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Review

Endogenous control mechanisms of FAK and PYK2 and their relevance to cancer development and therapy

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Abstract:

Focal adhesion kinase (FAK) and its close paralogue, proline-rich tyrosine kinase 2 (PYK2), are key regulators of aggressive spreading and metastasis of cancer cells. While targeted small-molecule inhibitors of FAK and PYK2 are showing promising antitumor activity, their clinical long-term efficacy may be undermined by the strong capacity of cancer cells to evade anti-kinase drugs. In healthy cells, the expression and/or function of FAK and PYK2 is tightly controlled through modulation of gene expression, competing alternatively spliced forms, non-coding RNAs, and proteins that directly or indirectly affect kinase activation or protein stability. The molecular factors involved are frequently deregulated in cancer cells. Here, we review the endogenous mechanisms controlling FAK and PYK2, and discuss how these mechanisms could inspire or improve anticancer therapies.

Keywords: dimerization; miRNA; motility; anoikis; chaperon; PTEN; FIP200; LKB1; PI3K; regulation

1. Introduction

Focal Adhesion Kinase (FAK) is a multi-domain non-receptor protein tyrosine kinase (PTK) found in metazoan, but also the unicellular eukaryote *Capsaspora owczarzaki* [1,2]. FAK is a multifunctional protein that integrates and transduces signals perceived through integrin or growth factor receptors into adapted cytoplasmic and nuclear responses. This mechanism allows FAK to functionally link adhesion, migration and survival of cells [3,4]. In particular, its ability to override apoptosis following cell detachment (anoikis) makes FAK an essential factor in embryogenesis and wound healing. While present in low levels in most adult tissues, FAK is overexpressed in most types of cancer in which its capacity to override anoikis and to drive cell motility promotes aggressive invasiveness and metastasis of cancer cells [5–8]. The observation that tumor-specific overexpression of FAK is linked to tumor invasiveness has made FAK a promising target for small-molecule inhibition [7,9]. Initial proof-of-principle studies have highlighted that inhibition of FAK not only reduces tumor spreading, but it also makes cancer cells more susceptible to chemotherapy [10,11]. However, most available inhibitors lack either specificity or efficacy, and their precise molecular mechanism remains in many cases poorly defined [10]. Moreover, proline-rich tyrosine kinase 2 (PYK2), a close paralogue to FAK in vertebrates [12], can functionally compensate for loss of FAK in some settings and may thus override inhibition of FAK [13,14]. FAK and PYK2 have different tissue distributions. Whereas FAK is widely expressed, expression of PYK2 is more restricted with major occurrences and roles in the brain, osteoclasts, macrophages and lymphocytes [15]. Both can have different, or even opposing, cellular roles; for example, FAK activation promotes cell cycle

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progression and survival, whereas PYK2 generally inhibits such events [5,16–21]. Hence, (tissue-) specific inhibition of FAK or PYK2 might be required for an inhibitor to achieve desired therapeutic effects.

In this review, we summarize current understanding of the endogenous mechanisms used by cells to contain aberrant FAK and PYK2 activity. Thorough understanding of these mechanisms might lead to the design of new or improved therapeutic interventions that could block aggressive spreading of cancer cells. The multitude of cellular functions and locations of FAK and PYK2 have been comprehensively described in recent reviews [22–25]. We thus only briefly outline the general molecular mechanism for FAK and PYK2 activation. We focus our review on how this mechanism is controlled by cellular factors, including alternatively spliced forms, interacting proteins and non-coding RNAs. We also discuss if and how the current knowledge of endogenous control mechanisms of FAK and/or PYK2 could assist the development anti-cancer strategies.

1.1 Mechanistic bases for activation of FAK and PYK2

PYK2 evolved from FAK through gene duplication and subsequent specialization in vertebrates [12]. In humans, both proteins share 46% sequence identity (65% similarity) and have the same three-domain organization [25–27]: an N-terminal band 4.1, ezrin, radixin, moesin (FERM) domain, a central catalytic kinase domain and a C-terminal focal adhesion targeting (FAT) domain (reviewed in detail by [4,23,25]. The domains are connected by long linkers that also contain important ligand binding sites (**Figure 1**) [25,28,29]. Owing to the common origin and high sequence similarity of FAK and PYK2, the basic principles for their (in)activation likely remain conserved.

Both proteins harbor binding sites for many ligands (more than 50 have been described for FAK [23]) and nuclear localization and export signals [30,31], allowing them to function as molecular scaffolds in various locations in the cell. Enzymatic kinase activity is required only for a subset of actions. In FAK, the FERM domain can directly bind to the kinase domain and inhibit its function [28]. Key residues for the FERM:kinase interaction are conserved between FAK and PYK2 (in particular FAK F596/PYK2 F599; [23]), suggesting that this FERM:kinase association also exists in PYK2. Experimental evidence for this association in PYK2 is however lacking [32–34]. In both proteins, kinase activity is generally initiated by autophosphorylation of a tyrosine situated in the FERM-kinase linker (FAK Y397 and PYK2 Y402). Together with a proline-rich region (PR1) situated upstream in the same linker, the phosphotyrosine (pY) forms a high-affinity binding site that recruits and activates Src kinases *via* a dual interaction with their SH2 and SH3 domains [25]. The bound and activated Src kinase is responsible for much of the kinase activity associated with FAK or PYK2. Importantly, autophosphorylation can only proceed *in trans*, and it requires FAK/PYK2 dimerization and/or clustering. In FAK, dimers are formed through FERM:FERM and FERM:FAT interactions (**Figure 2**). In PYK2, FERM domains form crystallographic dimers that are identical to the FAK FERM:FERM domains (PDB 4EKU), suggesting that the FERM:FERM mechanism might be conserved. Self-association of FAK and PYK2 is controlled by ligands. In FAK, trans-autophosphorylation, and hence kinase-dependent functions, occur mostly at focal adhesions to which FAK is recruited, enriched and primed for dimerization *via* interactions between its FAT domain and the leucine-aspartic acid (LD) motifs of paxillin [35]. Additionally, clustering of FAK is promoted through interactions between the FERM domain and membrane-associated phospholipids [36]. Although the FAT domain in PYK2 can bind to paxillin [37], PYK2 is not as strongly localized to focal adhesions as FAK is in most cell types. Conceptually similar recruitment/clustering mechanisms appear to lead to activation of FAK and PYK2 at other transmembrane receptor complexes, such as T-cell immune synapses [38,39], or to activation of PYK2 in post-synaptic densities [40]. In addition, FAK and PYK2 play roles in various other cellular locations, including endosomes, adherens junctions, the microtubule organizing center and the nucleus [3,26]. Some of these functions (e.g. at nascent adhesions or in the nucleus) are kinase-independent.

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A major difference between FAK and PYK2 is that the latter can be activated by calcium ions (Ca^{2+}). The exact molecular mechanism for activation of PYK2 by Ca^{2+} remains unclear and controversial. The current view is that Ca^{2+} does not bind directly to PYK2, but binds through calmodulin (which has been reported to bind to either the FERM or kinase domain of PYK2) or through Ca^{2+}-activated PYK2-modifying kinases (PKC, CaMKII, PKA), phosphatases (calcineurin, PP1) or Ca^{2+}-activated ligands (PSD-95) [23,41].

In summary, FAK and PYK2 are versatile protein scaffolds with kinase-dependent and kinase-independent functions. Although the details may differ, the underlying mechanism for the control and activation of kinase activity appears to be preserved between FAK and PYK2. Activation of the kinase function requires trans-autophosphorylation. Cellular ligands can trigger trans-autophosphorylation by stabilizing a weak intrinsic propensity for self-association of FAK or PYK2 and/or by increasing the local protein concentration. Consequently, endogenous inhibitory mechanisms can prevent kinase activation by lowering FAK and/or PYK2 protein concentrations, by reinforcing inactivating autoinhibitory interactions, by competing with activating ligands, by dephosphorylating the Src-recruiting tyrosine or by displacing FAK/PYK2 from their sites of activation (**Figure 1**).

2. FRNK and PRNK

Both the FAK and PYK2 genes (*PTK2* and *PTK2B*, respectively) can produce shorter protein forms that lack the FERM and kinase domains [42,43]. These autonomously expressed non-cataytic C-terminal portions presumably originate from alternative transcription initiation sites [44] and are called the FAK-related non-kinase (FRNK) and the PYK2-related non-kinase (PRNK). Starting at residues 693 and 780 of the canonical FAK and PYK2 isoforms, respectively, they include ligand binding sites (proline-rich sites PR2 and PR3 for FRNK and PR3 for PRNK), sites of phosphorylation (most prominently Y861 and S910 in FAK, Y849 and S866 in PYK2) and the FAT domain (**Figure 1**). PR2 and PR3 mediate the binding of different SH3-containing proteins, such as p130cas or Graf [43,45–49]. The FAT domain is sufficient to localize to focal adhesions, through interactions mainly with paxillin, but also with cofactors or alternative ligands, such as talin or Rgnef/p190RhoGef [50–52]. Alternative interactions can also localize FAT to other structures, such as the T-cell receptor complex [1,38,53], or the growth cone of developing axons [54]. FRNK and PRNK maintain these functions, although without any regulatory influence from the other FAK/PYK2 regions. The production of these truncated forms was therefore suggested to lead to a direct competition of FRNK or PRNK with FAK and/or PYK2 at sites that attract their FAT domains. Indeed, immunostaining showed that FRNK and PRNK localize to focal adhesions [42,43]. By competitively displacing FAK or PYK2, the alternative spliced variants lower the local concentrations of FAK and PYK2 and hence lower the propensity for trans-autophosphorylation of FAK and, possibly, PYK2. Accordingly, western-blot analysis indicates a lowering of FAK Y397 phosphorylation, a key step in FAK activation, at increasing FRNK levels [55,56]. Moreover, by displacing FAK or PYK2 and by forming incomplete signalling complexes, FRNK and PRNK promote the disassembly of focal adhesions. Indeed, the expression of FRNK and PRNK correlates with focal adhesion turnover [42,43,57].

In addition to displacing FAK from common binding sites, FRNK may also act by directly binding to FAK. In rat aortic smooth muscle cells, FRNK co-immunoprecipitates with FAK [56]. This association is decreased by phosphorylation of FRNK S217 by extracellular signal-regulated kinase (ERK) and increased five-fold by the S217A mutation. In cells, autophosphorylation of FAK was reduced by expression of FRNK, and even more so by expression of the S217A mutant form of FRNK [56]. The molecular basis for this phosphorylation-dependent interaction is unknown, but it might involve competition of FRNK with the intramolecular FERM:FAT interaction that promotes FAK for autophosphorylation by reinforcing FAK dimers. In size exclusion chromatography, dimerization of recombinant FAK was indeed weakened by the presence of recombinant FAT domains [35]. FRNK S217 corresponds to Erk-phosphorylated S910 in the canonical FAK isoform. This serine is situated five residues upstream of the four-helix FAT domain. The S910PPP motif weakly interacts with the

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FAT domain [58] and, when phosphorylated on S910, constitutes a proline-isomerase binding site [56,59]. Phopshorylation of FRNK S217 could therefore affect the FRNK:FAK association in various direct or indirect ways.

The possibility that a similar mechanism of competitive binding also exists for PRNK and PYK2 has not been tested. If this mechanism does exist, then this association would be regulated differently, because the PYK2 serine 866, that is also phosphorylated and located just N-terminal of its FAT domain, is not situated in an Erk phosphorylation motif [60].

3. Proteases

FAK and PYK2 are cleaved by several proteases. Some of the resulting fragments resemble FRNK or PRNK.

3.1. Caspases

Cysteine aspartate-specific proteases (caspases) play an important role in programed cell death (apoptosis). During apoptosis, caspases cleave critical repair and structural proteins that bolster cell survival. It has been shown that FAK is cleaved in the early stages of myc-induced apoptosis [61–63]. Sensitivity profiling of protease inhibitors showed that FAK is cleaved by caspase-3, 6, 7 and 8, with caspase-7 by far the most active [64,65]. Caspase-3, 7 and 8 generated 85 kDa and 33 kDa fragments [64,65] .Caspase-6 cleaved FAK with low efficiency, generating a 77 kDa fragment [65]. Generated cleavage products and cleavage site consensus suggested that the 85-kDa cleavage happened after D772 and that the 77 kDa cleavage occurred after D704 (**Figure 1**) [65]. Accordingly, the D772A mutation was not cleavable by caspase-3 [65]. It is expected that the separation from the FAT domain weakens the attachment of the FERM-kinase domain fragment to focal adhesions (and other FAT-targeted sites), leading to turnover of these structures. The 35 kDa fragment generated from FAK cleavage resembles FRNK and may act similarly. Indeed, western-blot analysis indicated a decrease in FAK Y397 phosphorylation levels in HeLa cells transfected with the 35 kDa fragment [65]. Direct evidence for caspase cleavage of PYK2 is currently lacking.

3.2. Calpain

Calpain, a calcium-dependent cysteine protease, cleaves both FAK and PYK2 [66,67]. When either purified calpain I or calpain II were added to FAK, four fragments were generated with sizes of 90 kDa, 50 kDa, 40 kDa, and 35 kDa, and the cleavage was blocked by the calpain inhibitor, calpastatin [67]. Antibodies against the FAK kinase domain reacted with the 90-kDa and 50-kDa bands while antibodies against the C-terminus of FAK bound to the 35-kDa band [67]. An 80 kDa and a 75 kDa PYK2 cleavage products were observed in human platelets, and when treated with the calpain inhibitor calpeptin, the bands were no longer present [68].

4. Regulation through post-translational modifications

In addition to irreversible protease cleavage, FAK and PYK2 are subjected to several types of post-translational modifications. FAK, but not PYK2, has been reported to be acetylated on residue A2 [60] and SUMOylated on K152 in the FERM domain [69]. SUMOylation was mediated by the SUMO ligase protein inhibitor of activated STAT1 (PIAS1) that interacted with FAK in cells and *in vitro*. SUMOylation was increased in suspended cells and correlated with increased nuclear presence of FAK and increased FAK Y397 phosphorylation independently of cell adhesion [69]. The mechanistic bases for this effect remain to be determined, but since K157 is in the vicinity of the FERM:kinase interaction site [28], its SUMOylation might interfere with the 'closed' autoinhibitory FAK conformation.

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The major post-translational modification however is (de)phosphorylation [70]. According to the UniProt database, FAK and PYK2 are phosphorylated on serines (8 on FAK; 6 on PYK2), threonines (FAK: 2; PYK2: 2) and tyrosines (FAK: 8; PYK2: 8). Regulation through phosphorylation was previously reviewed in detail [25,60,71,72]. Here, we present a very broad overview. Tyrosine phosphorylation is generally associated with activating characteristics, starting of course with the autophosphorylation of the FERM-kinase linker tyrosine (Y397 and Y402 in FAK and PYK2, respectively). Src-mediated phosphorylation of a tyrosine in the first FAT helix (FAK-Y925 and PYK2-Y881) triggers activation of mitogen-activated protein (MAP) kinases *via* the phosphotyrosine-binding adapter protein Grb2 [73–76]. In FAK, phosphorylation of S910 by Erk recruits the proline-isomerase PIN1 and subsequently the protein tyrosine phosphatase (PTP-PEST), which then counteracts FAK phosphorylation and activation, thus closing a negative feedback loop [59]. In PYK2, a nuclear export signal in its kinase-FAT linker is activated by phosphorylation of S778 by protein kinase A (PKA) and deactivated by the phosphatase calcineurin [31]. Conversely, PKC-mediated phosphorylation of FAK on S722 promoted nuclear localization [77]. (De)phosphorylation therefore regulates nuclear localization of PYK2 and FAK, although based on different mechanisms. Phosphorylation can also alter the protein structure, as seen for the case of the FAK activation loop tyrosines. Their phosphorylation not only increases the kinase catalytic activity by restructuring the activation loop, but it also prevents the kinase domain from forming an inhibitory interaction with the FERM domain [28].

While most kinases or phosphatases probably form only transient or short-lived complexes with FAK and/or PYK2, the tyrosine-kinases Src and Fyn durably attach to the Y397-phosphorylated FERM-kinase linker. They are associated with a kinase-active state [25,71,78–80]. In the following, we highlight one other kinase and one phosphatase that also have lasting associations with FAK or PYK2 and show inhibitory effects.

4.1. Liver kinase β1 (LKB1)

The liver kinase β1 (LKB1) or STK11 is a multifunctional serine/threonine kinase that links energy sensing and cell polarity [81–83]. In particular, LKB1 stabilizes cell polarity under energy stress. Given that loss of cell polarity increases undirectional 'exploratory' migration, LKB1 controls cell movements during embryogenesis and wound healing [81–83]. LKB1 also counteracts cancer cell metastasis and is the third most frequently mutated gene in lung adenocarcinoma [84–86]. Rather than through phosphosylation of its activation loop, LKB1 (**Figure 3**) is activated allosterically through an association with the pseudo-kinase STRADα, which is tethered to LKB1 by the scaffolding protein MO25α [87]. In addition, LKB1 activity and localization are affected by post-translational modification [81,83]. SUMOlyation of LKB1 K178, in response to energy stress, promotes the interaction between LKB1 and the AMP-activated protein kinase (AMPK). The resulting phosphorylation and activation of AMPK help to maintain the energy balance in a cell during energy stress [88]. Furthermore, live cell imaging experiments show that farnesylation on C430 within the LKB1 C-terminus is required for its co-localization with actin at the leading edges of migrating cells. The LKB1 kinase activity is required to stabilize the actin colocalization of LKB1 and to promote mesenchymal polarization and directed cell migration [82,83].

Initially, LKB1 was linked to FAK through the observation that the FAK adhesion pathway is upregulated in LKB1-/- mice in a mutant Kirsten rat sarcoma (KRAS) background [89]. Subsequent analyses confirmed that the presence and activation of LKB1 represses phosphorylation of FAK Y397, Y861 and Y925 [82,90]. The current model proposes that farnesylation colocalizes LKB1 with actin at the leading cell edge of migratory cells, where the LKB1 kinase activity indirectly decreases FAK activation. Inactivation of FAK decreases focal-adhesion turnover and hence stabilizes focal adhesions at the leading edge. Conversely, loss of LKB1 farnesylation or kinase activity results in FAK hyperphosphorylation and leads to cells that present a more exploratory behaviour with loss of directional persistance [81,82].

