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Adhesion-linked kinases in cancer; emphasis on Src, focal adhesion kinase and PI 3-kinase

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Abstract

Our understanding of the complex signal transduction pathways involved in signalling within cancer cells, between cancer cells and between cancer cells and their environment has increased dramatically in recent years. Here we concentrate on three non-receptor kinases: Src, focal adhesion kinase (FAK) and phosphatidylinositol 3-kinase (PI 3-kinase). These form part of a complex network of intracellular signals which is thought to be important in regulating cancer cells. © 2000 Elsevier Science Ltd. All rights reserved.

Keywords: Src; Focal adhesion kinase (FAK); Phosphatidylinositol 3-kinase (PI 3-kinase); Adhesion; Cell signalling

1. Src family kinases

The Src family of protein tyrosine kinases, part of a complex network of intracellular signals (Fig. 1), comprises a number of related signalling molecules whose precise biological functions appear as diverse as they are poorly understood. c-Src itself is the prototype member of the family and, despite early descriptions of its oncogenic properties in avian systems, its role in human cancer has never been established experimentally. However Src is implicated in a variety of cellular processes that are linked to cancer invasion and metastasis, making it an intriguing potential target for intervention.

In 1911 Rous demonstrated that an avian sarcoma could be transmitted between chickens by a non-cellular element [1]. The responsible agent was subsequently found to be the retrovirus now known as Rous sarcoma virus and the transforming viral oncogene to be *v-src* [2]. The cellular homologue of *v-src* is *c-src* and its protein product, c-Src, is a member of a larger family of related tyrosine kinases that includes Fyn, Yes, Lck, Blk, Lyn, Hck, Yrk and Fgr. c-Src, Fyn and c-Yes are ubiquitously expressed in mammalian cells whilst the others are more restricted in their expression. The following discussion is concerned with v-Src and c-Src although other members of the family may also be important in human cancers.

1.1. Molecular structure and regulation of Src

Src is located in the cytoplasm, at cellular sites of integrin clustering (the so-called focal adhesions in fibroblasts) and at cadherin-mediated cell–cell adhesions in epithelial cells. Src structure is shown in schematic form in Fig. 2. Amino terminal myristylation is required for association with the plasma membrane. In its inactive state Src is located around the perinuclear region of the cell, most likely in association with endosomal membranes [3,4]. Relocation of Src from the cytoplasm to the cell periphery after activation requires an organised actin cytoskeleton controlled by the Rho family of small G proteins [4]. Amongst its substrates is Src itself (autophosphorylation is at tyrosine-416 [5]), the p85 regulatory subunit of phosphatidylinositol 3 kinase (PI 3-kinase) [6] and the focal adhesion kinase (FAK) (discussed below). These particular Src substrates, and others not discussed here, bind to Src via the Src homology (SH) domains, SH3 and SH2, that interact with proline-rich and phosphotyrosine-containing sequences respectively in these proteins (Fig. 2b). The carboxy-terminal tyrosine residue (tyrosine-527) is critical to the regulation of Src activity. When this residue is phosphorylated

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Src undergoes a conformational change whereby phosphotyrosine-527 interacts with its own SH2 domain rendering the Src protein catalytically inactive (Fig. 2a). It is loss of this terminal regulatory region that confers the key transforming properties of *v-src* [7]. c-Src is inactivated by a terminal tyrosine kinase, Csk, that phosphorylates tyrosine-527 [8]. The identity of the phosphatase(s) responsible for activating Src *in vivo* by dephosphorylation of this residue has not been established.

1.2. Activation of Src by extracellular stimuli

Src kinase is activated upon ligand binding of a variety of receptor tyrosine kinases (RTKs) and is involved in downstream signalling from such receptors [9]. In some cases, Src directly interacts with the RTK at the cell membrane [10] and studies using cells deficient in expression of the ubiquitous Src family kinases indicate that Src is essential for at least some of the biological consequences induced by growth factors [11], including cell motility induced by platelet derived growth factor (PDGF) [11].

