THE ROLE OF FOCAL-ADHESION KINASE IN CANCER — A NEW THERAPEUTIC OPPORTUNITY

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Abstract | Focal-adhesion kinase (FAK) is an important mediator of growth-factor signalling, cell proliferation, cell survival and cell migration. Given that the development of malignancy is often associated with perturbations in these processes, it is not surprising that FAK activity is altered in cancer cells. Mouse models have shown that FAK is involved in tumour formation and progression, and other studies showing that FAK expression is increased in human tumours make FAK a potentially important new therapeutic target.

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Focal-adhesion kinase (FAK) resides at sites of integrin clustering — the so-called focal adhesions — that are prominent in cells that are grown in tissue culture. FAK carries out protein–protein-interaction adaptor functions at sites of cell attachment to the extracellular matrix (ECM), thereby contributing to focal-adhesion 'scaffolding', and also transmits adhesion-dependent and growth-factor-dependent signals into the cell interior (FIG. 1). In the cancer context, the synergistic signalling between growth-factor receptors and FAK might be particularly relevant as both are often upregulated in tumour cells. Together, the action of FAK and signalling from growth-factor receptors might control the altered growth of tumour cells as well as their responses to autocrine or paracrine factors. In addition, FAK influences the dynamic regulation of integrin-associated adhesions, and the actin cytoskeleton that is tethered there, through diverse molecular interactions. This, in turn, regulates cell migration by controlling the focal-complex assembly/disassembly cycle at the leading lamellipodia of migrating cells, while also controlling adhesion disassembly at the trailing edge. As these processes are crucial components of cell migration, and therefore also of invasion by cancer cells, FAK might well be involved in the spread of cancer cells. Focal adhesions are places from which adhesion and actin dynamics are coordinately regulated with survival and growth signalling, at least in part, through FAK-dependent functions[1]. Therefore, it is timely to review whether — and, if so, how — FAK might contribute to the development of malignancy.

Several reports have linked FAK expression with cancer. *FAK* mRNA was found to be increased in 49 human tissue samples, including a wide range of paired normal and neoplastic tissue samples. In this survey, increased levels of FAK were found in 1 of 8 adenomatous tissues, in 17 of 20 invasive tumours, and in all 15 metastatic tumours of different origins. However, no *FAK* mRNA was detected in 6 normal tissue samples[2], although sub-detectable levels might have been present. Another study showed that FAK protein levels were increased in 100% of colon and 88% of breast tumour samples, with FAK protein expression often being associated with advanced disease[3].

Since these studies were published, there have been many reports documenting similar findings in a wide range of human malignancies. These studies, which are based mainly on immunohistochemical and immunoblotting analysis, have shown increased FAK levels in cancers of the thyroid, prostate, cervix, colon, rectum, oral epithelium and ovary[4–12]. Increased FAK expression and activity are frequently correlated with malignant or metastatic disease and poor patient prognosis[3,10,13,14]. Interestingly, a recent report has also

Summary

- Focal-adhesion kinase (FAK) is a non-receptor tyrosine kinase that provides signalling and scaffolding functions at sites of integrin adhesion. It is involved in the regulation of turnover of these adhesion sites, a process that is crucial in the control of cell migration.
- FAK is linked to the protection of cells from anoikis (suspension-induced cell death). This anti-apoptotic function is potentially linked to the ability of FAK to sequester receptor-interacting protein (RIP) from the death-receptor machinery.
- Substantial circumstantial evidence has accumulated linking overexpression of FAK to a wide range of human epithelial cancers. Levels of FAK expression correlate with the invasive potential of tumours.
- Using a mouse model of skin carcinogenesis, a direct requirement for FAK has now been shown during tumour progression *in vivo*. These observations are probably linked to the ability of FAK to protect cells from apoptosis.
- Inhibition of FAK function might provide an attractive anticancer target, however it is not yet clear what the most effective strategy would be. Potential intervention routes are inhibition of the kinase activity of FAK or disruption of crucial protein–protein interactions.

highlighted a possible correlation between FAK expression and clinical outcome. In this ovarian cancer study, FAK overexpression in primary tumour biopsy material was correlated with metastasis to lymph nodes and distant organs, as well as with reduced survival times[15]. These findings indicate that FAK protein levels might be an appropriate prognostic indicator. By contrast, there is also one report of reduced FAK expression in metastatic liver tumours, compared with their matched primary human colourectal adenocarcinoma samples[16]. So FAK might have different roles in different tumours, or during different stages of tumour progression.

Although the mechanisms that underlie the increased expression of FAK in tumour cells are not fully understood, amplification of the *FAK* gene has been reported in a few cancer cell lines, and gains in gene copy number are found in cells derived from head and neck cancer[17].

Despite the numerous impressive correlates, experimental proof for a causative role for FAK in cancer has been lacking. However, recent evidence implies that FAK promotes tumorigenesis in an animal model. In this review, we consider key cellular properties regulated by FAK that could mediate its tumorigenic activity. This is probably linked to its well-documented ability to control cell adhesion and migration, as well as to influence cell-survival pathways. FAK is itself regulated by a range of mechanisms, including tyrosine phosphorylation, serine/threonine phosphorylation and many protein-binding interactions (these have been reviewed in detail recently[14]).

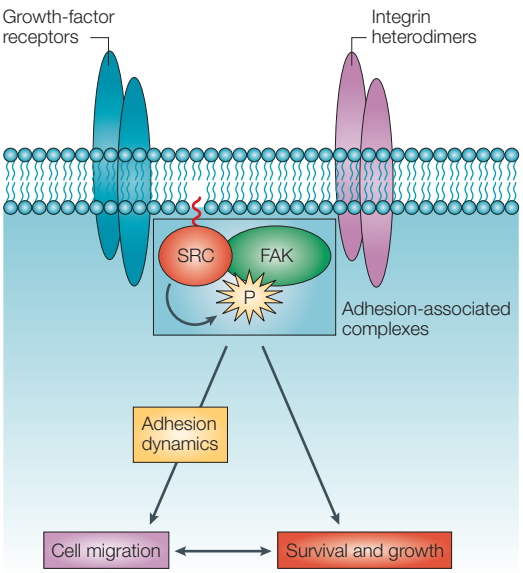

Figure 1 | **Focal-adhesion kinase as a signal integrator.** Focal-adhesion kinase (FAK) acts to integrate signals from extracellular cues, such as growth-factor receptors and integrins, and from the upstream SRC-family kinases, to control and coordinate adhesion dynamics/cell migration with survival signalling. SRC binds to growth-factor receptors and FAK to integrins (although, this has not been shown *in vivo*), and they bind to each other. SRC binding to growth-factor receptors is widely believed to be important, as is FAK signalling from integrins, regardless of whether the interaction between FAK and these transmembrane receptors is direct.