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The exact mechanisms by which LKB1 negatively affects FAK activation are unclear. FAK and LKB1 can co-immunoprecipitate each other, suggesting that they colocalize within the same protein complex. However, the FAK:LKB1 interaction may not be direct, as indicated by commercially available purified FAK and LKB1/MO25α/STRADα proteins not binding each other *in vitro* [82]. A possibility would be that the effect of LKB1 is mediated by its downstream substrates. Activation of AMPK or its family members (e.g., MARK1 and MARK4) correlates with inhibition of FAK phosphorylation in several systems, including human liver cancer cell lines and muscle cells [90–93]. The details of these inhibitory pathways through AMPK or MARKs remain incompletely established. Intriguingly, one study could recapitulate the FAK pY397-suppressing effect of LKB1 by expressing just the LKB1 N-terminal arm region (residues 1-47) in LKB1-null H157 cells, suggesting that, at least in this setting, LKB1 kinase activity was not needed [82]. The possiblity of an interaction between PYK2 and LKB1 has not yet been explored, although PYK2 activation has been observed following AMPK activation in muscle cells [94,95].

4.2. PTEN

The phosphatase and tensin homolog deleted on chromosome 10 (PTEN) (**Figure 3**) exerts a dual function as both a lipid and a protein phosphatase [96,97]]. As a lipid phosphatase, PTEN converts phosphoinositide(3,4,5)P3 to phosphoinositide(4,5)P2, and is therefore the antagonist of the phosphoinositide-3 kinase (PI3K). PI3K directly associates with FAK through its PR motifs [98] promoting cell survival via the activation of the PI3K/Akt pathway. This association has an important effect on maintaining cell adhesion to integrins, which is, in some tissues like endothelial cells, indispensible for cell survival [99].

PTEN affects cell migration and invasiveness by dampening this FAK/PI3K pathway. The most commonly observed effect of PTEN on FAK might be indirect through its lipid-phosphatase-based downregulation of the PI3K pathway [100]. As an additional indirect mechanism, PTEN was shown to inhibit FAK expression levels in myeloma and gastric cancers [101,102]. However, PTEN can also form a complex with Y397-phosphorylated FAK in glioma, breast cancer and colon carcinoma cells [103,104]. This interaction leads to dephosphoryation of pY397 following cell detachment in these cells. The FAK:PTEN association was compatible with the FAK:paxillin association, but it competed with binding of PI3K and Src to FAK [103,104]. Both the p85 subunit of the PI3K and the SH2-SH3 fragment of Src kinase bind to the Y397-phosphorylated FERM-linker fragment [25,71,78,79,105,106], suggesting that PTEN binding involves the same region. Moreover, it has been observed that FAK phosphorylates PTEN at Y336 with a positive effect on PTEN stability and phosphatase activity [107,108]. Y336 is located in the phospholipid-binding region of the C2 domain (**Figure 3**). In principle, the introduction of a negative charge in this position is expected to hamper association with negatively charged lipid headgroups. Future research is needed to clarify the underlying molecular mechanisms causing the multiple effects of the FAK:PTEN association.

5. Regulation through non-catalytic protein interactions

FAK or PYK2 also have a large number of interaction partners that do not post-translationally modify them. Many of these partners either control subcellular localization, or attach to FAK or PYK2 to form active signalling complexes [25,71,106]. So far, to our knowledge, only one non-catalytic protein has been identified as an inhibitor of FAK and PYK2 activation, namely FIP200.

5.1. RB1CC1 / FIP200

In vitro and *in vivo* protein interaction assays led to the discovery of a 200 kDa ligand for both FAK and PYK2, named the FAK family kinase-interacting protein (FIP200) [109]. The 1,594 residue FIP200 is predicted to contain an N-terminal ubiquitin-like domain (ULD) but would otherwise be mostly composed of loops and long helices, possibly forming coiled-coiled structural elements [109,110]

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(**Figure 3**). Accordingly, FIP200, also known as the RB1-inducible coiled-coil protein 1 (RB1CC1), is a non-catalytic scaffolding protein. It regulates a wide range of cellular events, such as growth, proliferation, apoptosis and autophagy, through interactions with signaling proteins (e.g. PYK2, FAK, ActA, p53, TSC1, ASK1, TRAF2 and Stathmin) [111].

Immunofluorescence analyses of FIP200 indicated mostly a diffuse localization in the cytoplasm [109]. But the presence of a consensus nuclear localization signal implies an alternative function for FIP200 in the nucleus, where it may act as a transcription factor [109,110,112,113]. Additionally, FIP200 polyclonal antibody pull-downs revelaled a partial colocalization of the protein in focal contacts where it may regulate FAK and vinculin [114]. FIP200 binding decreases the kinase activity of FAK and PYK2 [109,114] and the autophosphorylation levels of FAK [114]. During cell adhesion to fibronectin, FAK activation correlates with increased dissociation of FAK and FIP200 [111,114].

Given that FIP200 inhibits both FAK and PYK2, which can have opposing functions, for example in the promotion of the cell cycle and apoptosis, the outcome of their inhibition by FIP200 may be highly cell-type specific. For example, overexpression of FIP200 in glioblastoma cells (where PYK2 has dominant functions) leads to inhibition of apoptosis [115], whereas in breast cancer cells, FIP200 acts as a tumor suppressor gene [113].

Although the direct interaction with FIP200 appears to inhibit both FAK and PYK2 in a similar manner, the way in which FIP200 physically binds them might differ (**Figure 3**). In one study, the FIP C-terminal (CT-FIP; residues 1374-1591) interacts strongly with the PYK2 kinase domain. However, CT-FIP only binds to the N-terminal region of FAK, while the FAK kinase domain (403-672aa) binds to the FIP200 N-terminus (NT-FIP; 1-638aa) and the middle domain (MD-FIP; 639-1373) [114]. Conversely, NT-FIP and MD-FIP did not show affinity for PYK2. The apparent differences might however only be caused by a change in relative affinity of the fragments of FIP200 for FAK or PYK2. For example, although CT-FIP did not display significant binding to the FAK kinase, it could reduce its enzymatic activity when present at high concentrations in *in vitro* kinase assays [114,115]. The differences in binding affinity might have important functional implications in cases where FAK and PYK2 are present, but these functions might be different. For example, in retinoblastoma cells, the interaction of CT-FIP with PYK2 is stronger, and hence preferred, than the interaction of the other two domains of FIP200 with FAK [109,114,115].

6. Chaperones

Although the focus of this review is on endogenous inhibitory mechanisms, we also consider chaperones that enhance the activation of FAK. The housekeeping function of chaperones as protein stabilizers is deregulated in many cancers, and hence therapeutically lowering their action to normal levels may produce tumor-inhibiting effects [116].

Molecular chaperones can interact with exposed hydrophobic patches of misfolded proteins and help them (re)establish their native and functional three-dimensional structure. While chaperones commonly release refolded clients, some remain associated with structurally fragile proteins to sustain their function. Thus, chaperones maintain cellular proteins in their functional state and prevent their degradation [117,118]. Overexpression of chaperones, in particular of inducible heat shock proteins (HSPs), is prevalent in cancer cells where proteotoxic stress is endemic [116,119]. The resulting increase in protein stablity and function may enhance the action of overexpressed and/or mutated oncogenes and more generally help creating a protective and resistant cytoplasmic environment [120]. Inversely, a decrease in the expression of molecular chaperones is associated with accumulation of misfolded proteins and the onset of neurodegenerative diseases [120].

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In a systematic survey of the kinome, FAK and PYK2 were identified as 'weak clients' for HSP90 and its kinase-specific cochaperone, CDC37 [118]. Xiong et al. (2014) reported an interaction between HSP90β and FAK in breast cancer cells. FAK bound to the HSP90β middle domain (residues 233-620), in agreement with a structural analysis of the complex formed by HSP90, CDC37 and a client cyclin-dependent kinase (CDK4) [121]. The HSP90β interaction protects FAK from ubiquitylation-dependent proteasomal degradation. Inhibition of both HSP90β and FAK reduces tumor growth in breast cancer cells [122].

Chaperones can also have more indirect effects on FAK activity. Caino et al. showed that inhibition of mitochondrial HSP90 chaperones, including the tumor necrosis factor receptor-associated protein-1 (TRAP-1), leads to an energy stress-triggered activation of the LKB1-AMPK pathway [123]. AMPK phosphorylates and activates the Unc-51 like autophagy activating kinase (ULK1), which in turn phosphorylates FIP200. These events activate the autophagy-initiating complex between ULK1, FIP200 and autophagy related protein 13 (atg13), and they allow activated FIP200 to maintain FAK in an inactive unphosphorylated state [123]. Increased expression and function of mitochondrial HSP90, as observed in some cancers, lead to sufficient ATP production in nutrient-scarce tumor cells to avoid triggering activation of the LKB1-AMPK pathway. This releases the inhibitory effect of FIP200 on FAK, results in increased cytoskeletal dynamics and enhances tumor cell invasion to bone or liver in mice disease models [123].

Additionally, heat shock proteins HSP70 and Mamalian relative DNA J (MRJ, also named DNAJB6) exert an oncogenic effect on colon cancer cells. HSP70 and MRJ interact with the urokinase-type plasminogen activator receptor (uPAR) to activate FAK, c-Src, H-Ras, AKT and MAPK cell signaling pathways, regulating cell adhesion and migration [124]. Another example is the molecular chaperone Cosmc. Cosmc is necessary for the formation of the active T-synthase, which catalyses the production of T antigen. Increased expression of T antigen is correlated with tumor metastasis and poor prognosis in colorectal cancer. The forced expression of Cosmc in colon cancer cell lines increases T antigen expression resulting in increased activation of FAK, PI3K/Akt and MAPK kinase (MEK)/ERK signaling pathways [125]. Cosmc-induced tumor cell migration and invasion suggest that Cosmc may serve as a potential target for colorectal cancer. The eaxct mechanisms that lead to FAK Y397 phopshorylation through either HSP70/MRJ or Cosmc under these conditions have not yet been determined. Given that in both cases FAK activation involves the PI3K and MAPK pathways, the molecular routes may be overlapping or converging.

7. Transcriptional regulation of FAK and PYK2

The normal or alternative intronic promotors of the *PTK2* and *PTK2B* genes are controlled in a tissue and developmental stage-dependent manner [44]. Factors that activate the *PTK2* promotor include the nuclear factor-κB (NF-κB), argonaute 2 (AGO2), PEA3 and NANOG [12,126–128]. Overactivity of these *PTK2* transcription factors is associated with tumorigenesis, and their silencing can block tumorigenesis and metastasis [10]. The major reppressor of the human *PTK2* and *PTK2B* promotors is the stress-induced tumor suppressor p53 [129–131]. Two putative binding sites for p53 have been indentified in the *PTK2* promoter [132]. In turn, nuclear FAK promotes degradation of p53 by linking it to the E3 ubiquitin ligase Mdm2, using the FERM domain as a scaffold. FERM-triggered proteosomal degradation of p53 leads to cell proliferation and survival under cellular stress [133]. A strong correlation between FAK overexpression and p53 mutations has been observed in human breast cancer [134]. While loss or damage of p53 is closely associated with the development of tumors, forced overexpression can cause premature aging [135]. However, therapeutic restoration of a functional p53 pool at endogenous levels can have tumor-suppressive effects [136].

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8. non-coding RNA

Non-coding RNA (ncRNA) are RNA molecules that are not translated into proteins [137]. ncRNAs include transfer RNA (tRNA), ribosomal RNA (rRNA) and small RNAs. ncRNAs are further classified according to their length and function, and they include long non-coding RNA (lncRNA), micro-RNA (miRNA), small-interfering RNA (siRNA) and piwi-interacting RNA (piRNA). Small RNAs can regulate gene expression at the transcriptional and post-transcriptional level, but they can also affect protein function through direct binding and scaffolding. Many ncRNAs are abnormally expressed in cancer, leading to a large-scale deregulation of protein genes, most prominently the tumor suppressor p53 [138]. Here, we focus on those ncRNAs that directly alter expression and function of FAK or PYK2; we note that many other ncRNAs have pleiotropic effects that may also affect FAK or PYK2.

8.1. microRNA

miRNAs contain about 20 nucleotides and are transcribed from miRNA genes or coding gene introns. miRNAs post-transcriptionally downregulate target genes by either destabilizing and degrading their mRNA or by binding to the 3'-untranslated region (3'UTR) of their mRNA, thus inhibiting translation [139,140]. miRNAs are implicated in various cellular processes such as development, cell proliferation, and differentiation. They also regulate junction protein gene expression and ECM processes, thus helping to maintain the integrity of the cellular structure [141,142]. By inhibiting gene translation, many miRNAs act as tumor suppressors and their downregulation in cancer cells generally promotes cancer formation and metastasis [143]. Conversely, forced upregulation of miRNA can have tumor-suppressive effects, making miRNAs interesting therapeutic targets and prognostic markers for anti-cancer therapy.

8.1.1. Tumor-suppressor miRNAs acting directly on FAK

miR-7. One of the most studied microRNAs regulating FAK is miRNA-7 (miR-7). miR-7 is encoded by three DNA loci (9q21, 15q26 and 19q13) producing the same mature 23-nucleotide miR-7 sequence. miR-7 is enriched in various normal tissues and implicated in organ development and other biological processes (reviewed by [144]). miR-7 can directly bind to 3'UTR of FAK mRNA, repressing FAK protein expression [145]. miR-7 is also involved in the growth, migration, and invasion of many cancer types. miR-7 downregulates glioblastoma and colon cancer cell invasiveness by repressing FAK protein expression [145,146]. Forced expression of miR-7 significantly reduced endogenous FAK protein expression in breast cancer cell lines, inhibited primary breast tumor growth and invasiveness, and repressed metastatic migration of breast cancer xenografts [147]. In non-small-cell lung carcinoma (NSCLC) cells, miR-7 suppresses cell proliferation, migration and invasion by downregulating FAK expression and inhibiting the activation of the ERK/MAPK signaling pathway [148]. The inverse relation between miR-7 expression and tumor growth and invasiveness suggests that miR-7 can be used as a diagnostic marker for certain cancers [144]. miR-7 also positively affects radiation therapy outcomes as it increases cancer cell radiosensitivity [149], phenocopying an effect observed for FAK-targeting inhibitors [150–152].

miR-1298. miR-1298 was identified in a global miRNA functional screen as selectively lethal to cells expressing mutated KRAS, which is an oncogene mutated in 20% of human cancers, mainly NSCLC and colorectal cancers. miR-1298 acts a tumor suppressor in KRAS-driven NSCLC and colorectal cancer cells by directly targeting the mRNA of both FAK and the Laminin subunit beta 3 (LAMB3), lowering their protein expression levels [153]. Interestingly, only expression of LAMB3, but not FAK, was upregulated by KRAS; yet silencing of either FAK or LAMB3 recapitulated miR-1298-induced cell-death in KRAS-dependent cancer cells. However, FAK Y925 phosphorylation

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also links FAK activation via Grb2 to activation of the RAS pathway [154], and FAK signaling is a requirement for the maintenance of KRAS-dependent adenocarcinomas [155].

miR-543. miR-543 expression is decreased in tumorous endometrium tissue and associated with impaired cancer cell invasion *in vitro*. miR-543 binds to the 3'UTR of FAK and of the TWIST1 oncogene, decreasing their expression at both the mRNA and protein level. This repression resulted in impaired tumor cell proliferation, migration and invasion in cancer cell lines [156].

miR-379-5p. Underexpression of miR-379-5p in hepatocellular carcinoma (HCC) correlated with the tumor node-metastasis (TNM) stage and metastasis. miR-397-5p inhibits tumor migration and invasion in HCC cells by direct binding to the 3'UTR of FAK inhibiting FAK translation while the mRNA level is not affected. This inhibition suppresses AKT activation and hence inhibits the PI3K/AKT pathway. Introduction of miR-397-5p represses HCC metastasis and EMT *in vivo* [157].

miR-193b. A recent study showed that miR-193b is downregulated in liposarcoma while its reintroduction induces apoptosis in liposarcoma cells. miR-193b functions by directly targeting FAK mRNA regulating the oncogenic FAK/SRC/BCAR1 signaling pathway [158].

8.1.2 miRNAs indirectly regulating FAK.

Rather than directly suppressing FAK expression by binding to FAK mRNA, miRNAs can indirectly affect FAK targeting by activating upstream effectors. For instance, miR-205 and miR-940 negatively regulate Src kinase expression. miR-205 is suppressed in renal and prostate cancer. Its inhibition increases cell adhesion in normal keratinocytes, whereas its overexpression reduces tumor growth *in vivo* [159–161]. miR-205 binds to Src 3'UTR, degrading its mRNA, decreasing Src protein expression and hence activating FAK and paxillin [159–161]. The exosomal miR-940 also inhibits the expression of Src at the mRNA and protein levels in ovarian cancer, thus decreasing the activation of downstream proteins such as FAK, paxillin and Akt [162].

8.1.3. Oncogenic miRNAs acting on FAK

Although most FAK-targeting miRNAs act as tumor suppressors by lowering FAK expression and/or activation, some miRNAs show oncogenic effects by targeting cellular inhibitors of FAK [163,164]. The miR-130 family (miR-130b, miR-301a and miR-301b) is one example that is upregulated in bladder cancer tissues. The miR-130 family activates FAK and the Akt signaling pathway by targeting PTEN either directly through binding to its mRNA (miR-130b) or indirectly by repressing PTEN expression (miR-301a/b). High expression of miR-130 increased migration and invasion of bladder cancer cells [163] and the progression of malignant melanoma [164].

Several other miRNAs have been found to influence the activation of FAK by repressing or activating an upstream effector of FAK. Due to space limitations, these miRNAs are listed in Table 1 but not elaborated in the text.

8.1.4. miRNA regulators of PYK2

Not many miRNAs are known to regulate the expression of PYK2. Howerver, PYK2 is a direct target of miRNA-23b, which binds to its 515-522 3'UTR. Overexpression of miR-23b decreases the protein level of PYK2, but not of FAK, and results in reduced cell proliferation, migration and invasion of hepatocellular carcinoma and glioblastoma cells. Additionally, elevated expression of miR-517a and miR-517c resulted in decreased PYK2 expression, suggesting that these miRNAs play a tumor suppressor role in hepatocellular carcinoma [165].

