK signaling.

These reports show novel functions of FAK in cancer stem cells and cross-talk with main cancer stem cell signaling pathways, and also demonstrate that both functions of FAK, kinase-dependent and kinase-independent (scaffolding function) are critical for cancer stem cell functions and tumorigenesis.

5.2. FAK and cancer stem cell markers

Among transcription factors regulating cancer stem cell functions, Nanog was recently shown to induce FAK promoter activity(4) and increased Nanog protein expression caused increase of FAK expression and induced tumorigenesis (97). Both Nanog (98) and FAK (42) transcription are repressed by p53. It was shown that inactivation of p53 increased induced pluripotent stem cell generation (99-102), and p53 mutations and inactivation correlated with FAK overexpression in tumors (103). It will be interesting to study Nanog-FAK-p53 signaling in the regulation of cancer stem cell functioning and tumorigenesis. Another cancer stem cell marker and transcription factor Oct-4 was shown to promote migration and invasion of glioblastoma cells and up-regulated FAK expression (104). Thus, all these recent data show novel roles of FAK in the regulation of cancer stem cell functions, cancer stem cell markers and tumorigenesis and this novel area needs to be explored in future, as critical for cancer cell biology and therapeutics.

6. FAK AND TUMOR MICROENVIRONMENT

Interactions and cross-talk of signaling between cancer cells and tumor microenvironment is important for understanding mechanisms of metastasis and tumorigenesis. FAK is connected with tumor microenvironment components such as immune cells, and endothelial cells to regulate cancer cell functions. The direct role of FAK in cancer cell microenvironment is not explored in detail, and we will focus only on few main findings.

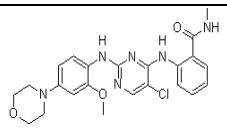
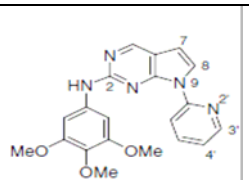
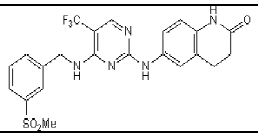
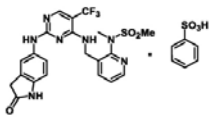
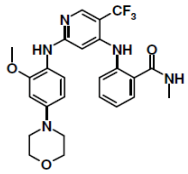
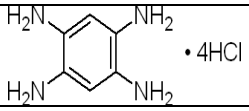
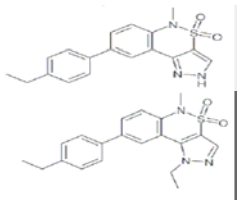
6.1. FAK, cytokines and immune cells

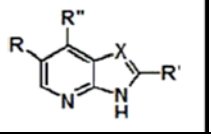
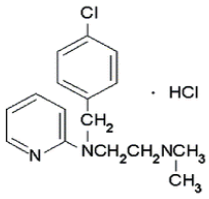
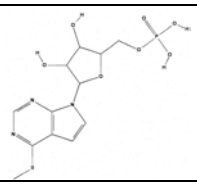
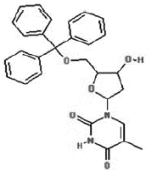
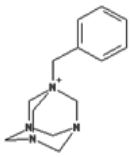
Immune cells of the tumor microenvironment play an important role in the inflammation associated with tumorigenesis (reviewed (105)). Activated FAK is known to play an important role in macrophage motility and invasion (106). The tumor-promoting cytokine TNF-alpha can induce NF-kappa B, which can induce FAK transcription in cancer cells (42) (Figure 5). In another study TNF-alpha also caused catalytic activation of FAK, which was required for MAPK activation, induced IL6 transcription and protein secretion in different cancer cells (breast, neuroblastoma, lung carcinoma, PC3 prostate carcinoma (107). FAK activation was not required for TNF alpha-mediated NF-kappa B activation (107), while Y397-FAK phosphorylation, FAK kinase activity and prolines 712/713 region were required for MAPK activation, increased IL-6 transcription and production in a Src-independent manner (107). All these studies demonstrate different mechanisms between cytokines, NF-kappa B and FAK in different cancer cells, which can affect inflammation, motility and invasion functions.

6.2. FAK and endothelial cells

The function of FAK in endothelial cell is connected with angiogenesis and lymphangiogenesis functions of FAK in cancer cells, discussed above. VEGF stimulates FAK tyrosine phosphorylation, increases focal adhesion formation (108) and induces Src-dependent phosphorylation of Y861 FAK (109) in endothelial cells. The direct role of FAK in endothelial cells to mediate angiogenesis was demonstrated when overexpression of FAK in endothelial cells promoted angiogenesis in transgenic mice model (5). Pyk-2 was able to compensate for FAK functions in angiogenesis in adult mice lacking endothelial FAK (110). Recently, the effect of FAK on angiogenesis was shown to be nonlinear, where homozygous deletion of endothelial FAK had inhibited angiogenesis (111), while FAK-heterozygous mice enhanced tumor angiogenesis associated with increase of phosphorylated AKT and Y861-FAK without Pyk-2 changes (112). This non-linear mechanism needs to be

Table 2. Pharmacological FAK inhibitors

Inhibitor	Chemical name	Structure	Company	Comment Reference
NVP-TAE226	2-((5-chloro-2-((2-methoxy-4-morpholinophenyl)amino)pyrimidin-4-yl)amino)-N-methylbenzamide		Novartis	Targets ATP-binding site region (115)
7H-pyrrolo[2,3-d]pyrimidines	Pyrrolopyrimidine		Novartis	Targets ATP-binding site region [116]
PF-573,228	6-(4-(3-(methylsulfonyl) benzylamino)-5-(trifluoromethyl)pyrimidin-2-ylamino)-3, 4-dihydro quinolin-2(1*H*)-one		Pfizer	Targets ATP-binding site Region(118)
PF-562,271	N-Methyl-N-(3-{[2-(2-oxo-2,3-dihydro-1H-indol-5-ylamino)-5-trifluoromethyl-pyrimidin-4-ylamino]-methyl}-pyridin-2-yl)-methanesulfonamide		Pfizer	Targets ATP-binding site, clinical trial inhibits FAK and Pyk-2 (119) Clinical trials results: (31)
PF-04554878 VS-6063	Unknown	Unknown	Pfizer Verastem	Second generation ATP-binding inhibitor with better PK profile (31)* ; in clinical trials
VS-4718	Unknown	Unknown	Verastem	Targets ATP-binding region; VS-4718 in clinical trial
VS-5095	Unknown	Unknown	Verastem	Targets ATP-binding region
PND-1186	2,4-diamino-pyridine-based scaffold		Poniard	Inhibits FAK kinase function, targets ATP binding site region [120],[121]
GSK-2256098	Unknown	Unknown	Glaxo Smith Kline	Inhibits FAK kinase; in clinical trials
Y15, FAK inhibitor 14	1,2,4,5-Benzenetetraamine tetrahydrochloride		Cure FAKtor Pharmaceuticals	Targets Y397 site (127), [143], (129),(130)
Allosteric FAK inhibitors Compounds 1 and 2	Compound 1: 8-(4-Ethylphenyl)-5-methyl-5-methyl-1,5-dihydropyrazolo[4,3-c][2,1]benzothiazine 4,4-dioxide Compound 2: 1-Thyl-8-(4-ethylphenyl0-5-methyl-1,5-dihydropyrazolo[4,3-c][2,1]benzothiazine 4,4-dioxide		Takeda	Allosteric FAK inhibitor ATP-non competitive inhibitors [133]
Imidazo- and pyrrolo-pyridines	1H-Pyrrolo[2,3-b]- and 3H-Imidazolo[4,5-b]-Pyridines		Merck Serono	Inhibitors induce DFG-loop conformation, bound to the hinge-region of FAK (117)

				
C4	Chloropyramine hydrochloride		Cure FAKtor Pharmaceuticals	Targets FAK-VEGFR pathway interaction (134)
INT2-31	7H-Pyrrolo[2,3-d]pyrimidine, 4-(methylthio)-7-(5-O-phosphono-.beta.-D-ribofuranosyl		CureFAKtor Pharmaceuticals	Targets FAK and IGFR-1 interaction(135), (136)
M13	5′-O-Tritylthymidine		Cure FAKtor Pharmaceuticals	Targets FAK and Mdm-2 interaction (137)
R2	1-benzyl-15,3,5,7-tetraazatricyclo [3.3.1.1~3,7~]decane		Cure FAKtor Pharmaceuticals	Targets FAK and p53 interaction (138)

In bold font: FAK inhibitors, which went to clinical trials

studied in future and is important for developing future therapies.

Thus, FAK is an ideal therapeutic target, which affects not only tumor cells, but also tumor microenvironment and has a high potential for future therapeutics.

7. TARGETING FAK WITH INHIBITORS

The initial attempts to inhibit FAK signaling in cancer cells were first performed with antisense FAK oligonucleotides (65) and adenoviral dominant-negative FAK-CD or FRNK (66) , then with FAK siRNA (67). Anti-sense FAK oligonucleotides, FAK-CD and FAK siRNA caused effective FAK down-regulation, decreased cancer cell viability, increased apoptosis and decreased tumorigenicity. Since these approaches have limitations for clinical research the first FAK small molecular pharmacological inhibitors were developed by several groups (Table 2). FAK small molecule inhibitors can be divided into two main groups: the first group includes inhibitors targeting enzymatic or kinase-dependent functions of FAK, such as inhibitors targeting the ATP-binding site domain and allosteric inhibitors that target other sites of FAK yet still block kinase activity, and the second group includes inhibitors that target the scaffolding function of FAK (113). We will provide a critical overview of different therapeutic approaches with small molecule inhibitors to target main FAK functions in cancer cells and in microenvironment.