Regulation of FAK in cancer cells

Given the evidence that high levels of FAK are associated with human cancer, it is surprising that the *FAK* gene promoter has not been more extensively studied. However, a recent report identified binding sites for the p53 tumour suppressor in the *FAK* promoter. These studies showed that p53 binding to this site was able to suppress expression of FAK[18]. This observation raises the intriguing possibility that transcriptional silencing by p53 might be involved in the normal control of FAK expression. However, it remains untested whether — and, if so, how — p53 loss or mutation contributes to altered FAK expression.

Another mode of FAK regulation that is well understood is phosphorylation, particularly tyrosine phosphorylation[14]. This has been reviewed thoroughly in other articles, (for example, see REF. 19). Autophosphorylation of FAK on a particular tyrosine (Y) residue, Y397, occurs in response to many stimuli, including integrin engagement, and this creates a high-affinity binding site for the SRC homology 2 (SH2) domain of several proteins including the upstream SRC kinase itself[20,21]. The association of SRC with FAK leads to a conformational change and activation of the kinase activity of SRC. The ensuing phosphorylation of FAK by SRC on Y576 and Y577 within the FAK catalytic domain is required for the full enzymatic activity of FAK[22]. SRC can further phosphorylate FAK on Y407, Y861 and Y925 with phosphorylated Y925 acting as a docking site for growth-factor-receptor-bound protein 2 (GRB2; REFS 22,23), which permits signalling to the RAS–MAPK (mitogen-activated protein kinase) cascade[24]. The FAK–SRC signalling complex acts to recruit and/or phosphorylate a number of signalling proteins and is involved in adhesion regulation and the motile and invasive phenotype, as well as in growth and survival signalling (FIG. 1).

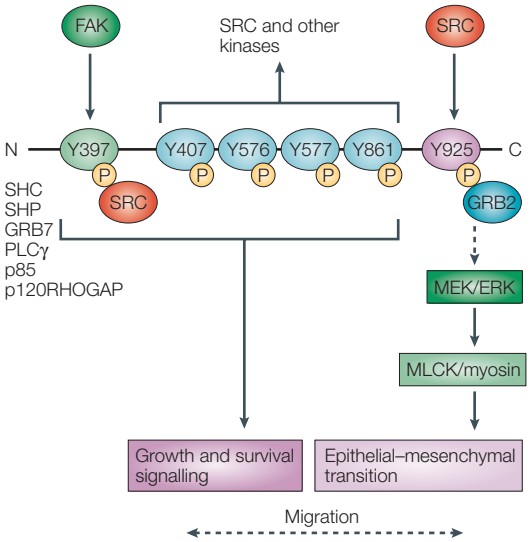

Figure 2 | **Focal-adhesion kinase tyrosine phosphorylation regulates downstream signalling events.** Phosphorylation of tyrosine (Y)397 is mainly due to autophosphorylation, although transphosphorylation by growth-factor receptors might also occur. This creates a high-affinity binding site for the SRC homology 2 (SH2) domain of SRC (and other SH2-domain-containing proteins), which can then phosphorylate focal-adhesion kinase (FAK) on additional tyrosine residues, of which Y925, in particular, appears to be a SRC-specific site. How signalling through increased phospho-FAK contributes to the behaviour of cancer cells is beginning to be understood. Phosphorylation of Y397 or Y925 might cause increased complex formation between FAK and its SH2-containing proteins. For example, SRC, SHC, p85 (a phosphatidylinositol 3-kinase regulatory subunit), phospholipase Cγ (PLCγ), growth factor receptor-bound protein 7 (GRB7), GRB2, p120RHOGAP and others. The lower bracket indicates phosphorylation sites that are likely to mediate growth and survival signalling, probably by creating binding sites for partner proteins. In the case of the SRC-specific FAK-Y925 phospho-acceptor site, phosphorylation at this site can cause FAK exclusion from focal adhesions[118]. This site is also proposed to link FAK to the RAS–MAPK (mitogen-activated protein kinase) pathway, which is associated with SRC-induced adhesion changes that cause an epithelial–mesenchymal transition[119] (FIG. 6). In this way, altered tyrosine phosphorylation of FAK in tumour cells could control subcellular localization, adhesion type predominance, growth and survival signalling, and cancer-cell behaviour. ERK, extracellular signal-regulated kinase; MEK, MAPK/ERK kinase; MLCK, myosin light-chain kinase.

FAK phosphorylation in cancer

Phosphorylation of FAK at specific sites has been reported to be associated with different tumour types. In ovarian tissue for example, phospho-FAK-Y397 (phosphorylation of FAK at Y397 in the FAK sequence) was found in invasive tumours, but not in normal epithelium[25]. Other studies have also shown increases in phospho-FAK-Y397 in different tumour types[13,26,27], although phosphorylation of FAK-Y397 might not necessarily reflect FAK kinase activity[28,29]. In colon cancer, phosphorylation of FAK has been linked to expression of gastrin-releasing peptide and tumour differentiation[30]. There is also evidence that FAK phosphorylation could regulate tumour-cell adhesion. In colon cancer cells, phosphorylation of FAK occurs on multiple sites (FIG. 2), although phosphorylation at FAK-Y925 is the major SRC-specific phosphorylation event that is associated with integrin adhesion dynamics and E-cadherin deregulation during SRC-induced epithelial–mesenchymal transition[28,31]. This indicates that FAK-Y925 could be important in some aspects of the cancer phenotype (FIG. 2). In addition, phosphorylation of FAK-Y861 promotes association of FAK with the $\alpha_v\beta_5$ integrin following vascular endothelial growth factor stimulation[32], and this could influence the tumour vasculature. Differential phosphorylation of individual FAK tyrosine residues after receipt of motogenic or oncogenic stimuli[29,32–35], combined with the likelihood kinases other than SRC can phosphorylate FAK[28,29], imply that more complexities will be unravelled.

FAK mechanisms of action

Cell migration. FAK is an important regulator of cell migration[14] — a function required for the invasion and metastasis of cancer cells. The latter requires individual cells, or probably small groups of cells, to initially move through three-dimensional (3D) ECM around the region of the primary tumour. This often, although not always, requires the combined action of matrix metalloproteinases (MMPs), to degrade ECM barriers. However, recent evidence implies that there are also proteolysis-independent mechanisms of invasion, and it is possible that FAK might be differentially required for distinct modes of tumour-cell invasion.