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8.2. lnRNAs

NcRNAs with more than 200 nucleotides, commonly called lncRNAs, are implicated in many biological and pathological processes [166]. The oncogenic lncRNA metastasis-associated lung adenocarcinoma transcript 1 (MALAT1) promotes vasculogenic mimicry and angiogenesis by increasing the expression of VE-cadherin and b-catenin on cell membranes, as well as by increasing levels of metalloproteases MMP2/9 and MT1-MMP and of phosphorylated ERK, FAK, and paxillin. These events promote gastric cancer tumor growth and metastasis [167]. LncRNA H19 is a competing endogenous RNA (ceRNA) for miR-138. Given that miR-138 targets FAK and suppresses its expression, the action of lncRNA H19 increases FAK expression and osteogenesis of bone marrow mesenchymal stem cells under tension [168]. LncRNA can also act by directly binding to proteins. Upregulation of the lncRNA CASC9 in esophageal squamous cell carcinoma (ESCC) was correlated with metastasis and poor prognosis. CASC9 upregulates the laminin subunit LAMC2 by recruiting the transcriptional coactivator CREB-binding protein (CBP) to the LAMC2 promotor. LAMC2 upregulation is associated with phospohrylation of FAK and activation of the PI3K/AKT pathway [169]. Phenotypically, CASC9 therefore produces opposing effects to those of miR-1298 (see above).

9. Discussion

By linking cell motility and survival, FAK and PYK2 promote cancer cell survival and invasiveness. In particular FAK, but also to some extend PYK2, have been recognized as anti-cancer targets, and first small-molecule inhibitors are currently in clinical trials. However, drug efficacy and specificity remains low in several cases, and their exact mechanism of action is not always well understood [10,170]. Moreover, the application of anti-cancer drugs designed to selectively inhibit single kinases can trigger large-scale signal network rewiring, leading to bypass mechanisms that render the drug ineffective [171,172].

Stringent endogenous control of kinase activity is essential for normal cellular function and homeostasis. Evoking naturally evolved endogenous control mechanisms for therapeutic purposes may trigger fewer escape manoeuvers in diseased cells than experienced by targeted kinase inhibitors. Endogenous mechanisms that (negatively) regulate the activity of FAK and PYK2 in normal or diseased cells might inspire improved or additional therapeutic interventions (**Figure 2**).

FAK and PYK2 have numerous kinase-dependent and kinase-independent biological roles (**Figure 4**), which can vary with cellular status, cell type and subcellular location. Among these roles, FAK and PYK2 can have opposing, redundant or synergistic effects, depending on the cell type and condition. Accordingly, cells use different general or specifically adapted mechanisms to control FAK and/or PYK2. The general mechanisms involve chaperones, proteases, and kinases/phosphatases (such as the Src, LKB1 or PTEN). Therapeutic strategies that target these regulatory mechanisms will likely provoke pleiotropic effects, which might not always be counterproductive in diseased cells, but would generally be difficult to predict and may adversely affect healthy tissue.

Inhibitors specifically targeting the p53 or Mdm2 binding site on the FERM domain of nuclear FAK are also promising in treating cancers without deleterious mutations of p53, because a first inhibitor of the putative p53 binding site on FAK FERM showed tumor-suppressive effects by dampening FAK-induced p53 degradation [173]. However, the structural basis for these interactions needs to be clarified to allow using them as templates for the rational design of inhibitory small-molecule mimicks.

An alternative strategy is to target the self-association mechanisms of FAK and PYK2 needed for their trans-autophosphorylation. Such allosteric protein-protein interaction inhibitors could directly manipulate the molecular control mechanisms of FAK or PYK2, without the risk of severe off-target

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side effects commonly associated with inhibitors of kinase domains. For FAK, such protein-protein interaction inhibitors could target the FERM or FAT domains, which are required for trans-autophosphorylation, or could stabilize the inhibitory FERM:kinase association [28,35,174]. Indeed, compounds have been developed that target these mechanisms, including molecules that block access to Y397 or inhibit interactions of the FAT or FERM domains [173,175–179]. Although these compounds lacked efficacy and required micromolar concentrations, they displayed tumor-suppressive activity in xenograft mouse models, and enhanced the effect of conventional chemotherapy. However, their specificity and mode of action remain poorly understood [175].

Another promising approach to utilizing endogenous mechanisms for controlling the spread and metastasis of cancer cells may be based on ncRNAs. Their mode of action through direct binding to mRNA is simple and sufficiently well understood, and detailed descriptions of their tumor-suppressive effects are rapidly accumulating. Therapeutic miRNAs or siRNAs are already emerging as next-generation biopharmaceuticals that can rapidly move from the bench to the clinic, with about 20 different molecules currently in clinical trials. Although none of these 20 ncRNAs are targeting FAK or PYK2, most of them are used to treat cancers, and two are specifically silencing kinases (SYK and PKN3) [180,181]. These proofs of concept for ncRNA-based therapeutics combined with the accruing evidence for tumor-suppressive effects of miRNA-mediated inhibition of FAK and/or PYK2 provide a strong incentive for the development of FAK- and/or PYK2-specific anti-cancer mi/siRNA therapies. Moreover, the biological capacities of the almost 20,000 potentially functional human lncRNAs are only beginning to emerge [182]. In particular, their capacity to act as ceRNA or to directly bind to proteins (see, for example [183]) might provide interesting regulatory effects with therapeutic potential. Although to date no lncRNA has been discovered that directly binds to FAK or PYK2, RNA aptamers can potently and selectively inhibit kinases through positioning of an adenine nucleotide into the ATP-binding pocket [184], and more than 40 kinases were identified as non-classical RNA binding proteins in a screen of the *Arabidopsis thaliana* proteome [185]. Efficient and specific *in vivo* delivery of ncRNAs faces many challenges, including poor tissue penetration, rapid degradation, immunotoxicity, reliance on cellular factors required for miRNA function and possible off-target effects [186]. Nonetheless, advances in tissue delivery methods and methods for ncRNA bioengineering and recombinant production (e.g., [187]), indicate that ncRNAs are promising nature-inspired agents that can enrich our toolkit of selective therapeutic inhibitors of FAK and PYK2.

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References

1. Alam, T.; Alazmi, M.; Naser, R.; Huser, F.; Momin, A. A.; Walkiewicz, K. W.; Canlas, C. G.; Huser, R. G.; Ali, A.; Merzaban, J.; Bajic, V. B.; Gao, X.; Arold, S. T. Proteome-level assessment of origin, prevalence and function of Leucine-Aspartic Acid (LD) motifs. *bioRxiv* **2018**, 278903, doi:10.1101/278903.
2. Sebé-Pedrós, A.; Roger, A. J.; Lang, F. B.; King, N.; Ruiz-Trillo, I. Ancient origin of the integrin-mediated adhesion and signaling machinery. *Proc. Natl. Acad. Sci.* **2010**, *107*, 10142–10147, doi:10.1073/pnas.1002257107.
3. Kleinschmidt, E. G.; Schlaepfer, D. D. Focal adhesion kinase signaling in unexpected places. *Curr. Opin. Cell Biol.* **2017**, *45*, 24–30, doi:10.1016/j.ceb.2017.01.003.
4. Carvalho, K.; Khalifat, N.; Maniti, O.; Nicolas, C.; Arold, S.; Picart, C.; Ramos, L. Phosphatidylinositol 4,5-Bisphosphate-Induced Conformational Change of Ezrin and Formation of Ezrin Oligomers. *Biochemistry (Mosc.)* **2010**, *49*, 9318–9327, doi:10.1021/bi101141d.
5. Xiong, W.; Parsons, J. T. Induction of Apoptosis after Expression of PYK2, a Tyrosine Kinase Structurally Related to Focal Adhesion Kinase. *J. Cell Biol.* **1997**, *139*, 529–539, doi:10.1083/jcb.139.2.529.
6. Lee, B. Y.; Timpson, P.; Horvath, L. G.; Daly, R. J. FAK signaling in human cancer as a target for therapeutics. *Pharmacol. Ther.* **2015**, *146*, 132–149, doi:10.1016/j.pharmthera.2014.10.001.
7. Yoon, H.; Dehart, J. P.; Murphy, J. M.; Lim, S.-T. S. Understanding the roles of FAK in cancer: inhibitors, genetic models, and new insights. *J. Histochem. Cytochem. Off. J. Histochem. Soc.* **2015**, *63*, 114–128, doi:10.1369/0022155414561498.
8. Tai, Y.-L.; Chen, L.-C.; Shen, T.-L. Emerging roles of focal adhesion kinase in cancer. *BioMed Res. Int.* **2015**, *2015*, 690690, doi:10.1155/2015/690690.
9. Golubovskaya, V. M.; Palma, N. L.; Zheng, M.; Ho, B.; Magis, A.; Ostrov, D.; Cance, W. G. 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.* **2013**, *13*, 532–545.
10. Sulzmaier, F. J.; Jean, C.; Schlaepfer, D. D. FAK in cancer: mechanistic findings and clinical applications. *Nat. Rev. Cancer* **2014**, *14*, 598–610, doi:10.1038/nrc3792.
11. Golubovskaya, V. M. Targeting FAK in human cancer: from finding to first clinical trials. *Front. Biosci. Landmark Ed.* **2014**, *19*, 687–706.
12. Corsi, J.-M.; Rouer, E.; Girault, J.-A.; Enslen, H. Organization and post-transcriptional processing of focal adhesion kinase gene. *BMC Genomics* **2006**, *7*, 198, doi:10.1186/1471-2164-7-198.
13. Sieg, D. J.; Ilić, D.; Jones, K. C.; Damsky, C. H.; Hunter, T.; Schlaepfer, D. D. Pyk2 and Src-family protein-tyrosine kinases compensate for the loss of FAK in fibronectin-stimulated signaling events but Pyk2 does not fully function to enhance FAK- cell migration. *EMBO J.* **1998**, *17*, 5933–5947, doi:10.1093/emboj/17.20.5933.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 8 May 2018 doi:10.20944/preprints201805.0125.v1

Peer-reviewed version available at Cancers 2018, 10, 196; doi:10.3390/cancers10060196

14. Weis, S. M.; Lim, S.-T.; Lutu-Fuga, K. M.; Barnes, L. A.; Chen, X. L.; Göthert, J. R.; Shen, T.-L.; Guan, J.-L.; Schlaepfer, D. D.; Cheresh, D. A. Compensatory role for Pyk2 during angiogenesis in adult mice lacking endothelial cell FAK. *J. Cell Biol.* **2008**, *181*, 43–50, doi:10.1083/jcb.200710038.

15. Beinke, S.; Phee, H.; Clingan, J. M.; Schlessinger, J.; Matloubian, M.; Weiss, A. Proline-rich tyrosine kinase-2 is critical for CD8 T-cell short-lived effector fate. *Proc. Natl. Acad. Sci.* **2010**, *107*, 16234–16239, doi:10.1073/pnas.1011556107.

16. Chauhan, D.; Hideshima, T.; Pandey, P.; Treon, S.; Teoh, G.; Raje, N.; Rosen, S.; Krett, N.; Husson, H.; Avraham, S.; Kharbanda, S.; Anderson, K. C. RAFTK/PYK2-dependent and -independent apoptosis in multiple myeloma cells. *Oncogene* **1999**, *18*, 6733–6740, doi:10.1038/sj.onc.1203082.

17. Zhao, J.; Zheng, C.; Guan, J. Pyk2 and FAK differentially regulate progression of the cell cycle. *J Cell Sci* **2000**, *113*, 3063–3072.

18. Zhao, J.; Pestell, R.; Guan, J.-L.; Solomon, M. J. Transcriptional Activation of Cyclin D1 Promoter by FAK Contributes to Cell Cycle Progression. *Mol. Biol. Cell* **2001**, *12*, 4066–4077, doi:10.1091/mbc.12.12.4066.

19. Zhao, J.; Bian, Z. C.; Yee, K.; Chen, B. P. C.; Chien, S.; Guan, J.-L. Identification of Transcription Factor KLF8 as a Downstream Target of Focal Adhesion Kinase in Its Regulation of Cyclin D1 and Cell Cycle Progression. *Mol. Cell* **2003**, *11*, 1503–1515, doi:10.1016/S1097-2765(03)00179-5.

20. Kuwabara, K.; Nakaoka, T.; Sato, K.; Nishishita, T.; Sasaki, T.; Yamashita, N. Differential Regulation of Cell Migration and Proliferation through Proline-Rich Tyrosine Kinase 2 in Endothelial Cells. *Endocrinology* **2004**, *145*, 3324–3330, doi:10.1210/en.2003-1433.

21. Ding, Q.; Grammer, J. R.; Nelson, M. A.; Guan, J.-L.; Stewart, J. E.; Gladson, C. L. p27Kip1 and Cyclin D1 Are Necessary for Focal Adhesion Kinase Regulation of Cell Cycle Progression in Glioblastoma Cells Propagated in Vitro and in Vivo in the Scid Mouse Brain. *J. Biol. Chem.* **2005**, *280*, 6802–6815, doi:10.1074/jbc.M409180200.

22. Schaller, M. D. Cellular functions of FAK kinases: insight into molecular mechanisms and novel functions. *J. Cell Sci.* **2010**, *123*, 1007–1013, doi:10.1242/jcs.045112.

23. Walkiewicz, K. W.; Girault, J.-A.; Arold, S. T. How to awaken your nanomachines: Site-specific activation of focal adhesion kinases through ligand interactions. *Prog. Biophys. Mol. Biol.* **2015**, *119*, 60–71, doi:10.1016/j.pbiomolbio.2015.06.001.

24. Hall, J. E.; Fu, W.; Schaller, M. D. Focal adhesion kinase: exploring Fak structure to gain insight into function. *Int. Rev. Cell Mol. Biol.* **2011**, *288*, 185–225, doi:10.1016/B978-0-12-386041-5.00005-4.

25. Hall, J. E.; Fu, W.; Schaller, M. D. Chapter Five - Focal Adhesion Kinase: Exploring FAK Structure to Gain Insight into Function. In *International Review of Cell and Molecular Biology*; Jeon, K. W., Ed.; Academic Press, 2011; Vol. 288, pp. 185–225.

26. Schaller, M. D. Cellular functions of FAK kinases: insight into molecular mechanisms and novel functions. *J Cell Sci* **2010**, *123*, 1007–1013, doi:10.1242/jcs.045112.

27. Lev, S.; Moreno, H.; Martinez, R.; Canoll, P.; Peles, E.; Musacchio, J. M.; Plowman, G. D.; Rudy, B.; Schlessinger, J. Protein tyrosine kinase PYK2 involved in Ca2+-induced regulation of ion channel and MAP kinase functions. *Nature* **1995**, *376*, 737–745, doi:10.1038/376737a0.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 8 May 2018 doi:10.20944/preprints201805.0125.v1

Peer-reviewed version available at Cancers 2018, 10, 196; doi:10.3390/cancers10060196

28. Lietha, D.; Cai, X.; Ceccarelli, D. F. J.; Li, Y.; Schaller, M. D.; Eck, M. J. Structural basis for the autoinhibition of Focal Adhesion Kinase. *Cell* **2007**, *129*, 1177–1187, doi:10.1016/j.cell.2007.05.041.

29. Arold, S. T.; Hoellerer, M. K.; Noble, M. E. M. The Structural Basis of Localization and Signaling by the Focal Adhesion Targeting Domain. *Structure* **2002**, *10*, 319–327, doi:10.1016/S0969-2126(02)00717-7.

30. Ossovskaya, V.; Lim, S.-T.; Ota, N.; Schlaepfer, D.; Ilic, D. FAK nuclear export signal sequences. *FEBS Lett.* **2008**, *582*, 2402–2406, doi:10.1016/j.febslet.2008.06.004.

31. Faure, C.; Ramos, M.; Girault, J.-A. Pyk2 cytonuclear localization: mechanisms and regulation by serine dephosphorylation. *Cell. Mol. Life Sci. CMLS* **2013**, *70*, 137–152, doi:10.1007/s00018-012-1075-5.

32. Dunty, J. M.; Gabarra-Niecko, V.; King, M. L.; Ceccarelli, D. F. J.; Eck, M. J.; Schaller, M. D. FERM Domain Interaction Promotes FAK Signaling. *Mol. Cell. Biol.* **2004**, *24*, 5353–5368, doi:10.1128/MCB.24.12.5353-5368.2004.

33. Lipinski, C. A.; Tran, N. L.; Dooley, A.; Pang, Y.-P.; Rohl, C.; Kloss, J.; Yang, Z.; McDonough, W.; Craig, D.; Berens, M. E.; Loftus, J. C. Critical role of the FERM domain in Pyk2 stimulated glioma cell migration. *Biochem. Biophys. Res. Commun.* **2006**, *349*, 939–947, doi:10.1016/j.bbrc.2006.08.134.

34. Riggs, D.; Yang, Z.; Kloss, J.; Loftus, J. C. The Pyk2 FERM regulates Pyk2 complex formation and phosphorylation. *Cell. Signal.* **2011**, *23*, 288–296, doi:10.1016/j.cellsig.2010.09.015.

35. Brami-Cherrier, K.; Gervasi, N.; Arsenieva, D.; Walkiewicz, K.; Boutterin, M.-C.; Ortega, A.; Leonard, P. G.; Seantier, B.; Gasmi, L.; Bouceba, T.; Kadaré, G.; Girault, J.-A.; Arold, S. T. FAK dimerization controls its kinase-dependent functions at focal adhesions. *EMBO J.* **2014**, *33*, 356–370, doi:10.1002/embj.201386399.

36. Goñi, G. M.; Epifano, C.; Boskovic, J.; Camacho-Artacho, M.; Zhou, J.; Bronowska, A.; Martín, M. T.; Eck, M. J.; Kremer, L.; Gräter, F.; Gervasio, F. L.; Perez-Moreno, M.; Lietha, D. Phosphatidylinositol 4,5-bisphosphate triggers activation of focal adhesion kinase by inducing clustering and conformational changes. *Proc. Natl. Acad. Sci. U. S. A.* **2014**, *111*, E3177-3186, doi:10.1073/pnas.1317022111.