7.1. Enzymatic FAK inhibitors and first clinical trials

Most FAK inhibitors - NVP-TAE-226 (114-115), developed by Novartis (116) and pyrrolopyrimidines developed by Merck (117); PF-573,228 (118), PF-562,271 (119), PF-04554878, developed by Pfizer; PND-1186 (120-121) developed by Poniard; and GSK2256098, developed by Glaxo Smith Kline, VS-6063, VS-4718, VS-5095 from *Verastem* target the FAK kinase domain with the ATP-binding site to inhibit FAK kinase enzymatic activity. The advantage of this approach is that it blocks the FAK enzymatic activity with high efficiency.

NVP-TAE-226 effectively inhibited *in vitro* kinase activity of recombinant FAK with an IC_{50} of 5.5 nM, caused apoptosis and decreased tumor growth in glioma and ovarian cancer xenograft models *in vivo*

(114),(122) . TAE226 not only caused tumor regression, but also affected tumor microenvironment, blocked production of VEGF and reduced microvessel density (122). TAE-226 also was shown to inhibit IGFR-1 at 120 nM (114). Since FAK and IGFR-1 were shown to interact and increase cancer cell survival (123), their dual targeting with TAE-226 inhibitor can be very effective. In fact, TAE226 inhibited pancreatic cell growth and also decreased phosphorylation of ERK and AKT (123). TAE226 also decreased 3D cell growth of human head and neck carcinoma cells (124).

Another inhibitor, PF-573,228 effectively blocked catalytic activity of recombinant FAK with IC_{50}= 4 nM (118). In different cancer cell lines this inhibitor decreased FAK phosphorylation at 30-100 nM. PF-573,228 did not induce apoptosis in prostate carcinoma PC3 cells and Fisher rat embryo REF52 and MDCK cells in contrast to FAK anti-sense oligonucleotides, which decreased FAK expression and caused apoptosis (125), but effectively decreased cancer cell migration (118), which indicates that non-cytotoxic FAK inhibitors can be developed to target FAK motility and metastasis in clinic.

Next Pfizer's inhibitor, PF-562,271 effectively inhibited *in vitro* FAK activity with IC_{50}= 1.5 nM, and also inhibited Pyk-2 kinase activity (IC_{50}=13 nM) (119). Since Pyk-2 was shown to compensate for FAK functions in angiogenesis, metastasis and tumorigenicity in FAK-deficient cells (110), (92), dual-targeting of FAK and Pyk-2 is beneficial for effective therapy. PF-562,271 effectively decreased tumor growth in many xenograft models (119), and also it inhibited pancreatic tumor growth, invasion and metastasis in an orthotopic murine model (126). This inhibitor blocked not only tumor growth and proliferation, and also inhibited pancreatic tumor microenvironment components such as tumor associated fibroblasts and macrophages (126).

The PND-1186 FAK inhibitor very effectively inhibits FAK activity *in vitro* (IC_{50} equal to 1.5 nM) and 100 nM in cultured breast carcinoma cells (120). PND-118 decreased subcutaneous breast tumor growth *in vivo*, which correlated with tumor cell apoptosis and activation of caspase-3. Moreover, addition of this inhibitor to the drinking water of mice inhibited ovarian carcinoma tumor growth associated with inhibition of Y397-FAK (120). Oral delivery of PND-1186 FAK inhibitor decreased breast tumor growth and inhibited spontaneous metastasis to lungs in orthotopic breast carcinoma models (121). Tumors from animals treated with PND-1186 exhibited increased apoptosis compared to vehicle-treated animals. The advantage of this inhibitor is that it also effectively affected tumor microenvironment, reduced inflammatory cell infiltration in primary 4T1 mammary carcinoma tumors, and inhibited TNF alpha-stimulated IL-6 secretion from 4T1 cells in a dose-dependent manner (121).

Recently, the first FAK inhibitor, PF-562,271 was tested clinical Phase I trial (31) (Table 2, marked by bold). At the dose of 125 mg orally twice a day with food PF-562271 was tolerable, with a manageable safety profile. 99 patients with advanced malignancies were treated with FAK inhibitor PF-562,271, and after treatment 31 patients experienced stable disease (end of cycle 2 at approximately 6 weeks) and 15 of those patients remained stable for six more cycles. PF-562271 displayed time and dose-dependent nonlinear pharmacokinetics profile. This first clinical trial study supported FAK as a promising therapeutic target (31).

The second generation Pfizer's inhibitor PF-04554878 (acquired by Verastem and named VS-6063) had a better pharmacodynamics profile (31) and currently is in clinical trial (clinical trial # NCT01778803, http://clinicaltrials.gov/).

Verastem's inhibitors VS-4718 and VS-5095 also effectively target FAK kinase activity. VS-4718 inhibitor is currently in clinical trials in subjects with metastatic non-hematologic malignancies (phase I clinical trial # NCT01849744, http://www.clinicaltrials.gov).

The recently developed FAK inhibitor, GSK2256098 was also being tested in clinical trial (clinical trial #NCT NCT01138033 http://clinicaltrials.gov/) (Table 2, marked by bold). The future clinical studies will demonstrate the efficacy of this inhibitor.

Another approach is to target the FAK autophosphorylation site, which was reported recently with allosteric FAK inhibitor 14 or compound Y15 to block tumor growth (127-130) . The inhibitor effectively inhibited FAK autophosphorylation activity at 25 nM-1 μM, did not inhibit Pyk-2, EGFR, Src, IGFR-1 and other enzymes *in vitro* and decreased tumor growth *in vivo* at 30 mg/kg by intraperitoneal delivery or at 120 mg/kg by oral delivery using breast, pancreatic, neuroblastoma, glioblastoma and colon cancer xenograft mice models. The inhibitor caused apoptosis and decreased proliferation in xenograft tumors *in vivo*. The advantage of this approach is the high specificity of targeting Y397, the main autophosphorylation site of FAK. This inhibitor also decreased Y418-phosphorylation of down-stream Src in colon cancer cells (130), which can be also benefitial for development of future therapies. FAK has additional functions independent of its autophosphorylation activity or kinase activity (131), which is evident from the different phenotypes of FAK-/- cells and cells with deleted Y397 site (132) or with knock-in point mutation (lysine 454 to arginine) (131), which is important for future therapeutic design of the enzymatic inhibitors.

Takeda also developed non-ATP-competitive FAK allosteric inhibitors that efficiently decreased FAK functions (133) (Table 2). The Takeda identified tricyclic sulfonamides (compounds 1 and 2) that targeted a novel allosteric site in the C-lobe of the kinase domain, caused conformational changes of the kinase domain and induced disruption of ATP pocket formation and inhibition of FAK kinase activity with IC_{50} 4.2 and 8.7 μM, respectively (133). Both, compounds required pre-incubation of kinase with inhibitor to cause allosteric ATP-noncompetitive inhibition. Both allosteric inhibitors were highly selective

among 288 kinases, with only 10 kinases other than FAK inhibited by >50% and Pyk-2 inhibited by 25%. The compound 2 was more selective than compound 1, as it did not inhibit 10 kinases >50% and did not inhibit Pyk-2 at 10 μM. This approach reveals novel allosteric and selective way of FAK inhibition. The *in vivo* studies in xenograft mice models need to demonstrate efficacy on these allosteric inhibitors.

7.2. Inhibitors targeting FAK scaffold

The novel approach of targeting of FAK scaffold with small molecule inhibitors was recently proposed and developed (38). Since FAK has so many binding partners such as Src, EGFR, Her-2, c-Met, PI-3K, disruption of these complexes is an additional therapeutic approach to disrupt signals that FAK integrates and effectively block FAK regulated functions. Since FAK blocks functions of tumor suppressor proteins, another approach is to disrupt FAK interaction with these proteins and reactivate their tumor-suppressor functions. The first approach to target the scaffolding function of FAK was performed by disrupting FAK and VEGFR-3(134) with C4, then disrupting FAK and IFGR1 interaction, FAK and c-Met with INT2-31 inhibitor (135-136); and then FAK and Mdm-2 interaction (137) with M13 inhibitor and recently FAK and p53 interaction with R2 inhibitor (138). These inhibitors effectively targeted the scaffolding function of FAK and inhibited cancer cell viability and tumor growth through disrupting angiogenesis, inhibition of AKT signaling or activating p53 signaling with activating down-stream targets of p53: p21, Bax and Mdm-2 (138). The inhibitors targeting FAK scaffolding function displayed efficacy at submicromolar and low micromolar doses *in vitro* and at 15-60 mg/kg *in vivo in mice* xenograft models. Since there are many other important scaffolding partners of FAK (Figure 2), disrupting FAK-protein interactions based on structural studies is a very perspective approach and will show the efficacy of this approach in future.