FAK controls the dynamic regulation of integrin-linked adhesions (or focal adhesions), cadherin-dependent cell–cell adhesions and peripheral actin structures, and so contributes to cell migration and invasion[14,36,37]. Its mechanism of action is complex and probably involves a web of downstream signalling connections. Recent advances in molecular intervention, combined with real-time fluorescence imaging of adhesion dynamics and actin remodelling, are beginning to imply mechanisms by which FAK can promote, and at times also suppress, cell migration.

FAK signalling might mediate the initial assembly of integrin-associated focal adhesions, which promote cell adhesion to the ECM[38], although maintenance of focal adhesions does not generally require FAK[38,39]. Migration also requires the regulated turnover, or disassembly, of these adhesions and FAK also controls this process, specifically by releasing points of attachment between the cell and the surrounding ECM[39–42]. Moreover, as the cycle of assembly and disassembly of these adhesions — that is, the ability to adhere and let go — controls the rate of cell movement, or indeed whether a cell can move or not, FAK is implicated as a key regulator of cell movement. In this regard, FAK-deficient cells exhibit larger focal adhesions at the cell periphery and migrate poorly in comparison to normal fibroblasts[41].

The recent development of live-cell imaging techniques used with fluorescently labelled focal-adhesion components confirms a role for FAK in promoting

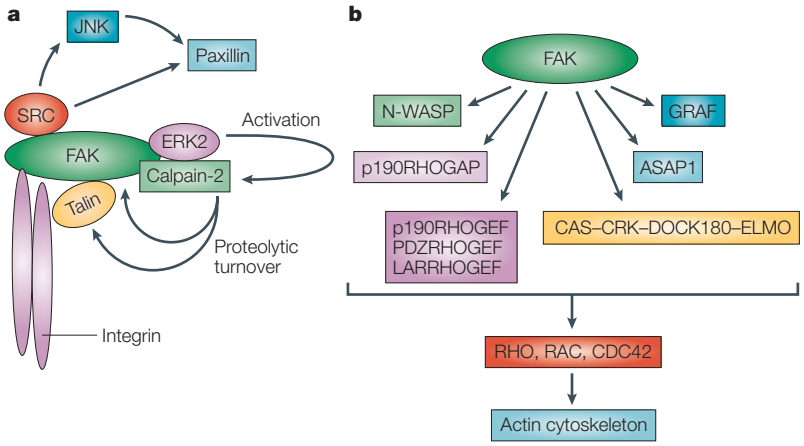

Figure 3 | **Focal-adhesion kinase influences cell migration by molecular signalling pathways. a** | During focal-adhesion turnover, integrin-mediated phosphorylation of focal-adhesion kinase (FAK) recruits a number of signalling and structural proteins, promoting the initial assembly of focal adhesions. Once phosphorylated by SRC, FAK functions as an efficient adaptor molecule that recruits both extracellular signal-regulated kinase 2 (ERK2) and calpain-2 to focal-adhesion sites. This facilitates ERK2-induced activation of calpain-2 and calpain-2-mediated cleavage of focal-adhesion components (for example, talin and FAK), resulting in focal-adhesion disassembly[40,47]. FAK also recruits JUN N-terminal kinase (JNK) to focal-adhesion sites, and JNK-mediated phosphorylation of paxillin on a specific serine residue promotes focal-adhesion remodelling and cell motility[56]. **b** | During actin remodelling, the RHO family of small GTPases — RHO, RAC and CDC42 — drive cell migration across two-dimensional substrates by controlling actin polymerization/de-polymerization and the actin-stress-fibre assembly/disassembly cycle. FAK both positively and negatively regulates the RHO-family GTPases by modulating various upstream regulators. FAK-mediated phosphorylation of the GTPase exchange factors RHO guanine nucleotide exchange factor of 190kDa (p190RHOGEF), PDZ-domain-containing RHOGEF (PDZRHOGEF) and LAR tyorinse phosphatase RHOGEF) correlates with enhanced RHOA activity[14,64,120]. FAK also modifies several GTPase-activating proteins including, ARF-GTPase-activating protein 1 (ASAP1), GTPase regulator associated with FAK (GRAF) and RHO-GTPase-activating protein of 190kDa (p190RHOGAP), which regulate activity of the RHO family[65,66]. In addition, FAK participates in the assembly of a complex consisting of CRK-associated substrate (CAS, also known as p130cas), CRK, dedicator of cytokinesis of 180kDa (DOCK180) and engulfment and cell motility protein (ELMO), where the association of DOCK180 with ELMO might locally stimulate RAC1 activity[68]. FAK-mediated phosphorylation of neuronal Wiscott–Aldrich syndrome protein (N-WASP) influences its subcellular localization and cell motility[69], probably by modifying the ability of N-WASP to promote actin polymerization through affecting the Arp2/3 complex.

focal-adhesion disassembly[39,42]. Quantitative measurements of rate constants for focal-adhesion turnover show that FAK signalling activates the MAPK signalling pathway, as well as the protease calpain-2, which promotes focal-adhesion turnover[39,43] (FIG. 3a). FAK therefore integrates several signalling responses that control focal-adhesion dynamics.

FAK expression, but not its kinase activity, is required for platelet-derived growth factor (PDGF)- and epidermal growth factor (EGF)-stimulated cell motility, indicating that FAK acts as an adaptor, or scaffold, to recruit molecules that promote adhesion turnover[29]. Such a role is supported by findings that FAK adaptor function promotes the assembly of a functional complex consisting of calpain-2, FAK, SRC and ERK2[1] (FIG. 3a). Calpain-2 is a ubiquitously expressed member of the calpain family of intracellular cysteine proteases, which were first implicated in cell migration by the use of pharmacological inhibitors. These inhibitors impair the release of cell–substrate interactions at the rear of migrating cells and thereby suppress cell movement[44].

HAPTOTACTIC MIGRATION
Migration of cells towards fixed attractants to which the cells bind. Often used to describe movement of cells towards extracellular-matrix components mediated by binding to specific integrins.