37. Lulo, J.; Yuzawa, S.; Schlessinger, J. Crystal structures of free and ligand-bound focal adhesion targeting domain of Pyk2. *Biochem. Biophys. Res. Commun.* **2009**, *383*, 347–352, doi:10.1016/j.bbrc.2009.04.011.

38. Garron, M.-L.; Arthos, J.; Guichou, J.-F.; McNally, J.; Cicala, C.; Arold, S. T. Structural Basis for the Interaction between Focal Adhesion Kinase and CD4. *J. Mol. Biol.* **2008**, *375*, 1320–1328, doi:10.1016/j.jmb.2007.11.040.

39. Chapman, N. M.; Houtman, J. C. D. Functions of the FAK family kinases in T cells: beyond actin cytoskeletal rearrangement. *Immunol. Res.* **2014**, *59*, 23–34, doi:10.1007/s12026-014-8527-y.

40. Bartos, J. A.; Ulrich, J. D.; Li, H.; Beazely, M. A.; Chen, Y.; MacDonald, J. F.; Hell, J. W. Postsynaptic Clustering and Activation of Pyk2 by PSD-95. *J. Neurosci.* **2010**, *30*, 449–463, doi:10.1523/JNEUROSCI.4992-08.2010.

41. Schaller, M. D. Calcium-dependent Pyk2 activation: a role for calmodulin? *Biochem. J.* **2008**, *410*, e3-4, doi:10.1042/BJ20080133.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 8 May 2018 doi:10.20944/preprints201805.0125.v1

Peer-reviewed version available at Cancers 2018, 10, 196; doi:10.3390/cancers10060196

42. Schaller, M. D.; Borgman, C. A.; Parsons, J. T. Autonomous expression of a noncatalytic domain of the focal adhesion-associated protein tyrosine kinase pp125FAK. *Mol. Cell. Biol.* **1993**, *13*, 785–791, doi:10.1128/MCB.13.2.785.
43. Xiong, W. C.; Macklem, M.; Parsons, J. T. Expression and characterization of splice variants of PYK2, a focal adhesion kinase-related protein. *J. Cell Sci.* **1998**, *111*, 1981–1991.
44. Nolan, K.; Lacoste, J.; Parsons, J. T. Regulated expression of focal adhesion kinase-related nonkinase, the autonomously expressed C-terminal domain of focal adhesion kinase. *Mol. Cell. Biol.* **1999**, *19*, 6120–6129.
45. Harte, M. T.; Hildebrand, J. D.; Burnham, M. R.; Bouton, A. H.; Parsons, J. T. p130Cas, a Substrate Associated with v-Src and v-Crk, Localizes to Focal Adhesions and Binds to Focal Adhesion Kinase. *J. Biol. Chem.* **1996**, *271*, 13649–13655, doi:10.1074/jbc.271.23.13649.
46. Polte, T. R.; Hanks, S. K. Interaction between focal adhesion kinase and Crk-associated tyrosine kinase substrate p130Cas. *Proc. Natl. Acad. Sci.* **1995**, *92*, 10678–10682.
47. Dikic, I.; Tokiwa, G.; Lev, S.; Courtneidge, S. A.; Schlessinger, J. A role for Pyk2 and Src in linking G-protein-coupled receptors with MAP kinase activation. *Nature* **1996**, *383*, 547–550, doi:10.1038/383547a0.
48. Avraham, S.; London, R.; Fu, Y.; Ota, S.; Hiregowdara, D.; Li, J.; Jiang, S.; Pasztor, L. M.; White, R. A.; Groopman, J. E.; Avraham, H. Identification and Characterization of a Novel Related Adhesion Focal Tyrosine Kinase (RAFTK) from Megakaryocytes and Brain. *J. Biol. Chem.* **1995**, *270*, 27742–27751, doi:10.1074/jbc.270.46.27742.
49. Sasaki, H.; Nagura, K.; Ishino, M.; Tobioka, H.; Kotani, K.; Sasaki, T. Cloning and Characterization of Cell Adhesion Kinase β, a Novel Protein-tyrosine Kinase of the Focal Adhesion Kinase Subfamily. *J. Biol. Chem.* **1995**, *270*, 21206–21219, doi:10.1074/jbc.270.36.21206.
50. Bellis, S. L.; Miller, J. T.; Turner, C. E. Characterization of Tyrosine Phosphorylation of Paxillin in Vitro by Focal Adhesion Kinase. *J. Biol. Chem.* **1995**, *270*, 17437–17441, doi:10.1074/jbc.270.29.17437.
51. Chen, H.-C.; Appeddu, P. A.; Parsons, J. T.; Hildebrand, J. D.; Schaller, M. D.; Guan, J.-L. Interaction of Focal Adhesion Kinase with Cytoskeletal Protein Talin. *J. Biol. Chem.* **1995**, *270*, 16995–16999, doi:10.1074/jbc.270.28.16995.
52. Zhai, J.; Lin, H.; Nie, Z.; Wu, J.; Cañete-Soler, R.; Schlaepfer, W. W.; Schlaepfer, D. D. Direct interaction of focal adhesion kinase with p190RhoGEF. *J. Biol. Chem.* **2003**, *278*, 24865–24873, doi:10.1074/jbc.M302381200.
53. Alam, T.; Alazmi, M.; Gao, X.; Arold, S. T. How to find a leucine in a haystack? Structure, ligand recognition and regulation of leucine–aspartic acid (LD) motifs. *Biochem. J.* **2014**, *460*, 317–329, doi:10.1042/BJ20140298.
54. Xu, S.; Liu, Y.; Li, X.; Liu, Y.; Meijers, R.; Zhang, Y.; Wang, J. The binding of DCC-P3 motif and FAK-FAT domain mediates the initial step of netrin-1/DCC signaling for axon attraction. *Cell Discov.* **2018**, *4*, doi:10.1038/s41421-017-0008-8.
55. Koshman, Y. E.; Kim, T.; Chu, M.; Engman, S. J.; Iyengar, R.; Robia, S. L.; Samarel, A. M. FRNK inhibition of focal adhesion kinase-dependent signaling and migration in vascular smooth muscle cells. *Arterioscler. Thromb. Vasc. Biol.* **2010**, *30*, 2226–2233, doi:10.1161/ATVBAHA.110.212761.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 8 May 2018 doi:10.20944/preprints201805.0125.v1

Peer-reviewed version available at Cancers 2018, 10, 196; doi:10.3390/cancers10060196

56. Zak, T. J.; Koshman, Y. E.; Samarel, A. M.; Robia, S. L. Regulation of Focal Adhesion Kinase through a Direct Interaction with an Endogenous Inhibitor. *Biochemistry (Mosc.)* **2017**, *56*, 4722–4731, doi:10.1021/acs.biochem.7b00616.

57. Richardson, A.; Parsons, J. T. A mechanism for regulation of the adhesion-associated protein tyrosine kinase pp125FAK. *Nature* **1996**, *380*, 538–540, doi:10.1038/380538a0.

58. Kadaré, G.; Gervasi, N.; Brami-Cherrier, K.; Blockus, H.; Messari, S. E.; Arold, S. T.; Girault, J.-A. Conformational Dynamics of the Focal Adhesion Targeting Domain Control Specific Functions of Focal Adhesion Kinase in Cells. *J. Biol. Chem.* **2015**, *290*, 478–491, doi:10.1074/jbc.M114.593632.

59. Zheng, Y.; Xia, Y.; Hawke, D.; Halle, M.; Tremblay, M. L.; Gao, X.; Zhou, X. Z.; Aldape, K.; Cobb, M. H.; Xie, K.; He, J.; Lu, Z. FAK phosphorylation by ERK primes ras-induced tyrosine dephosphorylation of FAK mediated by PIN1 and PTP-PEST. *Mol. Cell* **2009**, *35*, 11–25, doi:10.1016/j.molcel.2009.06.013.

60. Oppermann, F. S.; Gnad, F.; Olsen, J. V.; Hornberger, R.; Greff, Z.; Kéri, G.; Mann, M.; Daub, H. Large-scale Proteomics Analysis of the Human Kinome. *Mol. Cell. Proteomics* **2009**, *8*, 1751–1764, doi:10.1074/mcp.M800588-MCP200.

61. Crouch, D. H.; Fincham, V. J.; Frame, M. C. Targeted proteolysis of the focal adhesion kinase pp125 FAK during c-MYC-induced apoptosis is suppressed by integrin signalling. *Oncogene* **1996**, *12*, 2689–2696.

62. Nagata, S.; Golstein, P. The Fas death factor. *Science* **1995**, *267*, 1449–1456, doi:10.1126/science.7533326.

63. Pitti, R. M.; Marsters, S. A.; Ruppert, S.; Donahue, C. J.; Moore, A.; Ashkenazi, A. Induction of Apoptosis by Apo-2 Ligand, a New Member of the Tumor Necrosis Factor Cytokine Family. *J. Biol. Chem.* **1996**, *271*, 12687–12690, doi:10.1074/jbc.271.22.12687.

64. Gervais, F. G.; Thornberry, N. A.; Ruffolo, S. C.; Nicholson, D. W.; Roy, S. Caspases Cleave Focal Adhesion Kinase during Apoptosis to Generate a FRNK-like Polypeptide. *J. Biol. Chem.* **1998**, *273*, 17102–17108, doi:10.1074/jbc.273.27.17102.

65. Wen, L.-P.; Fahrni, J. A.; Troie, S.; Guan, J.-L.; Orth, K.; Rosen, G. D. Cleavage of Focal Adhesion Kinase by Caspases during Apoptosis. *J. Biol. Chem.* **1997**, *272*, 26056–26061, doi:10.1074/jbc.272.41.26056.

66. Marzia, M.; Chiusaroli, R.; Neff, L.; Kim, N.-Y.; Chishti, A. H.; Baron, R.; Horne, W. C. Calpain is required for normal osteoclast function and is down-regulated by calcitonin. *J. Biol. Chem.* **2006**, *281*, 9745–9754, doi:10.1074/jbc.M513516200.

67. Carragher, N. O.; Levkau, B.; Ross, R.; Raines, E. W. Degraded Collagen Fragments Promote Rapid Disassembly of Smooth Muscle Focal Adhesions That Correlates with Cleavage of Pp125FAK, Paxillin, and Talin. *J. Cell Biol.* **1999**, *147*, 619–630, doi:10.1083/jcb.147.3.619.

68. Raja, S.; Avraham, S.; Avraham, H. Tyrosine Phosphorylation of the Novel Protein-tyrosine Kinase RAFTK during an Early Phase of Platelet Activation by an Integrin Glycoprotein IIb-IIIa-independent Mechanism. *J. Biol. Chem.* **1997**, *272*, 10941–10947, doi:10.1074/jbc.272.16.10941.

69. Kadaré, G.; Toutant, M.; Formstecher, E.; Corvol, J.-C.; Carnaud, M.; Boutterin, M.-C.; Girault, J.-A. PIAS1-mediated Sumoylation of Focal Adhesion Kinase Activates Its Autophosphorylationn. *J. Biol. Chem.* **2003**, *278*, 47434–47440, doi:10.1074/jbc.M308562200.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 8 May 2018 doi:10.20944/preprints201805.0125.v1

Peer-reviewed version available at Cancers 2018, 10, 196; doi:10.3390/cancers10060196

70. Fonseca, C.; Voabil, P.; Carvalho, A. S.; Matthiesen, R. Tools for Protein Posttranslational Modifications Analysis: FAK, a Case Study. In *Mass Spectrometry Data Analysis in Proteomics*; Methods in Molecular Biology; Humana Press, Totowa, NJ, 2013; pp. 335–358 ISBN 978-1-62703-391-6.

71. Arold, S. T. How focal adhesion kinase achieves regulation by linking ligand binding, localization and action. *Curr. Opin. Struct. Biol.* **2011**, *21*, 808–813, doi:10.1016/j.sbi.2011.09.008.

72. Tomar, A.; Schlaepfer, D. D. A PAK-activated linker for EGFR and FAK. *Dev. Cell* **2010**, *18*, 170–172, doi:10.1016/j.devcel.2010.01.013.

73. Schlaepfer, D. D.; Hunter, T. Evidence for in vivo phosphorylation of the Grb2 SH2-domain binding site on focal adhesion kinase by Src-family protein-tyrosine kinases. *Mol. Cell. Biol.* **1996**, *16*, 5623–5633, doi:10.1128/MCB.16.10.5623.

74. Schlaepfer, D. D.; Jones, K. C.; Hunter, T. Multiple Grb2-mediated integrin-stimulated signaling pathways to ERK2/mitogen-activated protein kinase: summation of both c-Src- and focal adhesion kinase-initiated tyrosine phosphorylation events. *Mol. Cell. Biol.* **1998**, *18*, 2571–2585.

75. Shen, X.; Xi, G.; Radhakrishnan, Y.; Clemmons, D. R. Recruitment of Pyk2 to SHPS-1 signaling complex is required for IGF-I-dependent mitogenic signaling in vascular smooth muscle cells. *Cell. Mol. Life Sci.* **2010**, *67*, 3893–3903, doi:10.1007/s00018-010-0411-x.

76. Blaukat, A.; Ivankovic-Dikic, I.; Grönroos, E.; Dolfi, F.; Tokiwa, G.; Vuori, K.; Dikic, I. Adaptor Proteins Grb2 and Crk Couple Pyk2 with Activation of Specific Mitogen-activated Protein Kinase Cascades. *J. Biol. Chem.* **1999**, *274*, 14893–14901, doi:10.1074/jbc.274.21.14893.

77. Yi, X. P.; Zhou, J.; Huber, L.; Qu, J.; Wang, X.; Gerdes, A. M.; Li, F. Nuclear compartmentalization of FAK and FRNK in cardiac myocytes. *Am. J. Physiol.-Heart Circ. Physiol.* **2006**, *290*, H2509–H2515, doi:10.1152/ajpheart.00659.2005.

78. Schaller, M. D.; Hildebrand, J. D.; Shannon, J. D.; Fox, J. W.; Vines, R. R.; Parsons, J. T. Autophosphorylation of the focal adhesion kinase, pp125FAK, directs SH2-dependent binding of pp60src. *Mol. Cell. Biol.* **1994**, *14*, 1680–1688, doi:10.1128/MCB.14.3.1680.

79. Arold, S. T.; Ulmer, T. S.; Mulhern, T. D.; Werner, J. M.; Ladbury, J. E.; Campbell, I. D.; Noble, M. E. M. The Role of the Src Homology 3-Src Homology 2 Interface in the Regulation of Src Kinases. *J. Biol. Chem.* **2001**, *276*, 17199–17205, doi:10.1074/jbc.M011185200.

80. Mitra, S. K.; Schlaepfer, D. D. Integrin-regulated FAK–Src signaling in normal and cancer cells. *Curr. Opin. Cell Biol.* **2006**, *18*, 516–523, doi:10.1016/j.ceb.2006.08.011.

81. Wilkinson, S.; Hou, Y.; Zoine, J. T.; Saltz, J.; Zhang, C.; Chen, Z.; Cooper, L. a. D.; Marcus, A. I. Coordinated cell motility is regulated by a combination of LKB1 farnesylation and kinase activity. *Sci. Rep.* **2017**, *7*, 40929, doi:10.1038/srep40929.

82. Kline, E. R.; Shupe, J.; Gilbert-Ross, M.; Zhou, W.; Marcus, A. I. LKB1 Represses Focal Adhesion Kinase (FAK) Signaling via a FAK-LKB1 Complex to Regulate FAK Site Maturation and Directional Persistence. *J. Biol. Chem.* **2013**, *288*, 17663–17674, doi:10.1074/jbc.M112.444620.

83. Konen, J.; Wilkinson, S.; Lee, B.; Fu, H.; Zhou, W.; Jiang, Y.; Marcus, A. I. LKB1 kinase-dependent and -independent defects disrupt polarity and adhesion signaling to drive collagen remodeling during invasion. *Mol. Biol. Cell* **2016**, *27*, 1069–1084, doi:10.1091/mbc.E15-08-0569.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 8 May 2018 doi:10.20944/preprints201805.0125.v1

Peer-reviewed version available at Cancers 2018, 10, 196; doi:10.3390/cancers10060196

84. Ding, L.; Getz, G.; Wheeler, D. A.; Mardis, E. R.; McLellan, M. D.; Cibulskis, K.; Sougnez, C.; Greulich, H.; Muzny, D. M.; Morgan, M. B.; Fulton, L.; Fulton, R. S.; Zhang, Q.; Wendl, M. C.; Lawrence, M. S.; Larson, D. E.; Chen, K.; Dooling, D. J.; Sabo, A.; Hawes, A. C.; Shen, H.; Jhangiani, S. N.; Lewis, L. R.; Hall, O.; Zhu, Y.; Mathew, T.; Ren, Y.; Yao, J.; Scherer, S. E.; Clerc, K.; Metcalf, G. A.; Ng, B.; Milosavljevic, A.; Gonzalez-Garay, M. L.; Osborne, J. R.; Meyer, R.; Shi, X.; Tang, Y.; Koboldt, D. C.; Lin, L.; Abbott, R.; Miner, T. L.; Pohl, C.; Fewell, G.; Haipek, C.; Schmidt, H.; Dunford-Shore, B. H.; Kraja, A.; Crosby, S. D.; Sawyer, C. S.; Vickery, T.; Sander, S.; Robinson, J.; Winckler, W.; Baldwin, J.; Chirieac, L. R.; Dutt, A.; Fennell, T.; Hanna, M.; Johnson, B. E.; Onofrio, R. C.; Thomas, R. K.; Tonon, G.; Weir, B. A.; Zhao, X.; Ziaugra, L.; Zody, M. C.; Giordano, T.; Orringer, M. B.; Roth, J. A.; Spitz, M. R.; Wistuba, I. I.; Ozenberger, B.; Good, P. J.; Chang, A. C.; Beer, D. G.; Watson, M. A.; Ladanyi, M.; Broderick, S.; Yoshizawa, A.; Travis, W. D.; Pao, W.; Province, M. A.; Weinstock, G. M.; Varmus, H. E.; Gabriel, S. B.; Lander, E. S.; Gibbs, R. A.; Meyerson, M.; Wilson, R. K. Somatic mutations affect key pathways in lung adenocarcinoma. *Nature* **2008**, *455*, 1069–1075, doi:10.1038/nature07423.