7.3. Combination therapy

A combination therapy approach should be employed to overcome resistance of cancer cells to chemotherapy and increase efficacy of drugs, since cancer cells have many survival signaling pathways, such as FAK, Src, AKT, MAPK, PI3K, EGFR/HER-2, c-Met and others, which provide survival signals. The combination therapy approach using inhibitors of FAK with other signaling pathways showed increased efficacy of single inhibitors. For example, the dual inhibition of FAK with dominant-negative FAK-CD and EGFR inhibitor, AG-1478 or Src inhibitor, PP2 demonstrated increased efficacy compared with FAK inhibition alone, caused increased cell detachment, inhibition of AKT and ERK1/2 and Src, increased apoptosis, caused caspase-3 and 8 cleavage in breast and colon cancer cells (139, 140). The combination of FAK inhibitor, TAE-226 and docetaxel significantly decreased ovarian tumor growth, and targeted tumor microenvironment, induced apoptosis of tumor-associated endothelial cells, reduced microvessel density and prolonged survival in tumor-bearing mice (122). TAE-226 also increased radiosensitivity of head and neck cancer cells (124). The combination of PF-562,271 with sunitinib (SU11248) (an angiogenesis inhibitor) was very effective in decreasing tumor growth and inhibiting angiogenesis of human hepatocellular carcinoma in a rat xenograft model (141). The combination of PF-562,271 with gemcitabine decreased tumor-associated macrophages and fibroblasts compared with each agent alone, representing a novel approach in therapy by targeting the tumor microenvironment with FAK inhibitors (126). The combination of FAK inhibitor, Y15 with Src inhibitor PP2 inhibitor effectively decreased colon cancer viability , associated with decreased Y397-FAK and Y418-Src (130). In addition, recent study showed that Y15 inhibitor with sensitized colon cancer cells to 5-fluorouracil and oxaliplatin in vitro and to Y15 and 5-fluouracil in vivo (130). A recent study of a combination of the FAK inhibitor Y15 with gemcitabine was more effective than each inhibitor alone in decreasing pancreatic cancer xenograft tumor growth in mice (129). Another FAK inhibitor, C4, was highly effective in combination with doxorubicin to inhibit breast cancer xenograft tumor growth and angiogenesis in mice (134). Recently, a combination of FAK inhibitor Y15 and temozolomide was very effective in blocking U87 glioblastoma xenograft tumor growth (142). The combination of inhibitor targeting FAK and VEGFR-3 interaction C4 sensitized tumors to doxorubicin in subcutaneous breast cancer xenograft model (134). The combination of inhibitor R2 disrupting interaction of FAK with p53 and activating p53 signaling sensitized cancer cells to doxorubicin and 5-fluorouracil (138). Verastem starts a Phase I clinical trial of VS6063 FAK inhibitor in combination with paclitaxel in patients with advanced ovarian cancer (clinical trial # NCT01778803,http://clinicaltrials.gov/ct2/show/NCT01778803). These studies show high potential of combination therapy, where FAK inhibitor sensitizes cancer cells to chemotherapy.

8. CLINICAL CHALLENGES

The challenges that lie ahead are to know which tumors types will be most sensitive to FAK inhibitors; which subset of patients will respond more effectively to this therapy. To design a clinical trial we should apply the personalized approach to patients and accrue patients based on their molecular profile, which is a challenge. The discovery of biomarkers associated with FAK overexpression is a challenge and represents a future translational perspective. The most important clinical challenge is to correlate the various molecular biomarkers with the patient outcome, which will help to design clinical trials and allow selection of patient cohort benefiting from FAK-targeting therapies.

Another question is can we use FAK inhibitor as a single agent? It is the most challenging task to solve, since FAK is connected with so many signaling pathways and has many binding partners, which activate FAK signaling, and thus the combination therapy approach is the most feasible to effectively treat cancer patients. In fact, dual FAK-Src as a promising therapeutic target was proposed recently (143). The question is can Pyk-2 play

compensatory role to provide cancer cell survival? In this case the dual targeting of these two kinases can be used and novel Pyk-2-specific inhibitors should be developed to enter clinical trials.

9. CONCLUSIONS, PERSPECTIVES AND FUTURE DIRECTIONS

Thus, FAK has kinase-dependent enzymatic functions and kinase-independent scaffolding functions. The novel nuclear functions of FAK and its mechanism remain to be understood and discovered. FAK has important roles in motility, invasion, metastasis, angiogenesis, and novel functions in cancer stem cells and tumor microenvironment signaling. There has been major progress during the past few years in pharmacological targeting of FAK, and the first Phase I trials have been very promising. But many questions remain to be answered. What are the detail mechanisms of FAK up-regulation in tumors at early stages of cancer? We need to analyze molecular and biochemical mechanisms of FAK overexpression and correlate it with the patient outcome in different types of tumors and to develop biomarkers associated with FAK overexpression. It will be interesting to study the cross-signaling between FAK, WNT, Sonic Hedgehog, Gli, and Notch, and to study other cancer stem cell transcription factors and markers, which regulate FAK expression in cancer stem cells. Since FAK inhibits cancer stem cells through kinase-dependent and independent functions, the dissection of these functions is very important for the future therapy design. New preclinical and clinical studies will reveal detail mechanisms of targeting FAK in cancer stem cells and the tumor microenvironment. New studies on FAK signaling in endothelial cells and immune cells and regulation of cancer stem cells will also illuminate mechanisms of tumorigenesis. Can FAK expression or other signaling proteins regulating FAK be used as a biomarker for predicting patient prognosis, metastasis or treatment outcome? We will reveal which type of tumors and patients will benefit from FAK therapy. Beyond this, detailed mechanisms of FAK inhibition with small molecules and in combination with chemotherapy will need to be understood. The detail mechanism of enzymatic, allosteric and scaffolding inhibitors remain to be discovered, their on-target and off-target mechanisms at different doses need to de discovered to explain recently discovered nonlinear effects of FAK inhibitors on angiogenesis (112). The future dose and regiment of FAK inhibitors, mechanism of combination therapy, pharmacodynamics and toxicology studies will be important to conduct in clinical trials. Targeting FAK scaffold together with FAK enzymatic inhibitors and dissecting their functions is a future translational perspective.

In conclusion, the past twenty years of FAK research answered many questions about FAK-binding partners, the structure of its major domains and mechanisms of survival signaling, and left many new questions about FAK biology to answer in the future. Given the central role of FAK in cancer functions further refinements and understanding of its signaling pathways will lead to novel cancer therapy approaches.

10. ACKNOWLEDGEMENTS

The research is partly supported by NCI RO-1 grants CA65910 (W.G.Cance) and by the NCI Cancer Center Support grant to the Roswell Park Cancer Institute (CA 16056). We are grateful to Dr. A. Gordon for proofreading of the manuscript and colleagues from Roswell Park Cancer Institute and Department of Cellular and Molecular Biology at University of California, Berkeley, CA for helpful discussions and suggestions. We apologize to those whose papers could not be cited because of space limitations. *Competing interest statement:* Dr. Vita M. Golubovskaya is Co-Founder and shareholder of *CureFAKtor Pharmaceuticals*.

11. REFERENCES

1. M. D. Schaller, C. A. Borgman, B. S. Cobb, R. R. Vines, A. B. Reynolds and J. T. Parsons: pp125fak a structurally distinctive protein-tyrosine kinase associated with focal adhesions. *Proc Natl Acad Sci USA*, 89, 5192-5196 (1992)

2. M. Luo, H. Fan, T. Nagy, H. Wei, C. Wang, S. Liu, M. S. Wicha and J. L. Guan: Mammary epithelial-specific ablation of the focal adhesion kinase suppresses mammary tumorigenesis by affecting mammary cancer stem/progenitor cells. *Cancer Res*, 69, 466-474 (2009)

3. M. Schober and E. Fuchs: Tumor-initiating stem cells of squamous cell carcinomas and their control by TGF-beta and integrin/focal adhesion kinase (FAK) signaling. *Proc Natl Acad Sci U S A*, 108, 10544-10549 (2009)

4. B. Ho, G. Olson, S. Figel, I. Gelman, W. G. Cance and V. M. Golubovskaya: Nanog Increases Focal Adhesion Kinase (FAK) Promoter Activity and Expression and Directly Binds to FAK Protein to Be Phosphorylated. *J Biol Chem*, 287, 18656-18773 (2012)

5. X. Peng, H. Ueda, H. Zhou, T. Stokol, T. L. Shen, A. Alcaraz, T. Nagy, J. D. Vassalli and J. L. Guan: Overexpression of focal adhesion kinase in vascular endothelial cells promotes angiogenesis in transgenic mice. *Cardiovasc Res*, 64, 421-430 (2004)

6. C. Scheel and R. A. Weinberg: Phenotypic plasticity and epithelial-mesenchymal transitions in cancer and normal stem cells? *Int J Cancer*, 129, 2310-2314 (2011)

7. H. Fan, X. Zhao, S. Sun, M. Luo and J. L. Guan: Focal adhesion kinase scaffolding function to mediate endophilin A2 phosphorylation promotes epithelial-mesenchymal transition and mammary cancer stem cell activities *in vivo*. *J Biol Chem* 288, 3322-3333 (2013)

8. A. Serrels, M. Canel, V. G. Brunton and M. C. Frame: Src/FAK-mediated regulation of E-cadherin as a mechanism for controlling collective cell movement: insights from *in vivo* imaging. *Cell Adh Migr*, 5, 360-365 (2011)