Moreover, calpain-2-mediated cleavage of focal-adhesion components, such as talin, paxillin or FAK, often occurs in parallel with focal-adhesion turnover and reduced cell–matrix adhesiveness[45,46]. Indeed, recent work has shown that calpain-mediated cleavage of talin is a rate-limiting step in focal-adhesion turnover that is needed for cell migration[47]. Also, calpain-2 is directly phosphorylated and activated by EGF-induced MAPK, and EGF-induced de-adhesion and cell migration requires MAPK-induced activation of the calpain-2 isoform at the membrane[48]. Recent work (reviewed in REF. 1) has elucidated how the adaptor function of FAK promotes the motile response through MAPK and calpain-2. Such signalling events might have importance in invasion and metastasis, as increased expression and/or activity of EGFR (or its close relatives), SRC-family kinases, FAK, calpain-2 and MAPK have all been linked in some way to tumour invasion *in vivo*[49–54]. FAK expression can also promote recruitment of JUN N-terminal kinase (JNK) to focal-contact sites after integrin stimulation[55], and JNK-mediated phosphorylation of paxillin-S178 promotes cell migration[56] (FIG. 3a). This highlights the multiple ways in which FAK can induce focal-adhesion turnover and cell migration, and indeed these might not yet all have been uncovered. In this regard, the kinase activity of FAK could also influence focal-adhesion turnover by tyrosine phosphorylation of other focal-adhesion substrates, such as CRK-associated substrate (CAS, also known as p130cas) and paxillin[39,57–60]. Additionally, focal-adhesion dynamics are clearly also under the control of the RHO family of small GTPases (see below), which themselves are perturbed, and involved, in cancer.

So there is no doubt that FAK occupies an important position within the migration-regulatory signalling network, although it remains to be established whether the kinase activity of FAK is required in all situations, and whether FAK-dependent cell migration (as currently understood for cells in culture) is important for epithelial cancer cell migration *in vivo* (discussed in more detail below).

In addition to tumour-cell migration, FAK has been found to be expressed in angiogenic blood vessels of malignant astrocytomas, where it might contribute to angiogenesis by enabling HAPTOTACTIC MIGRATION towards ECM proteins[61]. Additionally, it is now known that FAK deficiency in endothelial cells (which causes vascular defects and lethality in null mice at day 8.5) impairs the normal organization of fibronectin, an important ECM component[62]. These data imply that FAK has important roles in the behaviour of endothelial cells, which, in turn, might affect tumour development.

Remodelling the actin cytoskeleton. Actin remodelling is another crucial element of the cell-motility process (key effectors of FAK in actin remodelling are depicted in FIG. 3b). Adhesion dynamics are tightly linked to control of actin assembly and disassembly and FAK contributes to both, the latter usually by influencing RHO-GTPase pathways. The contribution of RHO GTPases to actin remodelling is described in BOX 1. The fact that impaired

Box 1 | **The contribution of RHO GTPases to actin remodelling**

The initial stages of cell adhesion and focal-complex formation are associated with activation of RAC1 and CDC42, which stimulate lamellipodia and filopodia, respectively. These processes enable membrane protrusion and cell polarization in the direction of forward movement, whereas subsequent assembly of tension-inducing actin stress fibres and mature focal adhesions are controlled by RHOA and activation of downstream effectors such as DIA1/2 or RHO-associated kinase (ROCK). The activity of the RHO-family GTPases themselves is positively regulated by guanine nucleotide exchange factors (GEFs) and negatively by GTPase-activating proteins (GAPs). The contribution of focal-adhesion kinase to actin remodelling that is needed for cell migration is mediated through binding to RHO protein effectors and subsequent effects on RHOGTPases.

motility of FAK-deficient cells could be rescued by an inhibitor of ROCK, a downstream effector of RHOA, implies that FAK works, at least in part, through the modulation of RHO proteins[63]. Furthermore, the intrinsic activity of RHOA is increased in FAK-deficient cells[63], although re-expression of FAK in *Fak*$^{-/-}$ fibroblasts decreases RHOA activity[37,42]. These events promote actin remodelling and adhesion reorganization. However, in other cell types FAK-induced phosphorylation and activation of p190RHOGEF (guanine nucleotide exchange factor) is linked to RHOA activation[14,64], indicating that FAK-mediated control of RHO-GTPase regulators is complex, probably cell and context dependent, and perhaps spatially regulated. There are other examples of how FAK can influence actin remodelling. For example, FAK also interacts with ARF GTPase-activating protein 1 (ASAP1), which possesses GTPase-activating protein (GAP) activity for the ARF family of GTPases, and GTPase regulator associated with FAK (GRAF), which possesses GAP activity for RHOA and CDC42 (REFS 65,66) (FIG. 3b).

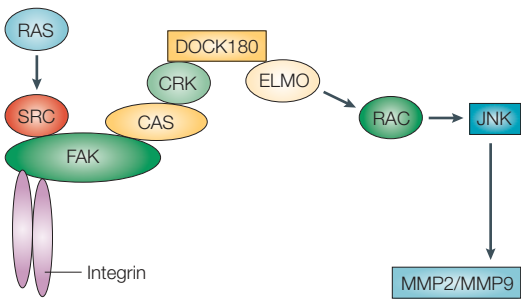

Figure 4 | **Focal-adhesion kinase influences cell migration through additional molecular signalling pathways.** Additional focal-adhesion kinase (FAK)-mediated signalling events induce the expression of genes encoding matrix metalloproteinases (MMPs)[121]. Once MMPs are secreted, they mediate the breakdown of surrounding extracellular-matrix substrates and promote cell invasion. Recent studies on RAS-transformed cells indicate that tyrosine phosphorylation of FAK on tyrosine (Y)861 promotes association with CRK-associated substrate (CAS, also known as p130cas) and recruitment of a CRK–DOCK180–ELMO complex (where DOCK180 is dedicator of cytokinesis of 180kDa and ELMO is engulfment and cell motility protein) that activates RAC1, subsequently activating JUN N-terminal kinase (JNK) that promotes MMP expression and cell invasion[35].

Another FAK function that is important for cell migration is the assembly of a complex between FAK, CAS and the proto-oncoprotein CRK, which serves to recruit the RAC1 GEF, dedicator of cytokinesis of 180kDa (DOCK180; reviewed in REF. 67). DOCK180, when associated with its binding partner engulfment and cell motility protein (ELMO), stimulates RAC1 activity[68]. So, FAK controls recruitment and activation of the CAS–CRK–DOCK180–ELMO complex, stimulating localized activation of RAC1, peripheral actin assembly and stabilization of focal complexes (FIG. 4 and reviewed in REF. 67). Moreover, as discussed below, this pathway contributes to the invasive process.