85. Carretero, J.; Medina, P. P.; Pio, R.; Montuenga, L. M.; Sanchez-Cespedes, M. Novel and natural knockout lung cancer cell lines for the LKB1/STK11 tumor suppressor gene. *Oncogene* **2004**, *23*, 4037–4040, doi:10.1038/sj.onc.1207502.

86. Sanchez-Cespedes, M.; Parrella, P.; Esteller, M.; Nomoto, S.; Trink, B.; Engles, J. M.; Westra, W. H.; Herman, J. G.; Sidransky, D. Inactivation of LKB1/STK11 is a common event in adenocarcinomas of the lung. *Cancer Res.* **2002**, *62*, 3659–3662.

87. Zeqiraj, E.; Filippi, B. M.; Deak, M.; Alessi, D. R.; van Aalten, D. M. F. Structure of the LKB1-STRAD-MO25 complex reveals an allosteric mechanism of kinase activation. *Science* **2009**, *326*, 1707–1711, doi:10.1126/science.1178377.

88. Ritho, J.; Arold, S. T.; Yeh, E. T. H. A Critical SUMO1 Modification of LKB1 Regulates AMPK Activity during Energy Stress. *Cell Rep.* **2015**, *12*, 734–742, doi:10.1016/j.celrep.2015.07.002.

89. Carretero, J.; Shimamura, T.; Rikova, K.; Jackson, A. L.; Wilkerson, M. D.; Borgman, C. L.; Buttarazzi, M. S.; Sanofsky, B. A.; McNamara, K. L.; Brandstetter, K. A.; Walton, Z. E.; Gu, T.-L.; Silva, J. C.; Crosby, K.; Shapiro, G. I.; Maira, M.; Ji, H.; Castrillon, D. H.; Kim, C. F.; García-Echeverría, C.; Bardeesy, N.; Sharpless, N. E.; Hayes, N. D.; Kim, W. Y.; Engelman, J. A.; Wong, K.-K. Integrative genomic and proteomic analyses identify targets for Lkb1 deficient metastatic lung tumors. *Cancer Cell* **2010**, *17*, 547–559, doi:10.1016/j.ccr.2010.04.026.

90. Goodwin, J. M.; Svensson, R. U.; Lou, H. J.; Winslow, M. M.; Turk, B. E.; Shaw, R. J. An AMPK-independent signaling pathway downstream of the LKB1 tumor suppressor controls Snail1 and metastatic potential. *Mol. Cell* **2014**, *55*, 436–450, doi:10.1016/j.molcel.2014.06.021.

91. Chan, K.-C.; Lin, M.-C.; Huang, C.-N.; Chang, W.-C.; Wang, C.-J. Mulberry 1-Deoxynojirimycin Pleiotropically Inhibits Glucose-Stimulated Vascular Smooth Muscle Cell Migration by Activation of AMPK/RhoB and Down-regulation of FAK. *J. Agric. Food Chem.* **2013**, *61*, 9867–9875, doi:10.1021/jf403636z.

92. Suzuki, A.; Kusakai, G.; Kishimoto, A.; Minegichi, Y.; Ogura, T.; Esumi, H. Induction of cell-cell detachment during glucose starvation through F-actin conversion by SNARK, the fourth member of the AMP-activated protein kinase catalytic subunit family. *Biochem. Biophys. Res. Commun.* **2003**, *311*, 156–161.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 8 May 2018 doi:10.20944/preprints201805.0125.v1

Peer-reviewed version available at Cancers 2018, 10, 196; doi:10.3390/cancers10060196

93. Lassiter, D. G.; Nylén, C.; Sjögren, R. J. O.; Chibalin, A. V.; Wallberg-Henriksson, H.; Näslund, E.; Krook, A.; Zierath, J. R. FAK tyrosine phosphorylation is regulated by AMPK and controls metabolism in human skeletal muscle. *Diabetologia* **2018**, *61*, 424–432, doi:10.1007/s00125-017-4451-8.

94. Chen, H. C.; Bandyopadhyay, G.; Sajan, M. P.; Kanoh, Y.; Standaert, M.; Farese, R. V.; Farese, R. V. Activation of the ERK pathway and atypical protein kinase C isoforms in exercise- and aminoimidazole-4-carboxamide-1-beta-D-riboside (AICAR)-stimulated glucose transport. *J. Biol. Chem.* **2002**, *277*, 23554–23562, doi:10.1074/jbc.M201152200.

95. Kim, E.-K.; Park, J.-M.; Lim, S.; Choi, J. W.; Kim, H. S.; Seok, H.; Seo, J. K.; Oh, K.; Lee, D.-S.; Kim, K. T.; Ryu, S. H.; Suh, P.-G. Activation of AMP-activated protein kinase is essential for lysophosphatidic acid-induced cell migration in ovarian cancer cells. *J. Biol. Chem.* **2011**, *286*, 24036–24045, doi:10.1074/jbc.M110.209908.

96. Zhang, X. C.; Piccini, A.; Myers, M. P.; Van Aelst, L.; Tonks, N. K. Functional analysis of the protein phosphatase activity of PTEN. *Biochem. J.* **2012**, *444*, 457–464, doi:10.1042/BJ20120098.

97. Worby, C. A.; Dixon, J. E. PTEN. *Annu. Rev. Biochem.* **2014**, *83*, 641–669, doi:10.1146/annurev-biochem-082411-113907.

98. Zhang, J.; Gao, X.; Schmit, F.; Adelmant, G.; Eck, M. J.; Marto, J. A.; Zhao, J. J.; Roberts, T. M. CRKL Mediates p110β-Dependent PI3K Signaling in PTEN-Deficient Cancer Cells. *Cell Rep.* **2017**, *20*, 549–557, doi:10.1016/j.celrep.2017.06.054.

99. Lu, Q.; Rounds, S. Focal Adhesion Kinase and Endothelial Cell Apoptosis. *Microvasc. Res.* **2012**, *83*, 56–63, doi:10.1016/j.mvr.2011.05.003.

100. Haddadi, N.; Lin, Y.; Travis, G.; Simpson, A. M.; McGowan, E. M.; Nassif, N. T. PTEN/PTENP1: 'Regulating the regulator of RTK-dependent PI3K/Akt signalling', new targets for cancer therapy. *Mol. Cancer* **2018**, *17*, doi:10.1186/s12943-018-0803-3.

101. Zhang, L.-L.; Liu, J.; Lei, S.; Zhang, J.; Zhou, W.; Yu, H.-G. PTEN inhibits the invasion and metastasis of gastric cancer via downregulation of FAK expression. *Cell. Signal.* **2014**, *26*, 1011–1020, doi:10.1016/j.cellsig.2014.01.025.

102. Wang, S.-Y.; Hao, H.-L.; Deng, K.; Li, Y.; Cheng, Z.-Y.; Lv, C.; Liu, Z.-M.; Yang, J.; Pan, L. Expression levels of phosphatase and tensin homolog deleted on chromosome 10 (PTEN) and focal adhesion kinase in patients with multiple myeloma and their relationship to clinical stage and extramedullary infiltration. *Leuk. Lymphoma* **2012**, *53*, 1162–1168, doi:10.3109/10428194.2011.647311.

103. Tamura, M.; Gu, J.; Danen, E. H.; Takino, T.; Miyamoto, S.; Yamada, K. M. PTEN interactions with focal adhesion kinase and suppression of the extracellular matrix-dependent phosphatidylinositol 3-kinase/Akt cell survival pathway. *J. Biol. Chem.* **1999**, *274*, 20693–20703.

104. Haier, J.; Nicolson, G. L. PTEN regulates tumor cell adhesion of colon carcinoma cells under dynamic conditions of fluid flow. *Oncogene* **2002**, *21*, 1450–1460, doi:10.1038/sj.onc.1205213.

105. Chen, H. C.; Guan, J. L. Association of focal adhesion kinase with its potential substrate phosphatidylinositol 3-kinase. *Proc. Natl. Acad. Sci. U. S. A.* **1994**, *91*, 10148–10152.

106. Lim, S.-T.; Mikolon, D.; Stupack, D. G.; Schlaepfer, D. D. FERM control of FAK function: implications for cancer therapy. *Cell Cycle Georget. Tex* **2008**, *7*, 2306–2314, doi:10.4161/cc.6367.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 8 May 2018 doi:10.20944/preprints201805.0125.v1

Peer-reviewed version available at Cancers 2018, 10, 196; doi:10.3390/cancers10060196

884 107. Ross, A. H.; Gericke, A. Phosphorylation keeps PTEN phosphatase closed for business. *Proc.*
885 *Natl. Acad. Sci.* **2009**, *106*, 1297–1298, doi:10.1073/pnas.0812473106.
886 108. Tzenaki, N.; Aivaliotis, M.; Papakonstanti, E. A. Focal adhesion kinase phosphorylates the
887 phosphatase and tensin homolog deleted on chromosome 10 under the control of p110δ
888 phosphoinositide-3 kinase. *FASEB J.* **2015**, *29*, 4840–4852, doi:10.1096/fj.15-274589.
889 109. Ueda, H.; Abbi, S.; Zheng, C.; Guan, J. L. Suppression of Pyk2 kinase and cellular activities by
890 FIP200. *J. Cell Biol.* **2000**, *149*, 423–430.
891 110. Chano, T.; Ikegawa, S.; Saito-Ohara, F.; Inazawa, J.; Mabuchi, A.; Saeki, Y.; Okabe, H.
892 Isolation, characterization and mapping of the mouse and human RB1CC1 genes. *Gene* **2002**,
893 *291*, 29–34.
894 111. Gan, B.; Guan, J.-L. FIP200, a key signaling node to coordinately regulate various cellular
895 processes. *Cell. Signal.* **2008**, *20*, 787–794, doi:10.1016/j.cellsig.2007.10.021.
896 112. Chano, T.; Ikegawa, S.; Kontani, K.; Okabe, H.; Baldini, N.; Saeki, Y. Identification of RB1CC1,
897 a novel human gene that can induce RB1 in various human cells. *Oncogene* **2002**, *21*, 1295–
898 1298, doi:10.1038/sj.onc.1205171.
899 113. Chano, T.; Kontani, K.; Teramoto, K.; Okabe, H.; Ikegawa, S. Truncating mutations of *RB1CC1*
900 in human breast cancer. *Nat. Genet.* **2002**, *31*, 285–288, doi:10.1038/ng911.
901 114. Abbi, S.; Ueda, H.; Zheng, C.; Cooper, L. A.; Zhao, J.; Christopher, R.; Guan, J.-L. Regulation
902 of focal adhesion kinase by a novel protein inhibitor FIP200. *Mol. Biol. Cell* **2002**, *13*, 3178–
903 3191, doi:10.1091/mbc.E02-05-0295.
904 115. Wang, D.; Olman, M. A.; Stewart, J.; Tipps, R.; Huang, P.; Sanders, P. W.; Toline, E.; Prayson,
905 R. A.; Lee, J.; Weil, R. J.; Palmer, C. A.; Gillespie, G. Y.; Liu, W. M.; Pieper, R. O.; Guan, J.-L.;
906 Gladson, C. L. Downregulation of FIP200 induces apoptosis of glioblastoma cells and
907 microvascular endothelial cells by enhancing Pyk2 activity. *PloS One* **2011**, *6*, e19629,
908 doi:10.1371/journal.pone.0019629.
909 116. Dobbelstein, M.; Moll, U. Targeting tumour-supportive cellular machineries in anticancer
910 drug development. *Nat. Rev. Drug Discov.* **2014**, *13*, 179–196, doi:10.1038/nrd4201.
911 117. Calderwood, S. K.; Murshid, A. Molecular Chaperone Accumulation in Cancer and Decrease
912 in Alzheimer's Disease: The Potential Roles of HSF1. *Front Neurosci* **2017**, *11*, 192,
913 doi:10.3389/fnins.2017.00192.
914 118. Taipale, M.; Krykbaeva, I.; Koeva, M.; Kayatekin, C.; Westover, K. D.; Karras, G. I.; Lindquist,
915 S. Quantitative analysis of HSP90-client interactions reveals principles of substrate
916 recognition. *Cell* **2012**, *150*, 987–1001, doi:10.1016/j.cell.2012.06.047.
917 119. Taipale, M.; Jarosz, D. F.; Lindquist, S. HSP90 at the hub of protein homeostasis: emerging
918 mechanistic insights. *Nat. Rev. Mol. Cell Biol.* **2010**, *11*, 515–528, doi:10.1038/nrm2918.
919 120. Calderwood, S. K.; Gong, J. Heat Shock Proteins Promote Cancer: It's a Protection Racket.
920 *Trends Biochem Sci* **2016**, *41*, 311–323, doi:10.1016/j.tibs.2016.01.003.
921 121. Verba, K. A.; Wang, R. Y.; Arakawa, A.; Liu, Y.; Shirouzu, M.; Yokoyama, S.; Agard, D. A.
922 Atomic structure of Hsp90-Cdc37-Cdk4 reveals that Hsp90 traps and stabilizes an unfolded
923 kinase. *Science* **2016**, *352*, 1542–7, doi:10.1126/science.aaf5023.
924 122. Xiong, X.; Wang, Y.; Liu, C.; Lu, Q.; Liu, T.; Chen, G.; Rao, H.; Luo, S. Heat shock protein
925 90beta stabilizes focal adhesion kinase and enhances cell migration and invasion in breast
926 cancer cells. *Exp Cell Res* **2014**, *326*, 78–89, doi:10.1016/j.yexcr.2014.05.018.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 8 May 2018 doi:10.20944/preprints201805.0125.v1

Peer-reviewed version available at Cancers 2018, 10, 196; doi:10.3390/cancers10060196

123. Caino, M. C.; Chae, Y. C.; Vaira, V.; Ferrero, S.; Nosotti, M.; Martin, N. M.; Weeraratna, A.; O'Connell, M.; Jernigan, D.; Fatatis, A.; Languino, L. R.; Bosari, S.; Altieri, D. C. Metabolic stress regulates cytoskeletal dynamics and metastasis of cancer cells. *J Clin Invest* **2013**, *123*, 2907–20, doi:10.1172/JCI67841.

124. Lin, Y.; Peng, N.; Zhuang, H.; Zhang, D.; Wang, Y.; Hua, Z. C. Heat shock proteins HSP70 and MRJ cooperatively regulate cell adhesion and migration through urokinase receptor. *BMC Cancer* **2014**, *14*, 639, doi:10.1186/1471-2407-14-639.

125. Huang, J.; Che, M. I.; Lin, N. Y.; Hung, J. S.; Huang, Y. T.; Lin, W. C.; Huang, H. C.; Lee, P. H.; Liang, J. T.; Huang, M. C. The molecular chaperone Cosmc enhances malignant behaviors of colon cancer cells via activation of Akt and ERK. *Mol Carcinog* **2014**, *53 Suppl 1*, E62-71, doi:10.1002/mc.22011.

126. Cheng, N.; Li, Y.; Han, Z.-G. Argonaute2 promotes tumor metastasis by way of up-regulating focal adhesion kinase expression in hepatocellular carcinoma. *Hepatol. Baltim. Md* **2013**, *57*, 1906–1918, doi:10.1002/hep.26202.

127. Li, S.; Huang, X.; Zhang, D.; Huang, Q.; Pei, G.; Wang, L.; Jiang, W.; Hu, Q.; Tan, R.; Hua, Z.-C. Requirement of PEA3 for Transcriptional Activation of FAK Gene in Tumor Metastasis. *PLoS ONE* **2013**, *8*, doi:10.1371/journal.pone.0079336.

128. Ho, B.; Olson, G.; Figel, S.; Gelman, I.; Cance, W. G.; Golubovskaya, V. M. Nanog increases focal adhesion kinase (FAK) promoter activity and expression and directly binds to FAK protein to be phosphorylated. *J. Biol. Chem.* **2012**, *287*, 18656–18673, doi:10.1074/jbc.M111.322883.

129. Golubovskaya, V. M.; Finch, R.; Kweh, F.; Massoll, N. A.; Campbell-Thompson, M.; Wallace, M. R.; Cance, W. G. p53 regulates FAK expression in human tumor cells. *Mol. Carcinog.* **2008**, *47*, 373–382, doi:10.1002/mc.20395.

130. Takahashi, A.; Suzuki, H.; Omori, K.; Seki, M.; Hashizume, T.; Shimazu, T.; Ishioka, N.; Ohnishi, T. Expression of p53-regulated proteins in human cultured lymphoblastoid TSCE5 and WTK1 cell lines during spaceflight. *J. Radiat. Res. (Tokyo)* **2012**, *53*, 168–175.

131. Lim, S.-T.; Miller, N. L. G.; Nam, J.-O.; Chen, X. L.; Lim, Y.; Schlaepfer, D. D. Pyk2 inhibition of p53 as an adaptive and intrinsic mechanism facilitating cell proliferation and survival. *J. Biol. Chem.* **2010**, *285*, 1743–1753, doi:10.1074/jbc.M109.064212.

132. Golubovskaya, V.; Kaur, A.; Cance, W. Cloning and characterization of the promoter region of human focal adhesion kinase gene: nuclear factor kappa B and p53 binding sites. *Biochim. Biophys. Acta* **2004**, *1678*, 111–125, doi:10.1016/j.bbaexp.2004.03.002.

133. Lim, S.-T.; Chen, X. L.; Lim, Y.; Hanson, D. A.; Vo, T.-T.; Howerton, K.; Larocque, N.; Fisher, S. J.; Schlaepfer, D. D.; Ilic, D. Nuclear FAK promotes cell proliferation and survival through FERM-enhanced p53 degradation. *Mol. Cell* **2008**, *29*, 9–22, doi:10.1016/j.molcel.2007.11.031.

134. Golubovskaya, V. M.; Conway-Dorsey, K.; Edmiston, S. N.; Tse, C.-K.; Lark, A. A.; Livasy, C. A.; Moore, D.; Millikan, R. C.; Cance, W. G. FAK overexpression and p53 mutations are highly correlated in human breast cancer. *Int. J. Cancer* **2009**, *125*, 1735–1738, doi:10.1002/ijc.24486.