9. T. Fujii, K. Koshikawa, S. Nomoto, O. Okochi, T. Kaneko, S. Inoue, Y. Yatabe, S. Takeda and A. Nakao:

Focal adhesion kinase is overexpressed in hepatocellular carcinoma and can be served as an independent prognostic factor. *J Hepatol*, 41, 104-111 (2004)

10. S. Carelli, G. Zadra, V. Vaira, M. Falleni, L. Bottiglieri, M. Nosotti, A. M. Di Giulio, A. Gorio and S. Bosari: Up-regulation of focal adhesion kinase in non-small cell lung cancer. *Lung Cancer*, 53, 263-271 (2006)

11. S. Ocak, H. Chen, C. Callison, A. L. Gonzalez and P. P. Massion: Expression of focal adhesion kinase in small-cell lung carcinoma. *Cancer*, 118(5), 1293-1301 (2012)

12. A. L. Lark, C. A. Livasy, L. Dressler, D. T. Moore, R. C. Millikan, J. Geradts, M. Iacocca, D. Cowan, D. Little, R. J. Craven and W. Cance: High focal adhesion kinase expression in invasive breast carcinomas is associated with an aggressive phenotype. *Mod Pathol*, 18, 1289-1294 (2005)

13. K. Furuyama, R. Doi, T. Mori, E. Toyoda, D. Ito, K. Kami, M. Koizumi, A. Kida, Y. Kawaguchi and K. Fujimoto: Clinical significance of focal adhesion kinase in resectable pancreatic cancer. *World J Surg*, 30, 219-226 (2006)

14. T. P. Hecker, J. R. Grammer, G. Y. Gillespie, J. Stewart, Jr. and C. L. Gladson: Focal adhesion kinase enhances signaling through the Shc/extracellular signal-regulated kinase pathway in anaplastic astrocytoma tumor biopsy samples. *Cancer Res*, 62, 2699-2707 (2002)

15. A. Gutenberg, W. Bruck, M. Buchfelder and H. C. Ludwig: Expression of tyrosine kinases FAK and Pyk2 in 331 human astrocytomas. *Acta Neuropathol*, 108, 224-30 (2004)

16. L. V. Owens, L. Xu, R. J. Craven, G. A. Dent, T. M. Weiner, L. Kornberg, E. T. Liu and W. G. Cance: Overexpression of the focal adhesion kinase (p125FAK) in invasive human tumors. *Cancer Research*, 55, 2752-2755 (1995)

17. A. Schroder, G. Delling and E. A. Kaiser: [Expression analysis of protein tyrosine kinases of the FAK (focal adhesion kinase) family in osteosarcoma]. *Pathologe*, 23, 361-366 (2002)

18. P. L. Judson, X. He, W. G. Cance and L. Van Le: Overexpression of focal adhesion kinase, a protein tyrosine kinase, in ovarian carcinoma. *Cancer*, 86, 1551-1556 (1999)

19. M. H. Oktay, K. Oktay, D. Hamele-Bena, A. Buyuk and L. G. Koss: Focal adhesion kinase as a marker of malignant phenotype in breast and cervical carcinomas. *Hum Pathol*, 34, 240-245 (2003)

20. H. S. Moon, W. I. Park, E. A. Choi, H. W. Chung and S. C. Kim: The expression and tyrosine phosphorylation of E-cadherin/catenin adhesion complex, and focal adhesion kinase in invasive cervical carcinomas. *Int J Gynecol Cancer*, 13, 640-646 (2003)

21. A. L. Lark, C. A. Livasy, B. Calvo, L. Caskey, D. T. Moore, X. Yang and W. G. Cance: Overexpression of focal adhesion kinase in primary colorectal carcinomas and colorectal liver metastases: immunohistochemistry and real-time PCR analyses. *Clin Cancer Res*, 9, 215-222 (2003)

22. S. E. Theocharis, G. P. Kouraklis, J. D. Kakisis, H. G. Kanelli, F. E. Apostolakou, G. M. Karatzas and A. S. Koutselinis: Focal adhesion kinase expression is not a prognostic predictor in colon adenocarcinoma patients. *Eur J Surg Oncol*, 29, 571-574 (2003)

23. E. A. Beierle, N. A. Massoll, J. Hartwich, E. V. Kurenova, V. M. Golubovskaya, W. G. Cance, P. McGrady and W. B. London: Focal Adhesion Kinase Expression in Human Neuroblastoma: Immunohistochemical and Real-time PCR Analyses. *Clin Cancer Res*, 14, 3299-3305 (2008)

24. L. V. Owens, L. Xu, G. A. Dent, X. Yang, G. C. Sturge, R. J. Craven and W. G. Cance: Focal adhesion kinase as a marker of invasive potential in differentiated human thyroid cancer. *Annals of Surgical Oncology*, 3, 100-105 (1996)

25. L. Tremblay, W. Hauck, A. G. Aprikian, L. R. Begin, A. Chapdelaine and S. Chevalier: Focal adhesion kinase (pp125FAK) expression, activation and association with paxillin and p50CSK in human metastatic prostate carcinoma. *Int J Cancer*, 68, 164-171 (1996)

26. M. Canel, P. Secades, J.-P. Rodrigo, R. Cabanillas, A. Herrero, C. Suarez and M.-D. Chiara: Overexpression of Focal Adhesion Kinase in Head and Neck Squamous Cell Carcinoma Is Independent of fak Gene Copy Number. *Clin Cancer Res 1583*, 12, 3272-3279 (2006)

27. C. Recher, L. Ysebaert, O. Beyne-Rauzy, V. Mansat-De Mas, J.-B. Ruidavets, P. Cariven, C. Demur, B. Payrastre, G. Laurent and C. Racaud-Sultan: Expression of Focal Adhesion Kinase in Acute Myeloid Leukemia Is Associated with Enhanced Blast Migration, Increased Cellularity, and Poor Prognosis. *Cancer Res*, 64, 3191-3197 (2004)

28. H. M. Lightfoot, Jr., A. Lark, C. A. Livasy, D. T. Moore, D. Cowan, L. Dressler, R. J. Craven and W. G. Cance: Upregulation of focal adhesion kinase (FAK) expression in ductal carcinoma *in situ* (DCIS) is an early event in breast tumorigenesis. *Breast Cancer Res Treat*, 88, 109-116 (2004)

29. V. Gabarra-Niecko, M. D. Schaller and J. M. Dunty: FAK regulates biological processes important for the pathogenesis of cancer. *Cancer Metastasis Rev*, 22(4), 359-374 (2003)

30. G. W. McLean, E. Avizienyte and M. C. Frame: Focal adhesion kinase as a potential target in oncology. *Expert Opin Pharmacother*, 4, 227-234 (2003)

31. J. R. Infante, D. R. Camidge, L. R. Mileshkin, E. X. Chen, R. J. Hicks, D. Rischin, H. Fingert, K. J. Pierce, H. Xu, W. G. Roberts, S. M. Shreeve, H. A. Burris and L. L. Siu: Safety, pharmacokinetic, and pharmacodynamic phase I dose-escalation trial of PF-00562271, an inhibitor of focal

adhesion kinase, in advanced solid tumors. *J Clin Oncol*, 30, 1527-1533 (2012)

32. S. K. Hanks, M. B. Calalb, M. C. Harper and S. K. Patel: Focal adhesion protein-tyrosine kinase phosphorylated in response to cell attachment to fibronectin. *Proceedings of the National Academy of Sciences of the United States of America*, 89, 8487-8491 (1992)

33. G. S. Whitney, P. Y. Chan, J. Blake, W. L. Cosand, M. G. Neubauer, A. Aruffo and S. B. Kanner: Human T and B lymphocytes express a structurally conserved focal adhesion kinase, pp125FAK. *DNA & Cell Biology*, 12, 823-830 (1993)

34. T. M. Weiner, E. T. Liu, R. J. Craven and W. G. Cance: Expression of focal adhesion kinase gene and invasive cancer. *Lancet*, 342, 1024-1025 (1993)

35. R. H. Palmer, L. I. Fessler, P. T. Edeen, S. J. Madigan, M. McKeown and T. Hunter: DFak56 is a novel Drosophila melanogaster focal adhesion kinase. *J Biol Chem*, 274, 35621-35629 (1999)

36. J. Fujimoto, K. Sawamoto, M. Okabe, Y. Takagi, T. Tezuka, S. Yoshikawa, H. Ryo, H. Okano and T. Yamamoto: Cloning and characterization of Dfak56, a homolog of focal adhesion kinase, in Drosophila melanogaster. *J Biol Chem*, 274, 29196-29201 (1999)

37. G. L. Fox, I. Rebay and R. O. Hynes: Expression of DFak56, a Drosophila homolog of vertebrate focal adhesion kinase, supports a role in cell migration *in vivo*. *Proc Natl Acad Sci U S A*, 96, 14978-14983 (1999)

38. W. G. Cance, E. Kurenova, T. Marlowe and V. Golubovskaya: Disrupting the scaffold to improve focal adhesion kinase-targeted cancer therapeutics. *Sci Signal*, 6, pe10 (2013)

39. J. M. Corsi, E. Rouer, J. A. Girault and H. Enslen: Organization and post-transcriptional processing of focal adhesion kinase gene. *BMC Genomics*, 7, 198-201 (2006)