FAK probably also influences actin polymerization more directly by binding and inducing phosphorylation of the CDC42 effector protein neuronal Wiskott–Aldrich syndrome protein (N-WASP)[69]. N-WASP promotes actin polymerization by activation of the Arp2/3 complex. FAK-mediated phosphorylation of N-WASP at Y256 appears to influence its intracellular localization, but it is not yet clear whether, and if so how, FAK influences N-WASP-mediated Arp2/3 activity and actin assembly, or whether this occurs in cancer cells[69] (FIG. 3b).

Invasion. FAK regulates the invasive activity of both normal and SRC-transformed fibroblasts through reconstituted 3D MATRIX[37,70]. By contrast, a negative role for FAK during EGF-induced invasion of A431 epidermal carcinoma cells has been reported[71]. Although integrin-linked adhesion complexes still form in cells cultured within a 3D matrix (referred to as '3D-matrix adhesions'), these are structurally distinct from the large mature focal adhesions formed by cells on two-dimensional (2D) substrates[72] (reviewed in REF. 73). In addition, phosphorylation of FAK on Y397 in cells that are growing in 3D gels is substantially reduced when compared with cells on 2D substrates[72]. In addition, phosphorylation of FAK on Y397 contributes to CYTOTROPHOBLAST migration and invasion[74]. These findings indicate that FAK might be differently regulated, or have distinct roles, depending on whether cells are moving within a 3D matrix, or across a 2D planar substrate.

Although v-Src can promote transformation in *FAK*$^{-/-}$ cells[75], FAK kinase activity is required for the efficient invasion of v-Src-transformed fibroblasts through a 3D matrix *in vitro*[37]. Importantly, whereas FAK-deficient v-Src-transformed fibroblasts are not impaired in their ability to migrate across 2D substrates, they are defective in invading 3D matrices *in vitro*[37]. Moreover, dominant-negative forms of FAK suppress matrix metalloproteinase 2 (MMP2) expression and activity[70,76], whereas antisense-mediated reduction in FAK also decreases expression of MMPs in carcinoma cells[77], implying a general requirement for FAK for the production and activity of MMPs that have cancer-associated matrix-degrading activities. The FAK-promoted assembly of a SRC–CAS–CRK–DOCK180 complex in

3D MATRIX
Reconstituted cell growth matrix such as Matrigel or fibrillar collagen, which is designed to mimic the *in vivo* environment encountered by tumour cells and so provide a surrogate when they are invading *in vitro*. This allows monitoring of cancer cells in culture migrating through a 3D matrix environment.

CYTOTROPHOBLAST
Part of the mammalian placenta; that is, the inner cellular layer of the trophectoderm (trophoblast), between the syncitiotrophoblast and chorionic villus capillaries.

v-Src-transformed fibroblasts, results in activation of RAC1 and JNK, and consequent increased MMP2 and MMP9 expression[37] (FIG. 4). So, FAK regulates cell motility and invasion by distinct pathways; that is, by promoting the dynamic regulation of focal adhesions and peripheral actin structures during migration, as well as by MMP-mediated matrix degradation (reviewed in REF. 14).

Interestingly, it is now recognized that tumour cells can invade a 3D matrix by both MMP-dependent and MMP-independent mechanisms — referred to as mesenchymal-like and amoeboid-like mechanisms, respectively[78,79] (reviewed in REF. 80). The precise roles of FAK during mesenchymal-like and amoeboid-like mechanisms of invasion by tumour cells remain to be established, although, as mentioned above, FAK has been implicated in MMP expression, indicating that FAK is probably required for mesenchymal-like invasion. By contrast, amoeboid-like invasion might be independent of this FAK function, as this mode of invasion through a 3D matrix is less dependent on integrin-mediated adhesions within the matrix[81]. Further studies are needed to address whether tumour cells that invade by distinct mechanisms are indeed differently dependent on FAK. But if this is the case, and tumour cells can switch between these types of invasive mechanisms, this could go some way to explaining the apparently contradictory FAK requirement for invasion by different cell types[37,71].

FAK in crosstalk between integrin- and cadherin-mediated adhesion. Integrin-dependent cell–matrix adhesions and cadherin-mediated cell–cell contacts can communicate with each other. Such crosstalk contributes to an altered balance between the two adhesion types, and is likely to contribute to cancer progression. Usually, cells that assemble dynamic cell–matrix contacts are more migratory and have less robust cell–cell adhesions, whereas cells that predominantly form cadherin-mediated cell–cell contacts maintain polarized epithelial-cell morphology, and typically have less robust focal-adhesion complexes. FAK has emerged as a mediator of crosstalk between integrin-mediated focal adhesions and intercellular junctions. Inhibiting signalling through FAK, or decreasing FAK expression, can either promote assembly or disassembly of cadherin-mediated cell–cell adhesions, depending on cell context and cadherin type[31,82]. In one case, treatment of HeLa cells with short interfering RNA (siRNA) for FAK, led to aberrant membrane protrusion and deregulation of N-cadherin-mediated intercellular adhesions, both of which correlated with increased peripheral RAC1 activity[82].

The positive effect of FAK on intercellular adhesion might be through regulation of expression, or localization, of the cadherins themselves. For example, transforming growth factor-β (TGFβ) suppresses the malignant phenotype of TGFβ-responsive human colon adenocarcinoma cells (Moser cells) by inducing E-cadherin expression that parallels FAK activation[83]. However, in endothelial cells, FAK activation is required for proper localization of E-cadherin to the cell periphery and consequent strengthening of the barrier of endothelial cells[84]. So, in some cell types, FAK positively regulates cadherin-mediated cell–cell contact and maintenance of a non-migratory epithelial phenotype; however, in other cell types, as is the case with KM12C colon cancer cells that have retained E-cadherin, FAK can have a negative influence on cadherin-mediated intercellular adhesion[31]. This apparent discrepancy could be due to some cell- or context-dependent signalling from FAK to RAC1, depending perhaps on other upstream signalling inputs, such as SRC activity.