135. Tyner, S. D.; Venkatachalam, S.; Choi, J.; Jones, S.; Ghebranious, N.; Igelmann, H.; Lu, X.; Soron, G.; Cooper, B.; Brayton, C.; Park, S. H.; Thompson, T.; Karsenty, G.; Bradley, A.;

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 8 May 2018 doi:10.20944/preprints201805.0125.v1

Peer-reviewed version available at Cancers 2018, 10, 196; doi:10.3390/cancers10060196

969 Donehower, L. A. p53 mutant mice that display early ageing-associated phenotypes. *Nature*
970 **2002**, *415*, 45–53, doi:10.1038/415045a.
971 136. Ventura, A.; Kirsch, D. G.; McLaughlin, M. E.; Tuveson, D. A.; Grimm, J.; Lintault, L.;
972 Newman, J.; Reczek, E. E.; Weissleder, R.; Jacks, T. Restoration of p53 function leads to
973 tumour regression *in vivo*. *Nature* **2007**, *445*, 661–665, doi:10.1038/nature05541.
974 137. Mattick, J. S.; Makunin, I. V. Non-coding RNA. *Hum Mol Genet* **2006**, *15 Spec No 1*, R17-29,
975 doi:10.1093/hmg/ddl046.
976 138. Non-Coding RNAs and Epigenetic Regulation of Gene Expression: Drivers of Natural
977 Selection edited by Kevin V. Morris. *Q. Rev. Biol.* **2014**, *89*, 182–182, doi:10.1086/676081.
978 139. Bartel, D. P. MicroRNAs: genomics, biogenesis, mechanism, and function. *Cell* **2004**, *116*, 281–
979 297.
980 140. Filipowicz, W.; Bhattacharyya, S. N.; Sonenberg, N. Mechanisms of post-transcriptional
981 regulation by microRNAs: are the answers in sight? *Nat. Rev. Genet.* **2008**, *9*, 102–114,
982 doi:10.1038/nrg2290.
983 141. Kasinski, A. L.; Slack, F. J. Epigenetics and genetics. MicroRNAs en route to the clinic:
984 progress in validating and targeting microRNAs for cancer therapy. *Nat. Rev. Cancer* **2011**, *11*,
985 849–864, doi:10.1038/nrc3166.
986 142. Zhuang, Y.; Peng, H.; Mastej, V.; Chen, W. MicroRNA Regulation of Endothelial Junction
987 Proteins and Clinical Consequence. *Mediators Inflamm.* **2016**, *2016*, 5078627,
988 doi:10.1155/2016/5078627.
989 143. Kumar, M. S.; Lu, J.; Mercer, K. L.; Golub, T. R.; Jacks, T. Impaired microRNA processing
990 enhances cellular transformation and tumorigenesis. *Nat. Genet.* **2007**, *39*, 673–677,
991 doi:10.1038/ng2003.
992 144. Zhao, J.; Tao, Y.; Zhou, Y.; Qin, N.; Chen, C.; Tian, D.; Xu, L. MicroRNA-7: a promising new
993 target in cancer therapy. *Cancer Cell Int* **2015**, *15*, 103, doi:10.1186/s12935-015-0259-0.
994 145. Wu, D. G.; Wang, Y. Y.; Fan, L. G.; Luo, H.; Han, B.; Sun, L. H.; Wang, X. F.; Zhang, J. X.; Cao,
995 L.; Wang, X. R.; You, Y. P.; Liu, N. MicroRNA-7 regulates glioblastoma cell invasion via
996 targeting focal adhesion kinase expression. *Chin Med J Engl* **2011**, *124*, 2616–21.
997 146. Zeng, C. Y.; Zhan, Y. S.; Huang, J.; Chen, Y. X. MicroRNA7 suppresses human colon cancer
998 invasion and proliferation by targeting the expression of focal adhesion kinase. *Mol Med Rep*
999 **2016**, *13*, 1297–303, doi:10.3892/mmr.2015.4643.
1000 147. Kong, X.; Li, G.; Yuan, Y.; He, Y.; Wu, X.; Zhang, W.; Wu, Z.; Chen, T.; Wu, W.; Lobie, P. E.;
1001 Zhu, T. MicroRNA-7 inhibits epithelial-to-mesenchymal transition and metastasis of breast
1002 cancer cells via targeting FAK expression. *PLoS One* **2012**, *7*, e41523,
1003 doi:10.1371/journal.pone.0041523.
1004 148. Cao, Q.; Mao, Z. D.; Shi, Y. J.; Chen, Y.; Sun, Y.; Zhang, Q.; Song, L.; Peng, L. P. MicroRNA-7
1005 inhibits cell proliferation, migration and invasion in human non-small cell lung cancer cells
1006 by targeting FAK through ERK/MAPK signaling pathway. *Oncotarget* **2016**, *7*, 77468–77481,
1007 doi:10.18632/oncotarget.12684.
1008 149. Lee, K. M.; Choi, E. J.; Kim, I. A. microRNA-7 increases radiosensitivity of human cancer cells
1009 with activated EGFR-associated signaling. *Radiother Oncol* **2011**, *101*, 171–6,
1010 doi:10.1016/j.radonc.2011.05.050.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 8 May 2018 doi:10.20944/preprints201805.0125.v1

Peer-reviewed version available at Cancers 2018, 10, 196; doi:10.3390/cancers10060196

150. Taylor, K. N.; Schlaepfer, D. D. Adaptive Resistance to Chemotherapy, A Multi-FAK-torial Linkage. *Mol. Cancer Ther.* **2018**, *17*, 719–723, doi:10.1158/1535-7163.MCT-17-1177.

151. Hochwald, S. N.; Nyberg, C.; Zheng, M.; Zheng, D.; Wood, C.; Massoll, N. A.; Magis, A.; Ostrov, D.; Cance, W. G.; Golubovskaya, V. M. A novel small molecule inhibitor of FAK decreases growth of human pancreatic cancer. *Cell Cycle Georget. Tex* **2009**, *8*, 2435–2443, doi:10.4161/cc.8.15.9145.

152. Kurenova, E. V.; Liao, J.; He, D.; Hunt, D. L.; Chekhau, A.; Hochwald, S. N.; Ostrov, D. A.; Cance, W. G. Effect of a novel FAK inhibitor targeted to the binding site of VEGFR3 on pancreatic cancer proliferation in vitro and in vivo. *J. Clin. Oncol.* **2011**, *29*, 214–214, doi:10.1200/jco.2011.29.4_suppl.214.

153. Zhou, Y.; Dang, J.; Chang, K. Y.; Yau, E.; Aza-Blanc, P.; Moscat, J.; Rana, T. M. miR-1298 Inhibits Mutant KRAS-Driven Tumor Growth by Repressing FAK and LAMB3. *Cancer Res* **2016**, *76*, 5777–5787, doi:10.1158/0008-5472.CAN-15-2936.

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

155. Konstantinidou, G.; Ramadori, G.; Torti, F.; Kangasniemi, K.; Ramirez, R. E.; Cai, Y.; Behrens, C.; Dellinger, M. T.; Brekken, R. A.; Wistuba; Heguy, A.; Teruya-Feldstein, J.; Scaglioni, P. P. RHOA-FAK is a required signaling axis for the maintenance of KRAS-driven lung adenocarcinomas. *Cancer Discov* **2013**, *3*, 444–57, doi:10.1158/2159-8290.CD-12-0388.

156. Bing, L.; Hong, C.; Li-Xin, S.; Wei, G. MicroRNA-543 suppresses endometrial cancer oncogenicity via targeting FAK and TWIST1 expression. *Arch Gynecol Obstet* **2014**, *290*, 533–41, doi:10.1007/s00404-014-3219-3.

157. Chen, J. S.; Li, H. S.; Huang, J. Q.; Dong, S. H.; Huang, Z. J.; Yi, W.; Zhan, G. F.; Feng, J. T.; Sun, J. C.; Huang, X. H. MicroRNA-379-5p inhibits tumor invasion and metastasis by targeting FAK/AKT signaling in hepatocellular carcinoma. *Cancer Lett* **2016**, *375*, 73–83, doi:10.1016/j.canlet.2016.02.043.

158. Mazzu, Y. Z.; Hu, Y.; Soni, R. K.; Mojica, K. M.; Qin, L. X.; Agius, P.; Waxman, Z. M.; Mihailovic, A.; Socci, N. D.; Hendrickson, R. C.; Tuschl, T.; Singer, S. miR-193b-Regulated Signaling Networks Serve as Tumor Suppressors in Liposarcoma and Promote Adipogenesis in Adipose-Derived Stem Cells. *Cancer Res* **2017**, *77*, 5728–5740, doi:10.1158/0008-5472.CAN-16-2253.

159. Majid, S.; Saini, S.; Dar, A. A.; Hirata, H.; Shahryari, V.; Tanaka, Y.; Yamamura, S.; Ueno, K.; Zaman, M. S.; Singh, K.; Chang, I.; Deng, G.; Dahiya, R. MicroRNA-205 inhibits Src-mediated oncogenic pathways in renal cancer. *Cancer Res* **2011**, *71*, 2611–21, doi:10.1158/0008-5472.CAN-10-3666.

160. Wang, N.; Li, Q.; Feng, N. H.; Cheng, G.; Guan, Z. L.; Wang, Y.; Qin, C.; Yin, C. J.; Hua, L. X. miR-205 is frequently downregulated in prostate cancer and acts as a tumor suppressor by inhibiting tumor growth. *Asian J Androl* **2013**, *15*, 735–41, doi:10.1038/aja.2013.80.

161. Li, J.; Bai, H.; Zhu, Y.; Wang, X. Y.; Wang, F.; Zhang, J. W.; Lavker, R. M.; Yu, J. Antagomir dependent microRNA-205 reduction enhances adhesion ability of human corneal epithelial keratinocytes. *Chin Med Sci J* **2010**, *25*, 65–70.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 8 May 2018 doi:10.20944/preprints201805.0125.v1

Peer-reviewed version available at Cancers 2018, 10, 196; doi:10.3390/cancers10060196

162. Rashed, M. H.; Kanlikilicer, P.; Rodriguez-Aguayo, C.; Pichler, M.; Bayraktar, R.; Bayraktar, E.; Ivan, C.; Filant, J.; Silva, A.; Aslan, B.; Denizli, M.; Mitra, R.; Ozpolat, B.; Calin, G. A.; Sood, A. K.; Abd-Ellah, M. F.; Helal, G. K.; Berestein, G. L. Exosomal miR-940 maintains SRC-mediated oncogenic activity in cancer cells: a possible role for exosomal disposal of tumor suppressor miRNAs. *Oncotarget* **2017**, *8*, 20145–20164, doi:10.18632/oncotarget.15525.

163. Egawa, H.; Jingushi, K.; Hirono, T.; Ueda, Y.; Kitae, K.; Nakata, W.; Fujita, K.; Uemura, M.; Nonomura, N.; Tsujikawa, K. The miR-130 family promotes cell migration and invasion in bladder cancer through FAK and Akt phosphorylation by regulating PTEN. *Sci Rep* **2016**, *6*, 20574, doi:10.1038/srep20574.

164. Cui, L.; Li, Y.; Lv, X.; Li, J.; Wang, X.; Lei, Z.; Li, X. Expression of MicroRNA-301a and its Functional Roles in Malignant Melanoma. *Cell Physiol Biochem* **2016**, *40*, 230–244, doi:10.1159/000452540.

165. Liu, R. F.; Xu, X.; Huang, J.; Fei, Q. L.; Chen, F.; Li, Y. D.; Han, Z. G. Down-regulation of miR-517a and miR-517c promotes proliferation of hepatocellular carcinoma cells via targeting Pyk2. *Cancer Lett* **2013**, *329*, 164–73, doi:10.1016/j.canlet.2012.10.027.

166. Gutschner, T.; Diederichs, S. The hallmarks of cancer: a long non-coding RNA point of view. *RNA Biol* **2012**, *9*, 703–19, doi:10.4161/rna.20481.

167. Li, Y.; Wu, Z.; Yuan, J.; Sun, L.; Lin, L.; Huang, N.; Bin, J.; Liao, Y.; Liao, W. Long non-coding RNA MALAT1 promotes gastric cancer tumorigenicity and metastasis by regulating vasculogenic mimicry and angiogenesis. *Cancer Lett* **2017**, *395*, 31–44, doi:10.1016/j.canlet.2017.02.035.

168. Wu, J.; Zhao, J.; Sun, L.; Pan, Y.; Wang, H.; Zhang, W. B. Long non-coding RNA H19 mediates mechanical tension-induced osteogenesis of bone marrow mesenchymal stem cells via FAK by sponging miR-138. *Bone* **2018**, *108*, 62–70, doi:10.1016/j.bone.2017.12.013.

169. Liang, Y.; Chen, X.; Wu, Y.; Li, J.; Zhang, S.; Wang, K.; Guan, X.; Yang, K.; Bai, Y. LncRNA CASC9 promotes esophageal squamous cell carcinoma metastasis through upregulating LAMC2 expression by interacting with the CREB-binding protein. *Cell Death Differ* **2018**, doi:10.1038/s41418-018-0084-9.

170. Lv, P. C.; Jiang, A. Q.; Zhang, W. M.; Zhu, H. L. FAK inhibitors in Cancer, a patent review. *Expert Opin Ther Pat* **2018**, *28*, 139–145, doi:10.1080/13543776.2018.1414183.

171. Duncan, J. S.; Whittle, M. C.; Nakamura, K.; Abell, A. N.; Midland, A. A.; Zawistowski, J. S.; Johnson, N. L.; Granger, D. A.; Jordan, N. V.; Darr, D. B.; Usary, J.; Kuan, P.-F.; Smalley, D. M.; Major, B.; He, X.; Hoadley, K. A.; Zhou, B.; Sharpless, N. E.; Perou, C. M.; Kim, W. Y.; Gomez, S. M.; Chen, X.; Jin, J.; Frye, S. V.; Earp, H. S.; Graves, L. M.; Johnson, G. L. Dynamic Reprogramming of the Kinome in Response to Targeted MEK Inhibition in Triple-Negative Breast Cancer. *Cell* **2012**, *149*, 307–321, doi:10.1016/j.cell.2012.02.053.

172. Graves, L. M.; Duncan, J. S.; Whittle, M. C.; Johnson, G. L. The dynamic nature of the kinome. *Biochem. J.* **2013**, *450*, 1–8, doi:10.1042/BJ20121456.

173. Golubovskaya, V. M.; Ho, B.; Zheng, M.; Magis, A.; Ostrov, D.; Morrison, C.; Cance, W. G. Disruption of focal adhesion kinase and p53 interaction with small molecule compound R2 reactivated p53 and blocked tumor growth. *BMC Cancer* **2013**, *13*, 342, doi:10.1186/1471-2407-13-342.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 8 May 2018 doi:10.20944/preprints201805.0125.v1

Peer-reviewed version available at Cancers 2018, 10, 196; doi:10.3390/cancers10060196

174. Goñi, G. M.; Epifano, C.; Boskovic, J.; Camacho-Artacho, M.; Zhou, J.; Bronowska, A.; Martín, M. T.; Eck, M. J.; Kremer, L.; Gräter, F.; Gervasio, F. L.; Perez-Moreno, M.; Lietha, D. Phosphatidylinositol 4,5-bisphosphate triggers activation of focal adhesion kinase by inducing clustering and conformational changes. *Proc. Natl. Acad. Sci. U. S. A.* **2014**, *111*, E3177-3186, doi:10.1073/pnas.1317022111.

175. Sulzmaier, F. J.; Jean, C.; Schlaepfer, D. D. FAK in cancer: mechanistic findings and clinical applications. *Nat Rev Cancer* **2014**, *14*, 598–610, doi:10.1038/nrc3792.

176. Tomita, N.; Hayashi, Y.; Suzuki, S.; Oomori, Y.; Aramaki, Y.; Matsushita, Y.; Iwatani, M.; Iwata, H.; Okabe, A.; Awazu, Y.; Isono, O.; Skene, R. J.; Hosfield, D. J.; Miki, H.; Kawamoto, T.; Hori, A.; Baba, A. Structure-based discovery of cellular-active allosteric inhibitors of FAK. *Bioorg. Med. Chem. Lett.* **2013**, *23*, 1779–1785, doi:10.1016/j.bmcl.2013.01.047.

177. Heinrich, T.; Seenisamy, J.; Emmanuvel, L.; Kulkarni, S. S.; Bomke, J.; Rohdich, F.; Greiner, H.; Esdar, C.; Krier, M.; Grädler, U.; Musil, D. 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.* **2013**, *56*, 1160–1170, doi:10.1021/jm3016014.

178. Golubovskaya, V. M.; Figel, S.; Ho, B. T.; Johnson, C. P.; Yemma, M.; Huang, G.; Zheng, M.; Nyberg, C.; Magis, A.; Ostrov, D. A.; Gelman, I. H.; Cance, W. G. A small molecule focal adhesion kinase (FAK) inhibitor, targeting Y397 site: 1-(2-hydroxyethyl)-3, 5, 7-triaza-1-azoniatricyclo [3.3.1.1(3,7)]decane; bromide effectively inhibits FAK autophosphorylation activity and decreases cancer cell viability, clonogenicity and tumor growth in vivo. *Carcinogenesis* **2012**, *33*, 1004–1013, doi:10.1093/carcin/bgs120.

179. Cance, W. G.; Kurenova, E.; Marlowe, T.; Golubovskaya, V. Disrupting the scaffold to improve focal adhesion kinase-targeted cancer therapeutics. *Sci. Signal.* **2013**, *6*, pe10, doi:10.1126/scisignal.2004021.

180. Schultheis, B.; Strumberg, D.; Santel, A.; Vank, C.; Gebhardt, F.; Keil, O.; Lange, C.; Giese, K.; Kaufmann, J.; Khan, M.; Drevs, J. First-in-Human Phase I Study of the Liposomal RNA Interference Therapeutic Atu027 in Patients With Advanced Solid Tumors. *J. Clin. Oncol.* **2014**, *32*, 4141–4148, doi:10.1200/JCO.2013.55.0376.