40. M. Agochiya, V. G. Brunton, D. W. Owens, E. K. Parkinson, C. Paraskeva, W. N. Keith and M. C. Frame: Increased dosage and amplification of the focal adhesion kinase gene in human cancer cells [In Process Citation]. *Oncogene*, 18, 5646-5653 (1999)

41. H. Okamoto, K. Yasui, C. Zhao, S. Arii and J. Inazawa: PTK2 and EIF3S3 genes may be amplification targets at 8q23-q24 and are associated with large hepatocellular carcinomas. *Hepatology*, 38, 1242-1249 (2003)

42. V. Golubovskaya, A. Kaur and W. Cance: Cloning and characterization of the promoter region of human focal adhesion kinase gene: nuclear factor kappa B and p53 binding sites. *Biochim Biophys Acta*, 1678, 111-125 (2004)

43. M. D. Schaller, J. D. Hildebrand, J. D. Shannon, J. W. Fox, R. R. Vines and J. T. Parsons: Autophosphorylation of the focal adhesion kinase, pp125FAK, directs SH2-dependent binding of pp60src. *Molecular & Cellular Biology*, 14, 1680-1688 (1994)

44. C. R. Hauck, D. J. Sieg, D. A. Hsia, J. C. Loftus, W. A. Gaarde, B. P. Monia and D. D. Schlaepfer: Inhibition of focal adhesion kinase expression or activity disrupts epidermal growth factor-stimulated signaling promoting the migration of invasive human carcinoma cells. *Cancer Res*, 61, 7079-7090 (2001)

45. G. Kadare, M. Toutant, E. Formstecher, J. C. Corvol, M. Carnaud, M. C. Boutterin and J. A. Girault: PIAS1-mediated sumoylation of focal adhesion kinase activates its autophosphorylation. *J Biol Chem*, 278(48), 47434-47440 (2003)

46. T. Eskildsen, H. Taipaleenmaki, J. Stenvang, B. M. Abdallah, N. Ditzel, A. Y. Nossent, M. Bak, S. Kauppinen and M. Kassem: MicroRNA-138 regulates osteogenic differentiation of human stromal (mesenchymal) stem cells *in vivo*. *Proc Natl Acad Sci U S A*, 108, 6139-6144

47. V. M. Golubovskaya, B. Sumbler, B. Ho, M. Yemma and W. G. Cance: MiR-138 and MiR-135 Directly Target Focal Adhesion Kinase, Inhibit Cell Invasion, and Increase Sensitivity to Chemotherapy in Cancer Cells. *Anticancer Agents Med Chem* (2013)

48. X. Kong, G. Li, Y. Yuan, Y. He, X. Wu, W. Zhang, Z. Wu, T. Chen, W. Wu, P. E. Lobie and T. Zhu: MicroRNA-7 inhibits epithelial-to-mesenchymal transition and metastasis of breast cancer cells via targeting FAK expression. *PLoS One*, 7, e41523 (2012)

49. V. Ossovskaya, S.-T. Lim, N. Ota, D. D. Schlaepfer and D. Ilic: FAK nuclear export signal sequences. *FEBS Letters*, 582, 2402-2404 (2008)

50. V. M. Golubovskaya, R. Finch and W. G. Cance: Direct Interaction of the N-terminal Domain of Focal Adhesion Kinase with the N-terminal Transactivation Domain of p53. *J. Biol. Chem.*, 280, 25008-25021 (2005)

51. S. T. Lim, X. L. Chen, Y. Lim, D. A. Hanson, T. T. Vo, K. Howerton, N. Larocque, S. J. Fisher, D. D. Schlaepfer and D. Ilic: Nuclear FAK promotes cell proliferation and survival through FERM-enhanced p53 degradation. *Mol Cell*, 29, 9-22 (2008)

52. S. T. Lim: Nuclear FAK: a new mode of gene regulation from cellular adhesions. *Mol Cells, 36,* 1-6 (2013)

53. W. G. Cance and V. M. Golubovskaya: Focal adhesion kinase versus p53: apoptosis or survival? *Sci Signal*, 1, pe22 (2008)

54. S. T. Arold, M. K. Hoellerer and M. E. Noble: The structural basis of localization and signaling by the focal adhesion targeting domain. *Structure*, 10, 319-327 (2002)

55. I. Hayashi, K. Vuori and R. C. Liddington: The focal adhesion targeting (FAT) region of focal adhesion kinase is

a four-helix bundle that binds paxillin. *Nat Struct Biol*, 9, 101-106 (2002)

56. D. F. Ceccarelli, H. K. Song, F. Poy, M. D. Schaller and M. J. Eck: Crystal structure of the FERM domain of focal adhesion kinase. *J Biol Chem*, 281, 252-259 (2006)

57. J. Nowakowski, C. N. Cronin, D. E. McRee, M. W. Knuth, C. G. Nelson, N. P. Pavletich, J. Rogers, B. C. Sang, D. N. Scheibe, R. V. Swanson and D. A. Thompson: Structures of the cancer-related Aurora-A, FAK, and EphA2 protein kinases from nanovolume crystallography. *Structure*, 10, 1659-1667 (2002)

58. D. Lietha, X. Cai, D. F. Ceccarelli, Y. Li, M. D. Schaller and M. J. Eck: Structural basis for the autoinhibition of focal adhesion kinase. *Cell*, 129, 1177-1187 (2007)

59. J. T. Parsons: Focal adhesion kinase: the first ten years. *J Cell Sci*, 116, 1409-1416 (2003)

60. M. D. Schaller: Biochemical signals and biological responses elicited by the focal adhesion kinase. *Biochim Biophys Acta*, 1540, 1-21 (2001)

61. J. L. Guan: Integrin signaling through FAK in the regulation of mammary stem cells and breast cancer. *IUBMB Life*, 62, 268-276 (2010)

62. V. M. Golubovskaya and W. G. Cance: Focal adhesion kinase and p53 signaling in cancer cells. *Int Rev Cytol*, 263, 103-153 (2007)

63. D. D. Schlaepfer, C. R. Hauck and D. J. Sieg: Signaling through focal adhesion kinase. *Prog Biophys Mol Biol*, 71(3-4), 435-478 (1999)

64. S. M. Frisch, K. Vuori, E. Ruoslahti and P. Y. Chan-Hui: Control of adhesion-dependent cell survival by focal adhesion kinase. *Journal of Cell Biology*, 134, 793-799 (1996)

65. L. H. Xu, L. V. Owens, G. C. Sturge, X. Yang, E. T. Liu, R. J. Craven and W. G. Cance: Attenuation of the expression of the focal adhesion kinase induces apoptosis in tumor cells. *Cell Growth Differ*, 7, 413-418 (1996)

66. L.-h. Xu, X.-h. Yang, C. A. Bradham, D. A. Brenner, A. S. Baldwin, R. J. Craven and W. G. Cance: The focal adhesion kinase suppresses transformation-associated, anchorage-Independent apoptosis in human breast cancer cells. *J. Biol. Chem.*, 275, 30597-30604 (2000)

67. V. M. Golubovskaya, M. Zheng, L. Zhang, J. L. Li and W. G. Cance: The direct effect of focal adhesion kinase (FAK), dominant-negative FAK, FAK-CD and FAK siRNA on gene expression and human MCF-7 breast cancer cell tumorigenesis. *BMC Cancer*, 9, 280-285 (2009)

68. E. Kurenova, L.-H. Xu, X. Yang, A. S. Baldwin, Jr., R. J. Craven, S. K. Hanks, Z.-g. Liu and W. G. Cance: Focal Adhesion Kinase Suppresses Apoptosis by Binding to the Death Domain of Receptor-Interacting Protein. *Mol. Cell. Biol.*, 24, 4361-4371 (2004)

69. Y. Sonoda, Y. Matsumoto, M. Funakoshi, D. Yamamoto, S. K. Hanks and T. Kasahara: Anti-apoptotic role of focal adhesion kinase (FAK). Induction of inhibitor-of-apoptosis proteins and apoptosis suppression by the overexpression of FAK in a human leukemic cell line, HL-60. *J Biol Chem*, 275, 16309-16315 (2000)

70. J. Schwock, N. Dhani, M. P. Cao, J. Zheng, R. Clarkson, N. Radulovich, R. Navab, L. C. Horn and D. W. Hedley: Targeting focal adhesion kinase with dominant-negative FRNK or Hsp90 inhibitor 17-DMAG suppresses tumor growth and metastasis of SiHa cervical xenografts. *Cancer Res*, 69, 4750-4759 (2009)

71. E. Sandilands, B. Serrels, D. G. McEwan, J. P. Morton, J. P. Macagno, K. McLeod, C. Stevens, V. G. Brunton, W. Y. Langdon, M. Vidal, O. J. Sansom, I. Dikic, S. Wilkinson and M. C. Frame: Autophagic targeting of Src promotes cancer cell survival following reduced FAK signalling. *Nat Cell Biol*, 14, 51-60 (2012)

72. D. D. Schlaepfer, S. K. Mitra and D. Ilic: Control of motile and invasive cell phenotypes by focal adhesion kinase. *Biochim Biophys Acta*, 1692(2-3), 77-102 (2004)

73. D. J. Sieg, C. R. Hauck and D. D. Schlaepfer: Required role of focal adhesion kinase (FAK) for integrin-stimulated cell migration. *J Cell Sci*, 112, 2677-2691 (1999)