A role for FAK in the survival of tumour cells. Evidence linking FAK to cell survival was first reported by Frisch *et al.*, who showed that FAK was able to suppress suspension-induced cell death (known as 'anoikis') in kidney epithelial (MDCK) cells[85]. The ability of FAK to do this depends on both FAK-Y397 phosphorylation and kinase activity. Overexpression of FAK also protects MDCK cells from ultraviolet-light-induced cell death, and this is linked to its association with CAS[86]. Increased FAK expression in the HL60 leukaemia cell line has also been linked with suppression of apoptosis. This study showed that phosphorylation of FAK at Y397 and Y925, as well as the kinase activity of FAK, are required for the observed effects on cell survival[87,88]. In addition, attenuation of FAK activity by either antibody injection[89,90], antisense oligonucleotides[91] or expression of the isolated focal-adhesion targeting domain all lead to induction of apoptosis[92–94].

FAK inhibition has been shown to have synergistic effects with inhibition of EGF-receptor signalling, leading to apoptosis in breast cancer cells[95]. Also, simultaneous inhibition of SRC and FAK robustly induces apoptosis in some colon cancer cell lines, whereas inhibition of FAK function alone more modestly induces cell death[96]. The ability of FAK to protect cells from death also varies between cancer cell lines. In one breast cancer cell line, inhibition of FAK alone caused apoptosis[96], whereas two other studies have shown that expression of the FAK amino-terminus, which causes dephosphorylation of FAK-Y397 and activation of caspase-3, leads to apoptosis. However, expression of this portion of FAK in normal breast cells had no effect on cell survival[97,98], raising the intriguing possibility that suppressing FAK function blocks survival pathways only in malignant cells.

So, overexpression of FAK in human tumour cells might contribute to malignancy by promoting survival under conditions that would normally lead to cell death. Some recent work has elucidated a mechanism by which FAK might achieve this. Kurenova *et al.* showed that the ability of FAK to suppress apoptosis is mediated by binding to receptor-interacting protein (RIP)[99], a major component of the DEATH-RECEPTOR COMPLEX, which has been shown to interact with both FAS and tumour-necrosis factor. FAK could sequester RIP from the death-receptor

DEATH-RECEPTOR COMPLEX
A multiprotein complex involved in the cellular response to pro-apoptotic stimuli. It links cell-surface receptors to the intracellular signalling cascade that accompanies programmed cell death.

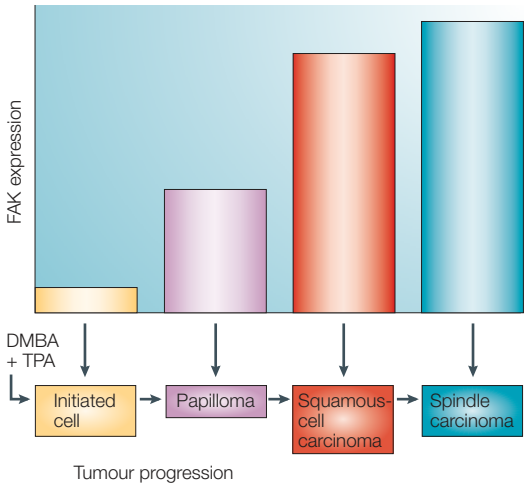

Figure 5 | **Expression of focal-adhesion kinase during tumour progression in the mouse skin carcinogenesis model.** Diagrammatic representation of the various stages in tumour progression that result from chemical initiation with 7,12-dimethylbenz[*a*]anthracene (DMBA) and promotion with 12-*O*-tetradecanoylphorbol-13-acetate (TPA). The relative focal-adhesion kinase (FAK) expression levels from cell lines generated from the various defined stages in tumour progression in the skin carcinogenesis model are depicted.

complex, suppressing apoptosis[99]. If this proves to be a common mechanism, disrupting the interaction between FAK and RIP might be a useful intervention strategy.

The importance of loss of FAK-mediated survival to the behaviour of tumour cells is primarily two-fold. First, adhesion-dependent survival, which is often lost in tumour cells, is mediated by FAK[85,91]. So, increased FAK expression might promote matrix-independent survival of tumour cells, which is required for invasion and metastasis. Support for this comes from studies in which FAK protein levels have been suppressed by RNA interference (RNAi), resulting in anoikis[100]. Second, the modulation of FAK protein levels influences the sensitivity of tumour cells to various chemotherapeutic agents[13,87,101] and ultraviolet irradiation[86]. Again, this could be linked to the ability of FAK to protect against anoikis. In support of this, signalling from β1-integrins is involved in matrix-dependent protection from drug-induced apoptosis in small-cell lung cancer cells[102]. In acute myeloid leukaemia cells, however, the increased sensitization of cells to daunorubicin following decreased FAK expression is not dependent on the matrix[13]. So, although the exact mechanisms by which FAK contributes to aberrant survival of tumour cells have yet to be worked out, the level of FAK protein in tumour cells might contribute to clinical outcome, particularly the response to therapeutic regimens[13].

A role for FAK in the growth of tumour cells. Several studies have indicated that FAK has a direct role in tumour growth. For example, inhibition of FAK signalling downstream of the urokinase receptor, by use of the well-characterized dominant-negative FAK variant, FAK-related non-kinase, suppresses proliferation of Hep3 cells *in vivo*[103]. Induction of dormancy correlates with reduced signalling through FAK, RAS and extracellular signal-regulated kinase (ERK)–MAPK, and dormancy can be reversed by expression of an active mutant of MEK1 (MAPK/ERK kinase 1). These findings indicate that FAK signalling through the ERK–MAPK pathway is required to maintain the growth of at least some tumour cells[103]. Interestingly, FAK signalling through SHC to the RAS and ERK–MAPK pathway has also been shown in anaplastic astrocytoma samples, and this correlates with proliferation of tumour cells[104], although in U251MG astrocytoma cells in culture, FAK forms a complex with p120RASGAP, leading to increased RAS activity associated with proliferation of tumour cells[105].

FAK levels affect carcinogenesis

Most of the evidence linking FAK to tumorigenesis has been circumstantial, positioning FAK 'at the scene of the crime'. However, there is now evidence that FAK mediates both tumour formation and malignant progression. In a study using the two stage 7,12-dimethylbenz[*a*]anthracene/12-*O*-tetradecanoylphorbol-13-acetate (DMBA/TPA) MOUSE CARCINOGENESIS MODEL (described in REF. 106), FAK expression is increased in a stepwise fashion in cell lines derived from various stages of the mouse skin carcinogenesis model (FIG. 5). Furthermore, phosphorylation of FAK at Y925, which has been identified as an important site for phosphorylation by SRC, is increased in several malignant tumours that were examined from this model (G.M., unpublished observations).