181. Watts, J. K.; Corey, D. R. Clinical status of duplex RNA. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 3203–3207, doi:10.1016/j.bmcl.2010.03.109.

182. Hon, C.-C.; Ramilowski, J. A.; Harshbarger, J.; Bertin, N.; Rackham, O. J. L.; Gough, J.; Denisenko, E.; Schmeier, S.; Poulsen, T. M.; Severin, J.; Lizio, M.; Kawaji, H.; Kasukawa, T.; Itoh, M.; Burroughs, A. M.; Noma, S.; Djebali, S.; Alam, T.; Medvedeva, Y. A.; Testa, A. C.; Lipovich, L.; Yip, C.-W.; Abugessaisa, I.; Mendez, M.; Hasegawa, A.; Tang, D.; Lassmann, T.; Heutink, P.; Babina, M.; Wells, C. A.; Kojima, S.; Nakamura, Y.; Suzuki, H.; Daub, C. O.; de Hoon, M. J. L.; Arner, E.; Hayashizaki, Y.; Carninci, P.; Forrest, A. R. R. An atlas of human long non-coding RNAs with accurate 5′ ends. *Nature* **2017**, *543*, 199–204, doi:10.1038/nature21374.

183. Li, T.; Mo, X.; Fu, L.; Xiao, B.; Guo, J. Molecular mechanisms of long noncoding RNAs on gastric cancer. *Oncotarget* **2016**, *7*, 8601–8612, doi:10.18632/oncotarget.6926.

184. Tesmer, V. M.; Lennarz, S.; Mayer, G.; Tesmer, J. J. G. Molecular mechanism for inhibition of G protein-coupled receptor kinase 2 by a selective RNA aptamer. *Struct. Lond. Engl. 1993* **2012**, *20*, 1300–1309, doi:10.1016/j.str.2012.05.002.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 8 May 2018 doi:10.20944/preprints201805.0125.v1

Peer-reviewed version available at Cancers 2018, 10, 196; doi:10.3390/cancers10060196

185. Marondedze, C.; Thomas, L.; Serrano, N. L.; Lilley, K. S.; Gehring, C. The RNA-binding protein repertoire of *Arabidopsis thaliana*. *Sci. Rep.* **2016**, *6*, 29766, doi:10.1038/srep29766.

186. Chen, Y.; Gao, D.-Y.; Huang, L. In vivo delivery of miRNAs for cancer therapy: challenges and strategies. *Adv. Drug Deliv. Rev.* **2015**, *81*, 128–141, doi:10.1016/j.addr.2014.05.009.

187. Ho, P. Y.; Duan, Z.; Batra, N.; Jilek, J. L.; Tu, M.-J.; Qiu, J.-X.; Hu, Z.; Wun, T.; Lara, P. N.; DeVere White, R. W.; Chen, H.-W.; Yu, A.-M. Bioengineered ncRNAs selectively change cellular miRNome profiles for cancer therapy. *J. Pharmacol. Exp. Ther.* **2018**, doi:10.1124/jpet.118.247775.

188. Tang, H.; Yao, L.; Tao, X.; Yu, Y.; Chen, M.; Zhang, R.; Xu, C. miR-9 functions as a tumor suppressor in ovarian serous carcinoma by targeting TLN1. *Int J Mol Med* **2013**, *32*, 381–8, doi:10.3892/ijmm.2013.1400.

189. Wang, Q.; Li, X.; Zhu, Y.; Yang, P. MicroRNA-16 suppresses epithelial-mesenchymal transitionrelated gene expression in human glioma. *Mol Med Rep* **2014**, *10*, 3310–4, doi:10.3892/mmr.2014.2583.

190. Du, W. W.; Li, X.; Li, T.; Li, H.; Khorshidi, A.; Liu, F.; Yang, B. B. The microRNA miR-17-3p inhibits mouse cardiac fibroblast senescence by targeting Par4. *J Cell Sci* **2015**, *128*, 293–304, doi:10.1242/jcs.158360.

191. Ren, Y.; Zhou, X.; Yang, J. J.; Liu, X.; Zhao, X. H.; Wang, Q. X.; Han, L.; Song, X.; Zhu, Z. Y.; Tian, W. P.; Zhang, L.; Mei, M.; Kang, C. S. AC1MMYR2 impairs high dose paclitaxel-induced tumor metastasis by targeting miR-21/CDK5 axis. *Cancer Lett* **2015**, *362*, 174–82, doi:10.1016/j.canlet.2015.03.038.

192. De Antonellis, P.; Carotenuto, M.; Vandenbussche, J.; De Vita, G.; Ferrucci, V.; Medaglia, C.; Boffa, I.; Galiero, A.; Di Somma, S.; Magliulo, D.; Aiese, N.; Alonzi, A.; Spano, D.; Liguori, L.; Chiarolla, C.; Verrico, A.; Schulte, J. H.; Mestdagh, P.; Vandesompele, J.; Gevaert, K.; Zollo, M. Early targets of miR-34a in neuroblastoma. *Mol Cell Proteomics* **2014**, *13*, 2114–31, doi:10.1074/mcp.M113.035808.

193. Zhang, D.; Zhou, J.; Dong, M. Dysregulation of microRNA-34a expression in colorectal cancer inhibits the phosphorylation of FAK via VEGF. *Dig Sci* **2014**, *59*, 958–67, doi:10.1007/s10620-013-2983-4.

194. Ma, G.; Jing, C.; Li, L.; Huang, F.; Ding, F.; Wang, B.; Lin, D.; Luo, A.; Liu, Z. MicroRNA-92b represses invasion-metastasis cascade of esophageal squamous cell carcinoma. *Oncotarget* **2016**, *7*, 20209–22, doi:10.18632/oncotarget.7747.

195. Cai, J. J.; Qi, Z. X.; Chen, L. C.; Yao, Y.; Gong, Y.; Mao, Y. miR-124 suppresses the migration and invasion of glioma cells in vitro via Capn4. *Oncol Rep* **2016**, *35*, 284–90, doi:10.3892/or.2015.4355.

196. Gao, F.; Wang, F. G.; Liu, R. R.; Xue, F.; Zhang, J.; Xu, G. Q.; Bi, J. H.; Meng, Z.; Huo, R. Epigenetic silencing of miR-130a ameliorates hemangioma by targeting tissue factor pathway inhibitor 2 through FAK/PI3K/Rac1/mdm2 signaling. *Int J Oncol* **2017**, *50*, 1821–1831, doi:10.3892/ijo.2017.3943.

197. Zhao, H.; Li, M.; Li, L.; Yang, X.; Lan, G.; Zhang, Y. MiR-133b is down-regulated in human osteosarcoma and inhibits osteosarcoma cells proliferation, migration and invasion, and promotes apoptosis. *PLoS One* **2013**, *8*, e83571, doi:10.1371/journal.pone.0083571.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 8 May 2018 doi:10.20944/preprints201805.0125.v1

Peer-reviewed version available at Cancers 2018, 10, 196; doi:10.3390/cancers10060196

198. Zha, R.; Guo, W.; Zhang, Z.; Qiu, Z.; Wang, Q.; Ding, J.; Huang, S.; Chen, T.; Gu, J.; Yao, M.; He, X. Genome-wide screening identified that miR-134 acts as a metastasis suppressor by targeting integrin beta1 in hepatocellular carcinoma. *PLoS One* **2014**, *9*, e87665, doi:10.1371/journal.pone.0087665.

199. Islam, M.; Datta, J.; Lang, J. C.; Teknos, T. N. Down regulation of RhoC by microRNA-138 results in de-activation of FAK, Src and Erk1/2 signaling pathway in head and neck squamous cell carcinoma. *Oral Oncol* **2014**, *50*, 448–56, doi:10.1016/j.oraloncology.2014.01.014.

200. Qu, B.; Xia, X.; Wu, H. H.; Tu, C. Q.; Pan, X. M. PDGF-regulated miRNA-138 inhibits the osteogenic differentiation of mesenchymal stem cells. *Biochem Biophys Res Commun* **2014**, *448*, 241–7, doi:10.1016/j.bbrc.2014.04.091.

201. Tanaka, K.; Kawano, M.; Itonaga, I.; Iwasaki, T.; Miyazaki, M.; Ikeda, S.; Tsumura, H. Tumor suppressive microRNA-138 inhibits metastatic potential via the targeting of focal adhesion kinase in Ewing's sarcoma cells. *Int J Oncol* **2016**, *48*, 1135–44, doi:10.3892/ijo.2016.3317.

202. Zhang, F.; Yang, R.; Zhang, G.; Cheng, R.; Bai, Y.; Zhao, H.; Lu, X.; Li, H.; Chen, S.; Li, J.; Wu, S.; Li, P.; Chen, X.; Sun, Q.; Zhao, G. Anticancer function of alpha-solanine in lung adenocarcinoma cells by inducing microRNA-138 expression. *Tumour Biol* **2016**, *37*, 6437–46, doi:10.1007/s13277-015-4528-2.

203. Chen, X.; Wang, X.; Ruan, A.; Han, W.; Zhao, Y.; Lu, X.; Xiao, P.; Shi, H.; Wang, R.; Chen, L.; Chen, S.; Du, Q.; Yang, H.; Zhang, X. miR-141 is a key regulator of renal cell carcinoma proliferation and metastasis by controlling EphA2 expression. *Clin Cancer Res* **2014**, *20*, 2617–30, doi:10.1158/1078-0432.CCR-13-3224.

204. Choi, S. K.; Kim, H. S.; Jin, T.; Hwang, E. H.; Jung, M.; Moon, W. K. Overexpression of the miR-141/200c cluster promotes the migratory and invasive ability of triple-negative breast cancer cells through the activation of the FAK and PI3K/AKT signaling pathways by secreting VEGF-A. *BMC Cancer* **2016**, *16*, 570, doi:10.1186/s12885-016-2620-7.

205. Lee, H. K.; Bier, A.; Cazacu, S.; Finniss, S.; Xiang, C.; Twito, H.; Poisson, L. M.; Mikkelsen, T.; Slavin, S.; Jacoby, E.; Yalon, M.; Toren, A.; Rempel, S. A.; Brodie, C. MicroRNA-145 is downregulated in glial tumors and regulates glioma cell migration by targeting connective tissue growth factor. *PLoS One* **2013**, *8*, e54652, doi:10.1371/journal.pone.0054652.

206. Cao, M.; Hou, D.; Liang, H.; Gong, F.; Wang, Y.; Yan, X.; Jiang, X.; Wang, C.; Zhang, J.; Zen, K.; Zhang, C. Y.; Chen, X. miR-150 promotes the proliferation and migration of lung cancer cells by targeting SRC kinase signalling inhibitor 1. *Eur J Cancer* **2014**, *50*, 1013–24, doi:10.1016/j.ejca.2013.12.024.

207. Hsu, K. W.; Fang, W. L.; Huang, K. H.; Huang, T. T.; Lee, H. C.; Hsieh, R. H.; Chi, C. W.; Yeh, T. S. Notch1 pathway-mediated microRNA-151-5p promotes gastric cancer progression. *Oncotarget* **2016**, *7*, 38036–38051, doi:10.18632/oncotarget.9342.

208. Ding, J.; Huang, S.; Wu, S.; Zhao, Y.; Liang, L.; Yan, M.; Ge, C.; Yao, J.; Chen, T.; Wan, D.; Wang, H.; Gu, J.; Yao, M.; Li, J.; Tu, H.; He, X. Gain of miR-151 on chromosome 8q24.3 facilitates tumour cell migration and spreading through downregulating RhoGDIA. *Nat Cell Biol* **2010**, *12*, 390–9, doi:10.1038/ncb2039.

209. Luedde, T. MicroRNA-151 and its hosting gene FAK (focal adhesion kinase) regulate tumor cell migration and spreading of hepatocellular carcinoma. *Hepatology* **2010**, *52*, 1164–6, doi:10.1002/hep.23854.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 8 May 2018 doi:10.20944/preprints201805.0125.v1

Peer-reviewed version available at Cancers 2018, 10, 196; doi:10.3390/cancers10060196

210. Chao, A.; Lin, C. Y.; Lee, Y. S.; Tsai, C. L.; Wei, P. C.; Hsueh, S.; Wu, T. I.; Tsai, C. N.; Wang, C. J.; Chao, A. S.; Wang, T. H.; Lai, C. H. Regulation of ovarian cancer progression by microRNA-187 through targeting Disabled homolog-2. *Oncogene* **2012**, *31*, 764–75, doi:10.1038/onc.2011.269.

211. Li, W.; Wang, H.; Zhang, J.; Zhai, L.; Chen, W.; Zhao, C. miR-199a-5p regulates beta1 integrin through Ets-1 to suppress invasion in breast cancer. *Cancer Sci* **2016**, *107*, 916–23, doi:10.1111/cas.12952.

212. Meng, X.; Chen, X.; Lu, P.; Ma, W.; Yue, D.; Song, L.; Fan, Q. MicroRNA-202 inhibits tumor progression by targeting LAMA1 in esophageal squamous cell carcinoma. *Biochem Biophys Res Commun* **2016**, *473*, 821–827, doi:10.1016/j.bbrc.2016.03.130.

213. Zhu, A.; Kang, N.; He, L.; Li, X.; Xu, X.; Zhang, H. MiR-221 and miR-26b Regulate Chemotactic Migration of MSCs Toward HGF Through Activation of Akt and FAK. *J Cell Biochem* **2016**, *117*, 1370–83, doi:10.1002/jcb.25428.

214. Fu, Q.; Song, X.; Liu, Z.; Deng, X.; Luo, R.; Ge, C.; Li, R.; Li, Z.; Zhao, M.; Chen, Y.; Lin, X.; Zhang, Q.; Fang, W. miRomics and Proteomics Reveal a miR-296-3p/PRKCA/FAK/Ras/c-Myc Feedback Loop Modulated by HDGF/DDX5/beta-catenin Complex in Lung Adenocarcinoma. *Clin Cancer Res* **2017**, *23*, 6336–6350, doi:10.1158/1078-0432.CCR-16-2813.

215. He, L.; Wang, X.; Kang, N.; Xu, J.; Dai, N.; Xu, X.; Zhang, H. MiR-375 inhibits the hepatocyte growth factor-elicited migration of mesenchymal stem cells by downregulating Akt signaling. *Cell Tissue Res* **2018**, doi:10.1007/s00441-017-2765-y.

216. Zhao, L. N.; Wang, P.; Liu, Y. H.; Cai, H.; Ma, J.; Liu, L. B.; Xi, Z.; Li, Z. Q.; Liu, X. B.; Xue, Y. X. MiR-383 inhibits proliferation, migration and angiogenesis of glioma-exposed endothelial cells in vitro via VEGF-mediated FAK and Src signaling pathways. *Cell Signal* **2017**, *30*, 142–153, doi:10.1016/j.cellsig.2016.09.007.

217. Fang, F.; Song, T.; Zhang, T.; Cui, Y.; Zhang, G.; Xiong, Q. MiR-425-5p promotes invasion and metastasis of hepatocellular carcinoma cells through SCAI-mediated dysregulation of multiple signaling pathways. *Oncotarget* **2017**, *8*, 31745–31757, doi:10.18632/oncotarget.15958.

218. Huang, W. C.; Chan, S. H.; Jang, T. H.; Chang, J. W.; Ko, Y. C.; Yen, T. C.; Chiang, S. L.; Chiang, W. F.; Shieh, T. Y.; Liao, C. T.; Juang, J. L.; Wang, H. C.; Cheng, A. J.; Lu, Y. C.; Wang, L. H. miRNA-491-5p and GIT1 serve as modulators and biomarkers for oral squamous cell carcinoma invasion and metastasis. *Cancer Res* **2014**, *74*, 751–64, doi:10.1158/0008-5472.CAN-13-1297.

219. Oneyama, C.; Morii, E.; Okuzaki, D.; Takahashi, Y.; Ikeda, J.; Wakabayashi, N.; Akamatsu, H.; Tsujimoto, M.; Nishida, T.; Aozasa, K.; Okada, M. MicroRNA-mediated upregulation of integrin-linked kinase promotes Src-induced tumor progression. *Oncogene* **2012**, *31*, 1623–35, doi:10.1038/onc.2011.367.

220. Cao, W.; Wei, W.; Zhan, Z.; Xie, D.; Xie, Y.; Xiao, Q. Regulation of drug resistance and metastasis of gastric cancer cells via the microRNA647-ANK2 axis. *Int J Mol Med* **2018**, *41*, 1958–1966, doi:10.3892/ijmm.2018.3381.

221. Cao, W.; Wei, W.; Zhan, Z.; Xie, D.; Xie, Y.; Xiao, Q. Role of miR-647 in human gastric cancer suppression. *Oncol Rep* **2017**, *37*, 1401–1411, doi:10.3892/or.2017.5383.