74. D. Illc, Y. Furuta, S. Kanazawa, N. Takeda, K. Sobue, N. Nakatsuji, S. Nomura, J. Fujimoto, M. Okada, T. Yamamoto and *et al.*: Reduced cell motility and enhanced focal adhesion contact formation in cells from FAK-deficient mice. *Nature*, 377, 539-544 (1995)

75. M. G. Yeo, M. A. Partridge, E. J. Ezratty, Q. Shen, G. G. Gundersen and E. E. Marcantonio: Src SH2 arginine 175 is required for cell motility: specific focal adhesion kinase targeting and focal adhesion assembly function. *Mol Cell Biol*, 26, 4399-4409 (2006)

76. H. R. Reiske, S. C. Kao, L. A. Cary, J. L. Guan, J. F. Lai and H. C. Chen: Requirement of phosphatidylinositol 3-kinase in focal adhesion kinase-promoted cell migration. *J Biol Chem*, 274, 12361-12366 (1999)

77. T. Shibue and R. A. Weinberg: Integrin beta1-focal adhesion kinase signaling directs the proliferation of metastatic cancer cells disseminated in the lungs. *Proc Natl Acad Sci U S A*, 106, 10290-10295 (2009)

78. S. M. Frisch, M. Schaller and B. Cieply: Mechanisms that link the oncogenic epithelial-mesenchymal transition to suppression of anoikis. *J Cell Sci*, 126, 21-29 (2013)

79. M. Canel, A. Serrels, M. C. Frame and V. G. Brunton: E-cadherin-integrin crosstalk in cancer invasion and metastasis. *J Cell Sci*, 126, 393-401 (2013)

80. E. Avizienyte, A. W. Wyke, R. J. Jones, G. W. McLean, M. A. Westhoff, V. G. Brunton and M. C. Frame: Src-induced de-regulation of E-cadherin in colon cancer cells requires integrin signalling. *Nat Cell Biol*, 4, 632-638 (2002)

81. C. R. Hauck, D. A. Hsia and D. D. Schlaepfer: The focal adhesion kinase--a regulator of cell migration and invasion. *IUBMB Life*, 53, 115-119 (2002)

82. D. Saito, S. Kyakumoto, N. Chosa, M. Ibi, N. Takahashi, N. Okubo, S. Sawada, A. Ishisaki and M. Kamo: Transforming growth factor-beta1 induces epithelial-mesenchymal transition and integrin alpha3beta1-mediated cell migration of HSC-4 human squamous cell carcinoma cells through Slug. *J Biochem*, 153, 303-315 (2013)

83. T. Lechertier and K. Hodivala-Dilke: Focal adhesion kinase and tumour angiogenesis. *J Pathol*, 226, 404-412 (2012)

84. R. Guinamard, M. Okigaki, J. Schlessinger and J. V. Ravetch: Absence of marginal zone B cells in Pyk-2-deficient mice defines their role in the humoral response. *Nat Immunol*, 1, 31-36 (2000)

85. S. K. Mitra, D. Mikolon, J. E. Molina, D. A. Hsia, D. A. Hanson, A. Chi, S. T. Lim, J. A. Bernard-Trifilo, D. Ilic, D. G. Stupack, D. A. Cheresh and D. D. Schlaepfer: Intrinsic FAK activity and Y925 phosphorylation facilitate an angiogenic switch in tumors. 25, 5969-5984 *Oncogene* (2006)

86. C. A. Garces, E. V. Kurenova, V. M. Golubovskaya and W. G. Cance: Vascular endothelial growth factor receptor-3 and focal adhesion kinase bind and suppress apoptosis in breast cancer cells. *Cancer Res*, 66, 1446-1454 (2006)

87. M. Dean, T. Fojo and S. Bates: Tumour stem cells and drug resistance. *Nat Rev Cancer*, 5, 275-284 (2005)

88. Y. Li and J. Laterra: Cancer stem cells: distinct entities or dynamically regulated phenotypes? *Cancer Res*, 72, 576-580 (2012)

89. G. H. Ashton, J. P. Morton, K. Myant, T. J. Phesse, R. A. Ridgway, V. Marsh, J. A. Wilkins, D. Athineos, V. Muncan, R. Kemp, K. Neufeld, H. Clevers, V. Brunton, D. J. Winton, X. Wang, R. C. Sears, A. R. Clarke, M. C. Frame and O. J. Sansom: Focal adhesion kinase is required for intestinal regeneration and tumorigenesis downstream of Wnt/c-Myc signaling. *Dev Cell*, 19, 259-269 (2010)

90. Y. Fonar, Y. E. Gutkovich, H. Root, A. Malyarova, E. Aamar, V. M. Golubovskaya, S. Elias, Y. M. Elkouby and D. Frank: Focal adhesion kinase protein regulates Wnt3a gene expression to control cell fate specification in the developing neural plate. *Mol Biol Cell*, 22, 2409-2421 (2011)

91. Y. Fonar and D. Frank: FAK and WNT signaling: the meeting of two pathways in cancer and development. *Anticancer Agents Med Chem*, 11, 600-606 (2011)

92. H. Fan and J. L. Guan: Compensatory function of Pyk2 protein in the promotion of focal adhesion kinase (FAK)-null mammary cancer stem cell tumorigenicity and metastatic activity. *J Biol Chem*, 286, 18573-15582 (2011)

93. M. Luo, X. Zhao, S. Chen, S. Liu, M. S. Wicha and J. L. Guan: Distinct FAK activities determine progenitor and mammary stem cell characteristics. *Cancer Res* 73, 5591-5602 (2013)

94. S. D. Wang, P. Rath, B. Lal, J. P. Richard, Y. Li, C. R. Goodwin, J. Laterra and S. Xia: EphB2 receptor controls proliferation/migration dichotomy of glioblastoma by interacting with focal adhesion kinase. *Oncogene* 31, 5132-5143 (2012)

95. J. S. Chen, X. H. Huang, Q. Wang, J. Q. Huang, L. J. Zhang, X. L. Chen, J. Lei and Z. X. Cheng: Sonic hedgehog signaling pathway induces cell migration and invasion through focal adhesion kinase/AKT signaling-mediated activation of matrix metalloproteinase (MMP)-2 and MMP-9 in liver cancer. *Carcinogenesis*, 34, 10-19 (2013)

96. J. Wang, L. Fu, F. Gu and Y. Ma: Notch1 is involved in migration and invasion of human breast cancer cells. *Oncol Rep*, 26(5), 1295-1303 (2011)

97. Y. L. Lin, Z. B. Han, F. Y. Xiong, L. Y. Tian, X. J. Wu, S. W. Xue, Y. R. Zhou, J. X. Deng and H. X. Chen: Malignant transformation of 293 cells induced by ectopic expression of human Nanog. *Mol Cell Biochem*, 351, 109-116 (2011)

98. T. Lin, C. Chao, S. Saito, S. J. Mazur, M. E. Murphy, E. Appella and Y. Xu: p53 induces differentiation of mouse embryonic stem cells by suppressing Nanog expression. *Nat Cell Biol*, 7, 165-71 (2005)

99. V. Krizhanovsky and S. W. Lowe: Stem cells: The promises and perils of p53. *Nature*, 460, 1085-1086 (2009)

100. H. Hong, K. Takahashi, T. Ichisaka, T. Aoi, O. Kanagawa, M. Nakagawa, K. Okita and S. Yamanaka: Suppression of induced pluripotent stem cell generation by the p53-p21 pathway. *Nature*, 460, 1132-1135 (2009)

101. T. Kawamura, J. Suzuki, Y. V. Wang, S. Menendez, L. B. Morera, A. Raya, G. M. Wahl and J. C. Izpisua Belmonte: Linking the p53 tumour suppressor pathway to somatic cell reprogramming. *Nature*, 460, 1140-1144 (2009)

102. R. M. Marion, K. Strati, H. Li, M. Murga, R. Blanco, S. Ortega, O. Fernandez-Capetillo, M. Serrano and M. A. Blasco: A p53-mediated DNA damage response limits

reprogramming to ensure iPS cell genomic integrity. *Nature*, 460, 1149-1153 (2009)

103. V. M. Golubovskaya, R. Finch, F. Kweh, N. A. Massoll, M. Campbell-Thompson, M. R. Wallace and W. G. Cance: p53 regulates FAK expression in human tumor cells. *Mol Carcinog*, 47, 373-382 (2008)

104. K. Kobayashi, H. Takahashi, A. Inoue, H. Harada, S. Toshimori, Y. Kobayashi, K. Goto, K. Sugimoto, H. Yano, T. Ohnishi and J. Tanaka: Oct-3/4 promotes migration and invasion of glioblastoma cells. *J Cell Biochem*, 113, 508-517 (2012)

105. S. I. Grivennikov, F. R. Greten and M. Karin: Immunity, inflammation, and cancer. *Cell*, 140, 883-899 (2010)

106. M. Y. Abshire, K. S. Thomas, K. A. Owen and A. H. Bouton: Macrophage motility requires distinct alpha5beta1/FAK and alpha4beta1/paxillin signaling events. *J Leukoc Biol*, 89, 251-257 (2011)

107. D. D. Schlaepfer, S. Hou, S. T. Lim, A. Tomar, H. Yu, Y. Lim, D. A. Hanson, S. A. Uryu, J. Molina and S. K. Mitra: Tumor necrosis factor-alpha stimulates focal adhesion kinase activity required for mitogen-activated kinase-associated interleukin 6 expression. *J Biol Chem*, 282, 17450-17459 (2007)