When *Fak*$^{+/-}$ mice are analysed in the same skin carcinogenesis model, the reduced FAK expression is sufficient to impair papilloma formation, compared with wild-type controls[107]. However, although this work demonstrated a clear link between the level of FAK protein expression and the propensity to form benign tumours, it was not possible to assess the role of FAK in the malignant progression of papillomas to squamous-cell carcinomas, because the levels of FAK protein were increased in papillomas from both *Fak*$^{+/-}$ and wild-type mice to a similar extent[107]. As a result, there was no visible difference in the conversion rates between *Fak*$^{+/-}$ and wild-type mice[107]. These findings indicated that there is a selection pressure that raises FAK expression during tumour formation.

Using an INDUCIBLE Cre–LOX SYSTEM to delete FAK expression specifically in epidermal cells and in the hair follicles[108,109], papilloma formation is significantly reduced. When papillomas did form in the FAK-deleted skin, conversion to squamous-cell carcinomas was also substantially reduced[109]. These findings provide compelling evidence that FAK contributes to both tumour formation, and the acquisition of malignancy, at least in this model[109].

DMBA/TPA MOUSE SKIN CARCINOGENESIS MODEL
Two-stage chemical carcinogenesis model that progresses from normal skin to benign papillomas to invasive tumours through several well-characterized stages. An initial treatment with DMBA serves as the tumour initiator followed by treatment with TPA as the promoter during tumour formation.

INDUCIBLE Cre–LOX SYSTEM
Method for the introduction of genetic modifications into specific genes by homologous recombination using Cre, a site-specific bacteriophage-P1-derived recombinase. The Cre recombinase cuts at the LOXP-tagged genes.

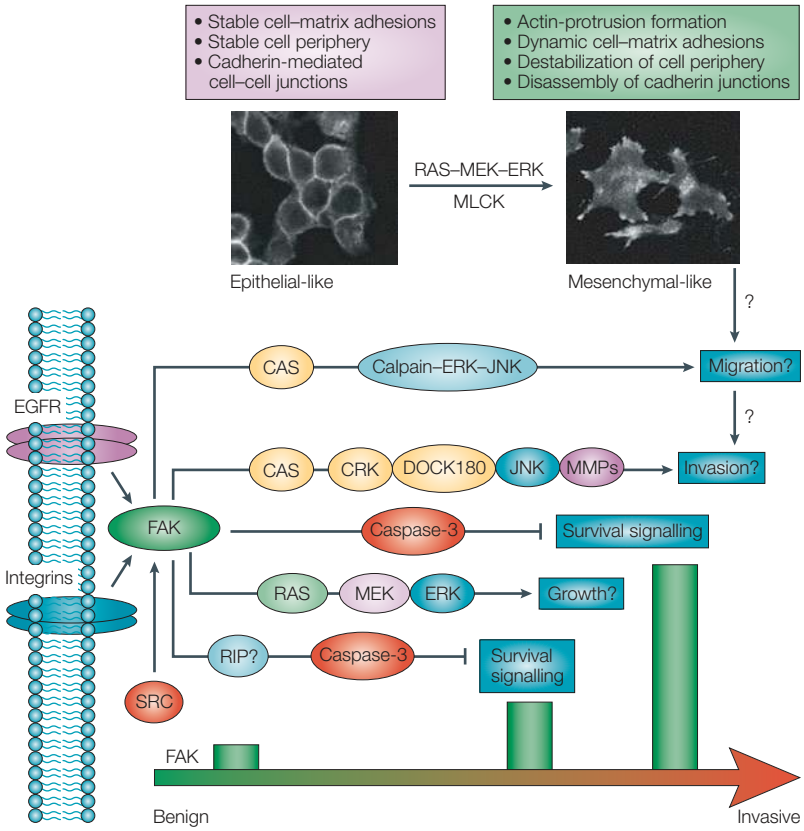

Figure 6 | **Model of the possible contributions of focal-adhesion kinase in cancer development.** Phosphorylation of focal-adhesion kinase (FAK) by SRC not only regulates its kinase activity and localization, but also the formation of phosphorylation-dependent protein complexes, integrin- and E-cadherin-mediated adhesions, and cellular motility and invasion. FAK-Y925 phosphorylation is particularly associated with SRC-induced adhesion changes associated with the epithelial–mesenchymal transition (FIG. 1). KM12C colon cancer cells stained with anti-E-cadherin (left panel) or anti-paxillin (right panel) indicate the morphological differences associated with phosphorylation of FAK[119]. These effects cause an epithelial–mesenchymal transition and could contribute to migration of cancer cells, perhaps at the tumour edges, although this has not been shown. Depicted is FAK-mediated induction of the invasive pathway involving signalling to RAC1 and JUN N-terminal kinase (JNK) and matrix metalloproteinases (MMPs). Shown also is the clear role of FAK in preventing apoptosis downstream of integrin or growth-factor-receptor signalling, as judged by its requirement to keep caspase-3 inactive, the latter associated with tumour formation and progression in a mouse model[109]. The role proposed for FAK in contributing to growth through the RAS–MAPK (mitogen-activated protein kinase) pathway is also shown. ERK, extracellular signal-regulated kinase; EGFR, epidermal growth factor receptor; DOCK180, dedicator of cytokinesis of 180kDa; MEK, MAPK/ERK kinase; MLCK, myosin light-chain kinase; RIP, receptor-interacting protein.

The role of FAK inskin carcinogenesis model

The role of FAK as a regulator of migration and cell survival prompted testing of which, if either, of these activities is associated with tumorigenisis in the skin carcinogenesis model[109]. These activities were investigated *in vitro* (using an inducible model of expression in mouse keratinocytes) and *in vivo* by examining mouse skin. *Fak*-null keratinocytes are unable to repopulate a disrupted monolayer like normal keratinocytes. This is consistent with reduced cell migration, and the tracking of individual FAK-deficient keratinocytes by time-lapse microscopy confirmed reduced rates of migration (by about 50%), although *Fak*-null cells were still visibly motile[109]. However, fewer *Fak*-null cells also remained in the monolayer, consistent with detachment and/or cell death. Collection of adherent and detached keratinocytes revealed that FAK loss of expression leads to aberrant cell-cycle profiles and cell death[109].

In mice, FAK-deficient keratinocytes are capable of re-forming the epithelium of wounded skin[109]. This observation is surprising, as it seems at odds with the extensive literature from *in vitro* experiments describing a key role for FAK in cell migration (reviewed in REFS 14,110) and, indeed, evidence from the same study that FAK-deficient keratinocytes display impaired migration *in vitro*[109]. These apparently paradoxical findings probably reflect a difference between cells migrating as isolated cells with no cell–cell contact, and the forward movement of epithelial sheets to repair wounded skin *in vivo*. In this regard, it is known that FAK can promote N-cadherin-mediated intercellular adhesion, and suppress migration of epithelial sheets, by downregulating peripheral RAC1 activity[82].