222. Ryu, S.; McDonnell, K.; Choi, H.; Gao, D.; Hahn, M.; Joshi, N.; Park, S.-M.; Catena, R.; Do, Y.; Brazin, J.; Vahdat, L. T.; Silver, R. B.; Mittal, V. Suppression of miRNA-708 by Polycomb

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 8 May 2018 doi:10.20944/preprints201805.0125.v1

Peer-reviewed version available at Cancers 2018, 10, 196; doi:10.3390/cancers10060196

Group Promotes Metastases by Calcium-Induced Cell Migration. *Cancer Cell* **2013**, *23*, 63–76, doi:10.1016/j.ccr.2012.11.019.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 8 May 2018 doi:10.20944/preprints201805.0125.v1

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1269 **Table 1.** FAK/PYK2 based endogenous inhibitors.

miRNA	Cell type	Target site(s)	Role	Ref
miR-9	Ovarian serous carcinoma	TLN1, FAK, Akt	Tumor suppressor	[188]
miR-16	Glioma	p-FAK, p-Akt expression, nuclear factor-κB, Slug	Tumor suppressor	[189]
miR-17-3p	Cardiac fibroblasts	Par4, CEBPB, FAK, N-cadherin, vimentin, Oct4, Sca-1, E-cadherin	Oncogene	[190]
miR-21	Breast cancer lymph node metastasis	CDK5RAP1, CDK5 activator p39, FAK	Oncogene	[191]
miR-34a	Neuroblastoma, colorectal cancer	VEGF, FAK	Tumor suppressor	[192,193]
miR-92b	Esophageal squamous cell carcinoma	ITGAV, FAK, Rac1	Tumor suppressor	[194]
miR-124	Glioma	Capn4, -FAK, MMP2, vimentin, N-cadherin	Tumor suppressor	[195]
miR-130a	Hemangioma	TFPI2, FAK, PI3K, Rac1, mdm2	Oncogene	[196]
miR-133b	Osteosarcoma	BCL2L2, MCL-1, IGF1R, MET, FAK, Akt	Tumor suppressor	[197]
miR-134	Hepatocellular carcinoma	ITGB1, FAK and RhoA	Tumor suppressor	[198]
miR-138	Ewing's sarcoma, head and neck squamous cell carcinoma	RhoC, FAK, Src, Erk(1/2)	Tumor suppressor	[168,199–202]
miR-141	Renal cell carcinoma	EphA2, p-FAK, p-AKT, MMP2/9	Tumor suppressor	[203]
miR-141/200c cluster	Breast cancer	VEGF-A, FAK, PI3K, Akt	Oncogene	[204]

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miR-145	Glioma	CTGF, SPARC, FAK	Tumor suppressor	[205]
miR-150	Lung cancer	Src, FAK, Ras, ERK	Oncogene	[206]
miR-151-5p	Gastric cancer, hepatocellular carcinoma	FAK (host genet), RhoGDIA, Rac1, Cdc42, Rho GTPases	Oncogene	[207–209]
miR-187	Ovarian cancer	Dab2, E-cadherin, vimentin, FAK	Tumor suppressor	[210]
miR-199a-5p	Breast cancer	Ets-1, FAK/Src/Akt/mTOR	Tumor suppressor	[211]
miR-202	Esophageal squamous cell carcinoma	LAMA1, FAK-PI3K-Akt	Tumor suppressor	[212]
MiR-221 and miR-26b	Mesenchymal stem cells	PTEN, FAK, PI3K, Akt		[213]
miR-296-3p	Lung Adenocarcinoma	PRKCA, FAK-Ras-c-Myc	Tumor suppressor	[214]
miR-375	Mesenchymal stem cells	FAK, paxillin, PDK1, Akt		[215]
miR-383	Glioma	VEGF/VEGFR2, FAK, Src	Tumor suppressor	[216]
miR-425-5p	Hepatocellular carcinoma	SCAI, integrin β1-Fak/Src-RhoA/CDC42, PTEN-AKT, TIMP2-MMP2/MMP9	Oncogene	[217]
miR-491-5p	Oral squamous cell carcinoma	GIT1, paxillin, FAK, EGF/EGFR- ERK1/2, MMP2/9	Tumor suppressor	[218]
miR-542-3p	Colon cancer	ILK, FAK/c-Src	Tumor suppressor	[219]
miR-647	Gastric cancer	ANK2, FAK, MMP2, MMP12, CD44, SNAIL1,	Tumor suppressor	[220,221]
miR-708	Metastatic breast cancer	Neuronatin, ERK, FAK	Tumor suppressor	[222]

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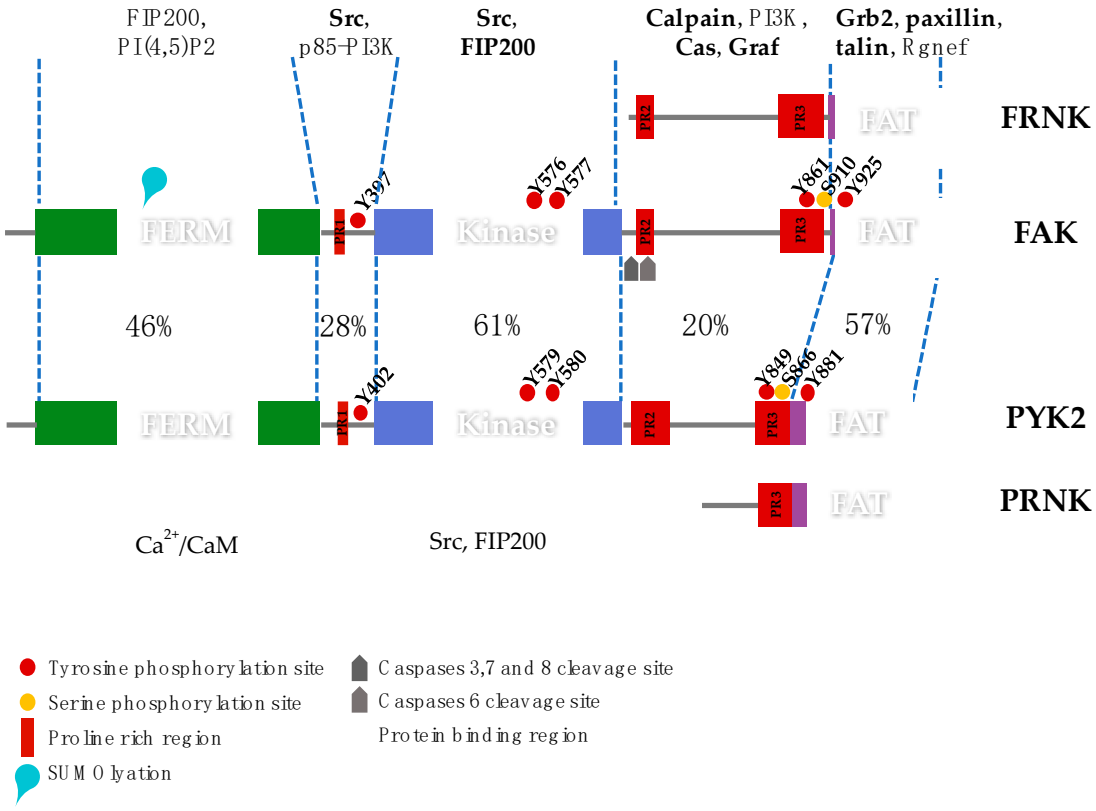

Figure 1. Schematic domain structure of FAK and PYK2. The three folded domains are shown in green, blue and magenta. Interaction motifs, sites of post-translational modification, and binding sites of ligands discussed in the text are shown. The % numbers give the sequence identity between corresponding regions of FAK and PYK2. The alternatively transcribed products FRNK and PRNK are schematically represented with respect to FAK and PYK2. Protein ligand names in bold indicate that these proteins were shown to bind to both, FAK and PYK2. Protein names not in bold have so far only been shown to be ligands for FAK (if above the FAK drawing) or PYK2 (Ca^{2+}/CaM).

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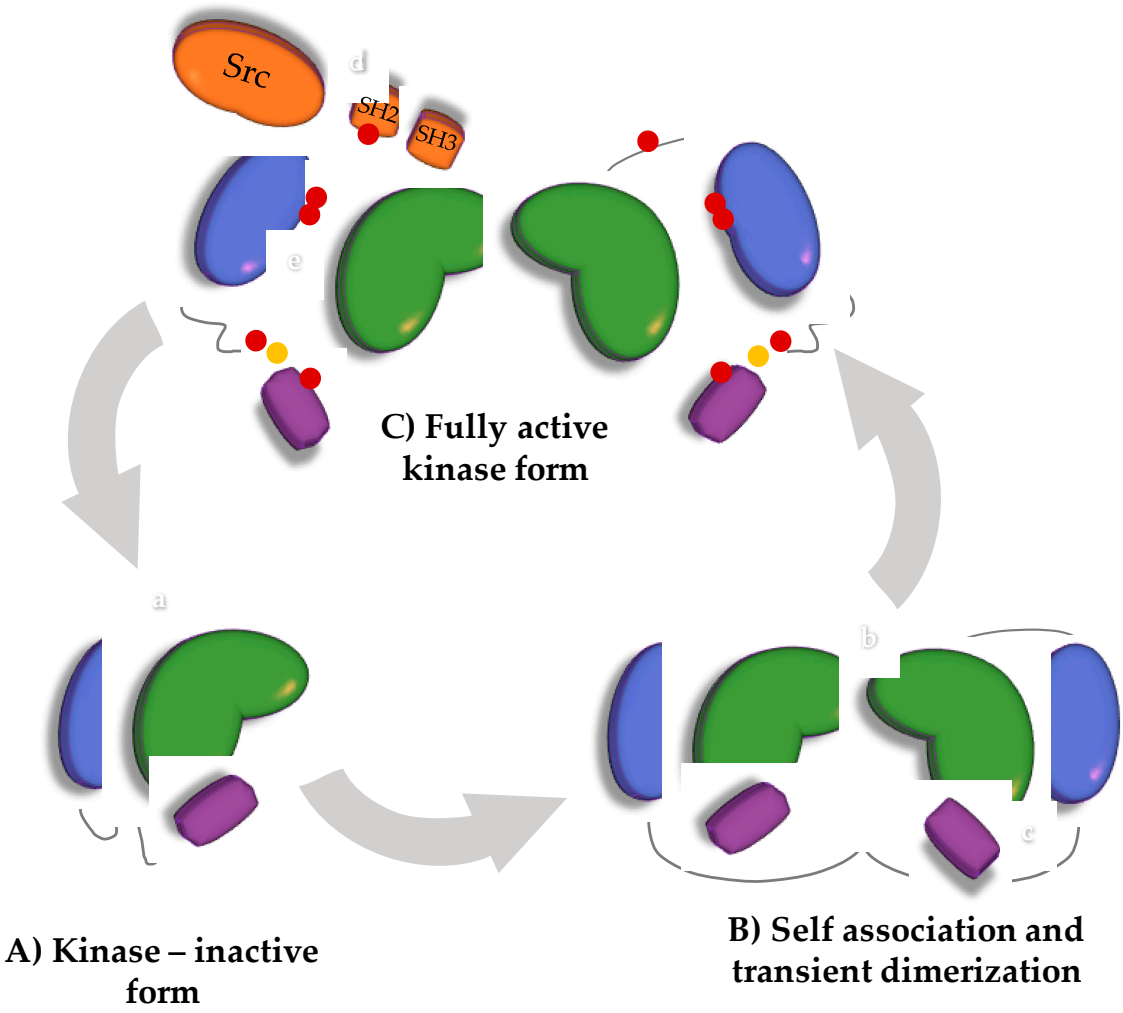

Figure 2. Canonical FAK activation scheme. (A) In the absence of integrin activation, an interaction between the FERM and kinase domain (indicated by a white lower-case 'a' in a red triangle) inhibits FAK kinase activity. Ligand-mediated recruitment and clustering of FAK at focal adhesions promotes transient dimerization by stabilizing weak FERM:FERM interactions (lower-case 'b' in triangle) and promoting FERM:FAT binding *in trans* (lower-case c). FAK clustering and self-association allows trans-autophosphorylation of Y397 (red dot) in the FERM-kinase linker. When phosphorylated, Y397 and PR1 form a bidentate binding site for the SH2 and SH3 domains of Src (lower-case d). Recruitment-activated Src phosphorylates the activation loop of the FAK kinase domain (lower-case e) and other tyrosines on FAK, resulting in an open FAK conformation and full enzymatic activity. Triggered signaling may result in additional FAK modifications (e.g. serine phosphorylation; yellow dot), and may ultimately lead to dephosphorylation and/or displacement of FAK from focal adhesions (back to the closed monomeric inactive conformation of A), or to proteolytic cleavage and degradation (not shown).

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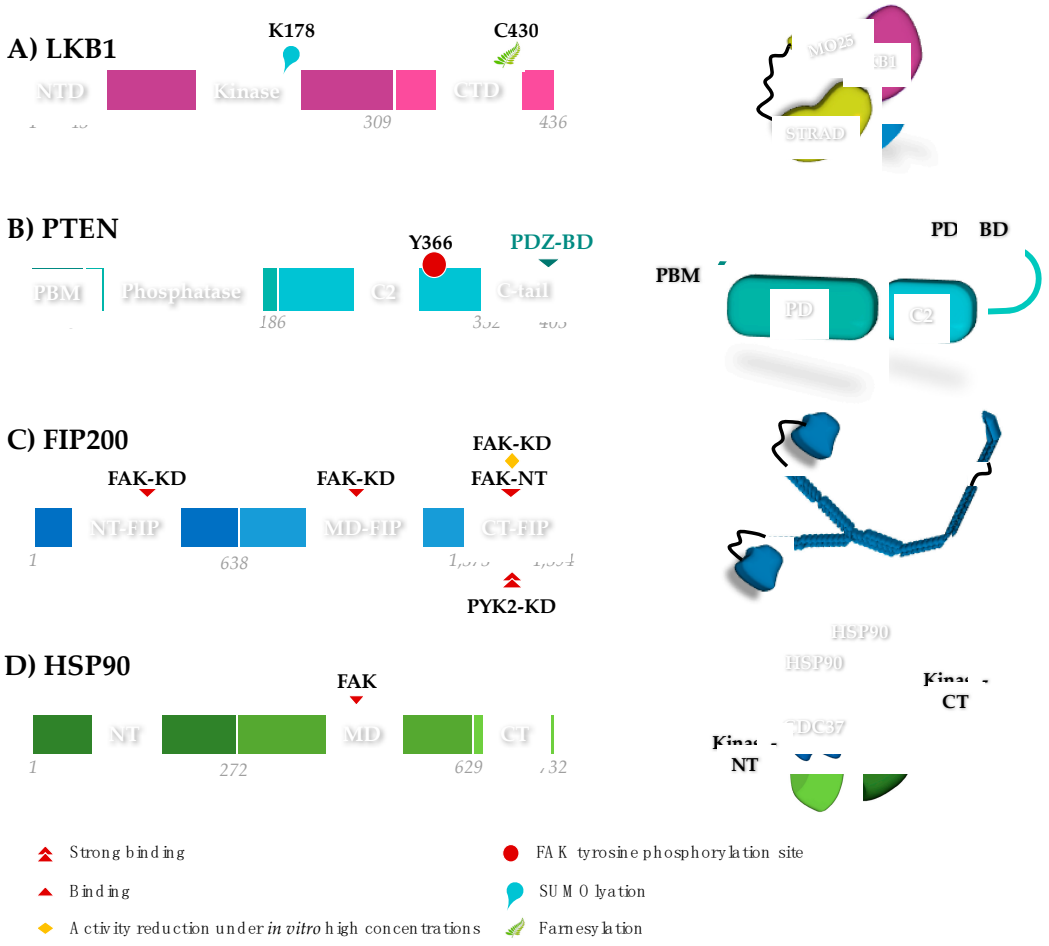

Figure 3. Schematic domain structures (left) and 3D architectures (right) of protein regulators of FAK: LKB1, PTEN and FIP200 are negative regulators. LKB1 is shown as part of the activating complex formed with MO25α and STRADα (according to PDB accession 2wtk). The presented 3-dimensional structure of FIP200 (blue) is highly speculative, based on secondary structure predictions and homology modeling. The stabilizing function of HSP90 is upregulated in cancers. HSP90 is displayed with the kinase-specific adaptor CDC37, maintaining the separated N-terminal (NT) and C-terminal (CT) lobe of a kinase domain (taken from PDB 5fwl). Binding sites and posttranscriptional modifications relevant for their interaction with FAK and PY2 are indicated. Abbreviations are: LKB1: NTD, N-terminal domain; CTD, C-terminal domain. PTEN: PD, phosphatase domain; PBM, PI(4,5)P2-binding module; PDZ-BD: PDZ binding domain. FIP200/HSP90: NT, N-terminal domain; MD: middle domain; CT, C-terminal domain. FAK: KD, kinase domain; NT, N-terminal fragment.

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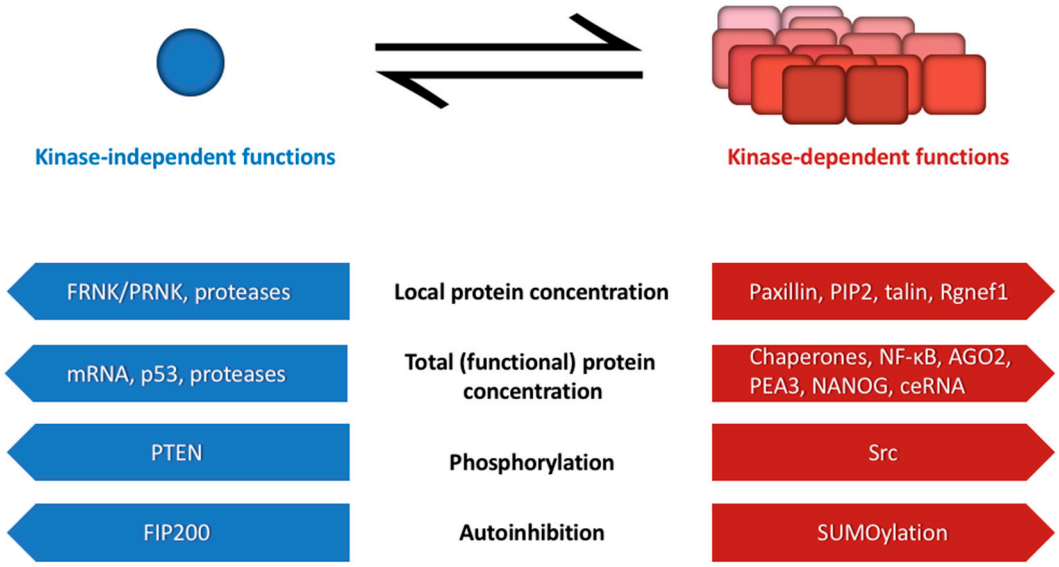

1308

1309 **Figure 4. Schematic overview of the endogenous mechanisms promoting either**
1310 **kinase-independent or kinase-dependent functions of FAK or PYK2.** *Top panel:* Kinase-inactive
1311 FAK/PYK2 is illustrated by a blue sphere. Kinase-active FAK/PYK2 are shown as clustered square
1312 dimers in shades of red. *Bottom panel:* Promoters of kinase-independent and kinase-dependent
1313 functions are presented in blue and red, respectively, next to the mechanism or function they affect.
1314 The direction of the arrowheads indicates decrease (left) or increase (right) of the indicated
1315 mechanism/function. This list is incomplete, as the factors with unknown molecular mechanism are
1316 not shown (e.g. LKB1).

1317