108. H. Abedi and I. Zachary: Vascular endothelial growth factor stimulates tyrosine phosphorylation and recruitment to new focal adhesions of focal adhesion kinase and paxillin in endothelial cells. *J Biol Chem*, 272, 15442-15551 (1997)

109. R. Abu-Ghazaleh, J. Kabir, H. Jia, M. Lobo and I. Zachary: Src mediates stimulation by vascular endothelial growth factor of the phosphorylation of focal adhesion kinase at tyrosine 861, and migration and anti-apoptosis in endothelial cells. *Biochem J*, 360, 255-264 (2001)

110. S. M. Weis, S.-T. Lim, K. M. Lutu-Fuga, L. A. Barnes, X. L. Chen, J. R. Gothert, T.-L. Shen, J.-L. Guan, D. D. Schlaepfer and D. A. Cheresh: Compensatory role for Pyk2 during angiogenesis in adult mice lacking endothelial cell FAK. *J. Cell Biol.*, 181, 43-50 (2008)

111. B. Tavora, S. Batista, L. E. Reynolds, S. Jadeja, S. Robinson, V. Kostourou, I. Hart, M. Fruttiger, M. Parsons and K. M. Hodivala-Dilke: Endothelial FAK is required for tumour angiogenesis. *EMBO Mol Med*, 2, 516-528 (2010)

112. V. Kostourou, T. Lechertier, L. E. Reynolds, D. M. Lees, M. Baker, D. T. Jones, B. Tavora, A. R. Ramjaun, G. M. Birdsey, S. D. Robinson, M. Parsons, A. M. Randi, I. R. Hart and K. Hodivala-Dilke: FAK-heterozygous mice display enhanced tumour angiogenesis. *Nat Commun*, 4, 2020 (2013)

113. V. M. Golubovskaya, C. Virnig and W. G. Cance: TAE226-induced apoptosis in breast cancer cells with overexpressed Src or EGFR. *Mol Carcinog*, 47, 222-234 (2008)

114. T.-J. Liu, T. LaFortune, T. Honda, O. Ohmori, S. Hatakeyama, T. Meyer, D. Jackson, J. de Groot and W. K. A. Yung: Inhibition of both focal adhesion kinase and insulin-like growth factor-I receptor kinase suppresses glioma proliferation *in vitro* and *in vivo*. *Mol Cancer Ther*, 6, 1357-1367 (2007)

115. Q. Shi, A. B. Hjelmeland, S. T. Keir, L. Song, S. Wickman, D. Jackson, O. Ohmori, D. D. Bigner, H. S. Friedman and J. N. Rich: A novel low-molecular weight inhibitor of focal adhesion kinase, TAE226, inhibits glioma growth. *Mol Carcinog*, 46, 488-496 (2007)

116. H. S. Choi, Z. Wang, W. Richmond, X. He, K. Yang, T. Jiang, D. Karanewsky, X. J. Gu, V. Zhou, Y. Liu, J. Che, C. C. Lee, J. Caldwell, T. Kanazawa, I. Umemura, N. Matsuura, O. Ohmori, T. Honda, N. Gray and Y. He: Design and synthesis of 7H-pyrrolo[2,3-d]pyrimidines as focal adhesion kinase inhibitors. Part 2. *Bioorg Med Chem Lett*, 16, 2689-2692 (2006)

117. T. Heinrich, J. Seenisamy, L. Emmanuvel, S. S. Kulkarni, J. Bomke, F. Rohdich, H. Greiner, C. Esdar, M. Krier, U. Gradler and D. Musil: Fragment-Based Discovery of New Highly Substituted 1H-Pyrrolo[2,3-b]- and 3H-Imidazolo[4,5-b]-Pyridines as Focal Adhesion Kinase Inhibitors. *J Med Chem* 56, 1160-1170 (2013)

118. J. K. Slack-Davis, K. H. Martin, R. W. Tilghman, M. Iwanicki, E. J. Ung, C. Autry, M. J. Luzzio, B. Cooper, J. C. Kath, W. G. Roberts and J. T. Parsons: Cellular characterization of a novel focal adhesion kinase inhibitor. *J Biol Chem*, 282, 14845-14852 (2007)

119. W. G. Roberts, E. Ung, P. Whalen, B. Cooper, C. Hulford, C. Autry, D. Richter, E. Emerson, J. Lin, J. Kath, K. Coleman, L. Yao, L. Martinez-Alsina, M. Lorenzen, M. Berliner, M. Luzzio, N. Patel, E. Schmitt, S. LaGreca, J. Jani, M. Wessel, E. Marr, M. Griffor and F. Vajdos: Antitumor activity and pharmacology of a selective focal adhesion kinase inhibitor, PF-562,271. *Cancer Res*, 68, 1935-1944 (2008)

120. I. Tanjoni, C. Walsh, S. Uryu, A. Tomar, J. O. Nam, A. Mielgo, S. T. Lim, C. Liang, M. Koenig, C. Sun, N. Patel, C. Kwok, G. McMahon, D. G. Stupack and D. D. Schlaepfer: PND-1186 FAK inhibitor selectively promotes tumor cell apoptosis in three-dimensional environments. *Cancer Biol Ther*, 9, 764-777 (2010)

121. C. Walsh, I. Tanjoni, S. Uryu, A. Tomar, J. O. Nam, H. Luo, A. Phillips, N. Patel, C. Kwok, G. McMahon, D. G. Stupack and D. D. Schlaepfer: Oral delivery of PND-1186 FAK inhibitor decreases tumor growth and spontaneous breast to lung metastasis in pre-clinical models. *Cancer Biol Ther*, 9, 778-790 (2010)

122. J. Halder, Y. G. Lin, W. M. Merritt, W. A. Spannuth, A. M. Nick, T. Honda, A. A. Kamat, L. Y. Han, T. J. Kim,

C. Lu, A. M. Tari, W. Bornmann, A. Fernandez, G. Lopez-Berestein and A. K. Sood: Therapeutic efficacy of a novel focal adhesion kinase inhibitor TAE226 in ovarian carcinoma. *Cancer Res*, 67, 10976-10983 (2007)

123. W. Liu, D. A. Bloom, W. G. Cance, E. V. Kurenova, V. M. Golubovskaya and S. N. Hochwald: FAK and IGF-IR interact to provide survival signals in human pancreatic adenocarcinoma cells. *Carcinogenesis*, 29, 1096-1107 (2008)

124. S. Hehlgans, I. Lange, I. Eke and N. Cordes: 3D cell cultures of human head and neck squamous cell carcinoma cells are radiosensitized by the focal adhesion kinase inhibitor TAE226. *Radiother Oncol*, 92, 371-378 (2009)

125. C. S. Smith, V. M. Golubovskaya, E. Peck, L. H. Xu, B. P. Monia, X. Yang and W. G. Cance: Effect of focal adhesion kinase (FAK) downregulation with FAK antisense oligonucleotides and 5-fluorouracil on the viability of melanoma cell lines. *Melanoma Res*, 15, 357-362 (2005)

126. J. B. Stokes, S. J. Adair, J. K. Slack-Davis, D. M. Walters, R. W. Tilghman, E. D. Hershey, B. Lowrey, K. S. Thomas, A. H. Bouton, R. F. Hwang, E. B. Stelow, J. T. Parsons and T. W. Bauer: Inhibition of focal adhesion kinase by PF-562,271 inhibits the growth and metastasis of pancreatic cancer concomitant with altering the tumor microenvironment. *Mol Cancer Ther*, 10, 2135-45 (2011)

127. V. M. Golubovskaya, C. Nyberg, M. Zheng, F. Kweh, A. Magis, D. Ostrov and W. G. Cance: A Small Molecule Inhibitor, 1,2,4,5-Benzenetetraamine Tetrahydrochloride, Targeting the Y397 Site of Focal Adhesion Kinase Decreases Tumor Growth. *J Med Chem* 51, *7405-7416* (2008)

128. E. A. Beierle, X. Ma, J. Stewart, C. Nyberg, A. Trujillo, W. G. Cance and V. M. Golubovskaya: Inhibition of focal adhesion kinase decreases tumor growth in human neuroblastoma. *Cell Cycle*, 9, 1005-1015 (2010)

129. S. N. Hochwald, C. Nyberg, M. Zheng, D. Zheng, C. Wood, N. A. Massoll, A. Magis, D. Ostrov, W. G. Cance and V. M. Golubovskaya: A novel small molecule inhibitor of FAK decreases growth of human pancreatic cancer. *Cell Cycle*, 8, 2435-2443 (2009)

130. M. Heffler, V. M. Golubovskaya, K. M. Dunn and W. Cance: Focal adhesion kinase autophosphorylation inhibition decreases colon cancer cell growth and enhances the efficacy of chemotherapy. *Cancer Biol Ther*, 14, 761-772 (2013)

131. S. T. Lim, X. L. Chen, A. Tomar, N. L. Miller, J. Yoo and D. D. Schlaepfer: Knockin mutation reveals an essential role for focal adhesion kinase (FAK) activity in blood vessel morphogenesis, cell motility-polarity, but not cell proliferation. *J Biol Chem* 285, 21526-21536 (2010)