Although there was no obvious effect of FAK deletion on the epithelial repair of wounds in mouse skin, we cannot rule out that FAK-dependent influences on some types of tumour-cell migration — perhaps of isolated cells or small groups of cells that have undergone a mesenchymal transition at the leading edges of migrating sheets, or at the edges of tumours — might have a role in invasion of malignant cells. However, these findings highlight the importance of developing physiologically relevant 3D *in vitro* culture systems, at least for the movement of epithelial sheets, and for testing the effects of molecular or pharmacological intervention in modulating the migration of cells *in vivo*.

In contrast to the disparity between the *in vitro* and *in vivo* migration results, FAK-deficient keratinocytes did have increased rates of cell death both *in vitro* and *in vivo*. Increased levels of activated caspase-3 staining, a reliable marker of apoptosis in tissue sections[111,112], are present in both skin and papillomas from FAK-deficient mice when compared to normal controls[109]. Interestingly, in skin, caspase-3 staining is strongest in cells of the hair follicles, where most target cells for DMBA-induced carcinogenesis probably reside[113]. Taken together, these data are consistent with FAK-dependent survival signalling being required for tumour formation and progression in the DMBA/TPA mouse skin carcinogenesis model[109].

Future directions

FAK has been causally implicated in tumour development *in vivo*, albeit in a single mouse carcinogenesis model (FIG. 5). Much more work needs to be done to fully determine the role of FAK in tumorigenesis, which might vary in different tumour types. For example, as discussed in this article, contributing effects could come from cell-cycle and survival signalling, effects on cadherins or other cell-adhesion molecules, on migration and invasion of mesenchymal-like cells, on MMP production, and on other cancer-associated processes (FIG. 6). The causal role of FAK in tumour development, combined with reports that increased FAK expression is associated with poor clinical outcome[13,114], indicate that FAK might be a useful therapeutic target[54].

There is considerable interest and effort now in the development of tyrosine-kinase inhibitors as cancer treatments. However, in the case of FAK, the requirement for kinase activity in the development of cancer has not been proven — therefore, it is not clear whether targeting this domain will have any therapeutic benefit. Indeed, studies in cell lines have demonstrated that the ability of FAK to regulate cell motility and invasion cannot be wholly attributed its kinase activity[29,71,115]. By contrast, the kinase activity of FAK does seem to be required for adhesion-regulated survival[85]. It is important now to determine whether this is required for the contribution of FAK to tumour development. Although no inhibitors of FAK are currently being evaluated in clinical trials, patents have been taken out on several FAK-inhibitory compounds; however, no animal studies using these compounds have been reported so far. These compounds are based largely on pyrimidine derivatives that are ATP competitors. It is also not clear whether continued FAK expression and activity is required for maintenance of the tumour phenotype — this is likely to vary between tumour types, and is an important factor in determining the clinical use of FAK kinase inhibitors.

In addition to its kinase activity, FAK is also an important molecular adaptor that regulates the dynamics of multiprotein complexes. So it might be possible to specifically block the interaction between FAK and its key binding partners as a therapeutic strategy. For example, the interaction between signalling effectors and important FAK phosphotyrosine residues, such as Y397 and Y925, or with the SH3-binding proline-rich sequences of FAK, could be targeted. Such approaches have been used for other signalling proteins, including SRC and GRB2. Inhibitors of the SRC SH2 and SH3 domains have been based on modifications of cognate peptide sequences, or have been identified by screening of non-peptide compounds based on the preferred binding sequences of the SRC SH2 and SH3 domains[116].

However, this approach has recognized problems, due to relatively low-affinity interactions, promiscuity, and problems with delivery and recognized redundancy in achieving signalling end points. For these reasons, such strategies do not yet have a high success rate, but the possibility of achieving specificity with limited toxicity means that these strategies should continue to be pursued. There have been some promising results with inhibitors of GRB2 protein–protein interactions. For example, SH2 inhibitors that suppress GRB2 binding to the EGF receptor prevent downstream activation of RAS signalling and growth of tumour cells[117]. By analogy, specifically targeting particular adaptor functions of FAK might be an interesting approach.

In the case of FAK, there is some evidence that RNAi might be a valuable therapeutic tool. Systemic administration of FAK siRNA to mice results in inhibition of metastasis in a model of pancreatic cancer[100]. Furthermore, FAK siRNA potentiates gemcitabine-induced cytotoxicity *in vivo*[101], raising the possibility that these therapeutic approaches could be potentially useful in combination with conventional cytotoxic agents.

Although current data implicate the survival function of FAK as an important contributor to tumour formation and progression in a mouse skin carcinogenesis model[109], there remains considerable circumstantial evidence implicating FAK as a regulator of cancer-cell motility and invasion. Indeed, it is likely that the kinase and/or adaptor functions of FAK contribute to both positive and negative outcomes regarding cancer-cell motility and invasion that are context dependent. These outcomes are perhaps controlled by tumour cell type or the localized production of motogenic growth factors, as well as production of the ECM substrates and expression and activities of the integrin receptors involved.

Further studies in additional tumour models are required to determine the range of physiological functions of FAK in different tumour types, and particularly to assess whether FAK has a role in metastasis. Increasing our knowledge of where, and when, the diverse functions of FAK are required during cancer progression, and pursuing the development of clinically relevant biomarkers of the biochemical and biological activities of FAK, will aid the design and future evaluation of therapeutic strategies that target FAK function.

Note added in proof

A very recently published paper by Van de Water and colleagues has demonstrated that FAK is required for metastasis in a syngeneic rat model[122]. Specifically, inducible expression of an inhibitory FAK protein, FAK-related non-kinase (FRNK), in MTLn3 mammary adenocarcinoma cells, suppressed the growth of primary tumours and blocked metastasis formation in the lungs. Importantly, in this case, FRNK expression was linked to adhesion and migration defects, rather than induction of apoptosis. This work implies that impaired signalling through FAK can eliminate the targeting of tumour cells to the lungs - most likely by blocking the adhesion and migration of breast cancer cells, processes that are probably required for establishment of distant metastasis - and confirms that FAK can contribute to tumour development in diverse ways.

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