132. J. M. Corsi, C. Houbron, P. Billuart, I. Brunet, K. Bouvree, A. Eichmann, J. A. Girault and H. Enslen: Autophosphorylation-independent and dependent functions of Focal Adhesion Kinase during development. *J Biol Chem* 284, 34769-34776 (2009)

133. M. Iwatani, H. Iwata, A. Okabe, R. J. Skene, N. Tomita, Y. Hayashi, Y. Aramaki, D. J. Hosfield, A. Hori, A. Baba and H. Miki: Discovery and characterization of novel allosteric FAK inhibitors. *Eur J Med Chem* 61, 49-60 (2012)

134. E. V. Kurenova, D. L. Hunt, D. He, A. T. Magis, D. A. Ostrov and W. G. Cance: Small Molecule Chloropyramine Hydrochloride (C4) Targets the Binding Site of Focal Adhesion Kinase and Vascular Endothelial Growth Factor Receptor 3 and Suppresses Breast Cancer Growth *in vivo*. *J Med Chem* 52, 4716-4724 (2009)

135. D. A. Ucar, E. Kurenova, T. J. Garrett, W. G. Cance, C. Nyberg, A. Cox, N. Massoll, D. A. Ostrov, N. Lawrence, S. M. Sebti, M. Zajac-Kaye and S. N. Hochwald: Disruption of the protein interaction between FAK and IGF-1R inhibits melanoma tumor growth. *Cell Cycle*, 11, 3250-3259 (2012)

136. D. A. Ucar, A. T. Magis, D. H. He, N. J. Lawrence, S. M. Sebti, E. Kurenova, M. Zajac-Kaye, J. Zhang and S. N. Hochwald: Inhibiting the interaction of cMET and IGF-1R with FAK effectively reduces growth of pancreatic cancer cells *in vitro* and *in vivo*. *Anticancer Agents Med Chem*, 13, 595-602 (2013)

137. V. Golubovskaya, N. L. Palma, M. Zheng, B. Ho, A. Magis, D. Ostrov and W. G. Cance: A Small-Molecule Inhibitor, 5'-O-Tritylthymidine, Targets FAK And Mdm-2 Interaction, And Blocks Breast And Colon Tumorigenesis *In vivo*. *Anticancer Agents Med Chem* 13, 532-545 (2013)

138. V. M. Golubovskaya, B. Ho, M. Zheng, A. Magis, D. Ostrov, C. Morrison and W. G. Cance: Disruption of focal adhesion kinase and p53 interaction with small molecule compound R2 reactivated p53 and blocked tumor growth. *BMC Cancer*, 13, 342-346 (2013)

139. V. Golubovskaya, L. Beviglia, L. H. Xu, H. S. Earp, 3rd, R. Craven and W. Cance: Dual inhibition of focal adhesion kinase and epidermal growth factor receptor pathways cooperatively induces death receptor-mediated apoptosis in human breast cancer cells. *J Biol Chem*, 277, 38978-38987 (2002)

140. V. M. Golubovskaya, S. Gross, A. S. Kaur, R. I. Wilson, L. H. Xu, X. H. Yang and W. G. Cance: Simultaneous inhibition of focal adhesion kinase and SRC enhances detachment and apoptosis in colon cancer cell lines. *Mol Cancer Res*, 1, 755-764 (2003)

141. C. M. Bagi, J. Christensen, D. P. Cohen, W. G. Roberts, D. Wilkie, T. Swanson, T. Tuthill and C. J. Andresen: Sunitinib and PF-562,271 (FAK/Pyk2 inhibitor) effectively block growth and recovery of human hepatocellular carcinoma in a rat xenograft model. *Cancer Biol Ther*, 8, 856-865 (2009)

142. V. Golubovskaya, G. Huang, B. Ho, M. Yemma, C. D. Morrison, J. Lee, B. Eliceiri and W. G. Cance: Pharmacological blockade of FAK autophosphorylation decreases human glioblastoma tumor growth and synergizes with temozolomide. *Mol Cancer Ther* 12, 162-172 (2013)

143. V. Bolos, J. M. Gasent, S. Lopez-Tarruella and E. Grande: The dual kinase complex FAK-Src as a promising therapeutic target in cancer. *Onco Targets Ther*, 3, 83-97 (2010)

144. Z. Xing, H. C. Chen, J. K. Nowlen, S. J. Taylor, D. Shalloway and J. L. Guan: Direct interaction of v-Src with the focal adhesion kinase mediated by the Src SH2 domain. *Mol Biol Cell*, 5, 413-421 (1994)

145. H. C. Chen and J. L. Guan: Association of focal adhesion kinase with its potential substrate phosphatidylinositol 3-kinase. *Proc Natl Acad Sci U S A*, 91, 10148-52 (1994)

146. D. D. Schlaepfer, S. K. Hanks, T. Hunter and P. van der Geer: Integrin-mediated signal transduction linked to Ras pathway by GRB2 binding to focal adhesion kinase. *Nature*, 372, 786-791 (1994)

147. S. Avraham, R. London, Y. Fu, S. Ota, D. Hiregowdara, J. Li, S. Jiang, L. M. Pasztor, R. A. White, J. E. Groopman and *et al.*: Identification and characterization of a novel related adhesion focal tyrosine kinase (RAFTK) from megakaryocytes and brain. *J Biol Chem*, 270, 27742-27751 (1995)

148. S. Lev, H. Moreno, R. Martinez, P. Canoll, E. Peles, J. M. Musacchio, G. D. Plowman, B. Rudy and J. Schlessinger: Protein tyrosine kinase PYK2 involved in Ca(2+)-induced regulation of ion channel and MAP kinase functions. *Nature*, 376(6543), 737-745 (1995)

149. H. Sasaki, K. Nagura, M. Ishino, H. Tobioka, K. Kotani and T. Sasaki: Cloning and characterization of cell adhesion kinase beta, a novel protein-tyrosine kinase of the focal adhesion kinase subfamily. *J Biol Chem*, 270, 21206-21219 (1995)

150. J. D. Hildebrand, M. D. Schaller and J. T. Parsons: Paxillin, a tyrosine phosphorylated focal adhesion-associated protein binds to the carboxyl terminal domain of focal adhesion kinase. *Molecular Biology of the Cell*, 6, 637-647 (1995)

151. M. B. Calalb, T. R. Polte and S. K. Hanks: Tyrosine phosphorylation of focal adhesion kinase at sites in the catalytic domain regulates kinase activity: a role for Src family kinases. *Molecular & Cellular Biology*, 15, 954-963 (1995)

152. M. T. Harte, J. D. Hildebrand, M. R. Burnham, A. H. Bouton and J. T. Parsons: p130cas, a substrate associated with v-Src and v-Crk, localizes to focal adhesions and binds to focal adhesion kinase. *Journal of Biological Chemistry*, 271, 13649-13655 (1996)

153. A. Richardson, R. K. Malik, J. D. Hildebrand and J. T. Parsons: Inhibition of cell spreading by expression of the C-terminal domain of focal adhesion kinase (FAK) is rescued by coexpression of Src or catalytically inactive FAK: a role for paxillin tyrosine phosphorylation. *Mol Cell Biol*, 17, 6906-6914 (1997)

154. J. M. Taylor, J. D. Hildebrand, C. P. Mack, M. E. Cox and J. T. Parsons: Characterization of graf, the GTPase-activating protein for rho associated with focal adhesion kinase. Phosphorylation and possible regulation by mitogen-activated protein kinase. *J Biol Chem*, 273, 8063-8070 (1998)

155. J. Zhao, R. Pestell and J. L. Guan: Transcriptional activation of cyclin D1 promoter by FAK contributes to cell cycle progression. *Mol Biol Cell*, 12, 4066-4077 (2001)

Abbreviations: FAK, Focal Adhesion Kinase; MiR, micro RNA; FRNK, FAK related non kinase. FAT, focal adhesion targeting domain; GRAF, GAP for Rho associated with Grb-2, Growth factor receptor-bound protein 2; IGFR, insulin growth factor receptor; Mdm-2, Mouse double minute 2 homolog; NF kappa B, nuclear factor kappa B; Pyk-2, CAK-beta, RAFTK, non receptor tyrosine kinase homologue of FAK; Src, non receptor tyrosine kinase Src; VEGFR, vascular endothelial growth factor receptor. APC, adenomatous polyposis coli; DLL, Delta-like ligand; Dsh, disheveled protein; GSK 3β, glycogen synthase kinase-3 beta; JAG, Jagged jigand; RP5/6, low density lipoprotein receptor-related protein 5/6; mTOR, mammalian target of rapamycin; TCF/LEF, T-cell factor/lymphoid enhancer factor. ERK, extracellular signal related kinase; IKK, I kappa B kinase; IkBα, I kappa B alpha; NF-kappa B, nuclear factor kappa B; MAPK, mitogen activated protein kinase; TNF alpha, tumor necrosis factor alpha; TNFR, tumor necrosis factor receptor; VEGF, vascular endothelial growth factor.

Key Words: Focal Adhesion Kinase, Cancer, Metastasis, Small molecule inhibitors, Angiogenesis, Survival, Review

Send correspondence to: Vita M. Golubovskaya, Department of Surgical Oncology, Roswell Park Cancer Institute, Buffalo, NY, Tel: 716-845-3920, Fax: 716-845-3944 E-mail: Vita.Golubovskaya@roswellpark.org