Src can also be activated by extracellular matrix (ECM) contact and this is mediated by the integrin receptors [12]. Integrins form the fundamental subunits of focal adhesions and are largely responsible for cell–matrix adhesion. Although Src family proteins are apparently not essential for integrin-mediated 'outside-in' signalling in response to cell–ECM interactions [11], Src is involved in the turnover of focal adhesions that is needed for cell migration most likely in conjunction with FAK [13]. Src's role in the process of cell migration could be one way in which its activity influences cancer cell invasion.

In epithelial cells the ubiquitous Src family kinases co-localise with E-cadherin at cell–cell adhesions [14]. Analogous to Src's role in regulating focal adhesion dynamics in mesenchymal cells the catalytic activity of Src induces the disassembly of cadherin-dependent cell–cell adhesions and is required for epithelial cell scattering [15]. This particular function of Src might contribute to cancer cell release from epithelial tumours that have retained functional E-cadherin. In this regard, inhibitors of Src catalytic activity suppress both the release of E-cadherin-expressing colon cancer cells from an epithelial sheet and ECM-dependent invasion *in vitro* (Fig. 3).

1.3. Role of Src in cell growth and survival

The precise downstream events following Src activation are not clear but it has a role in signal transduction pathways which result in a variety of endpoints that might be important for the development of malignancy including cell growth, cell survival, invasion and metastasis (see Table 1).

Src has been implicated in the control of gene expression. One of its downstream effectors, the mitogen activated protein (MAP) kinase pathway, for example, is involved in the regulation of the activator protein (AP)-1 transcription complex which is intimately involved in invasion [16]. Furthermore, Src is needed for growth factor-induced cell cycle progression, at least under some conditions, suggesting a role in growth control [17]. In this context *v-src* transforms avian and mammalian fibroblasts conferring altered proliferation and

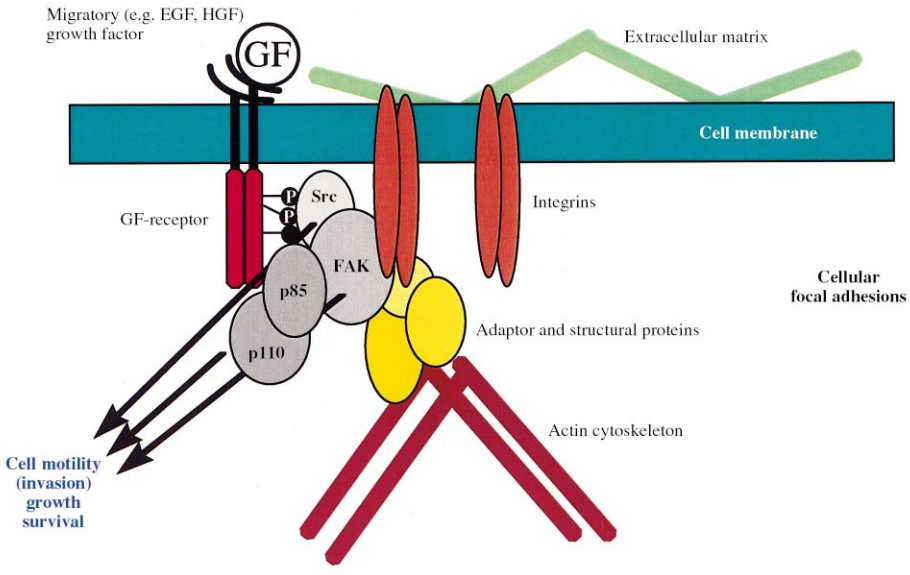

Fig. 1. Kinase-mediated control of cancer cell behaviour from integrin adhesions. GF, growth factor; EGF, epidermal growth factor; HGF, hepatocyte growth factor; FAK, focal adhesion kinase.

anchorage-independent colony growth [18,19]. In addition, Src is activated during mitosis and a mitosis-specific substrate, SAM68, has been identified [20,21].

Fibroblasts deficient in c-Src display reduced motility in tissue culture and also display an impaired ability to spread on plastic [12]. In addition, Src appears to have a role in fibroblast adhesion to the ECM [22]. *In vitro* studies also suggest that Src can confer upon colonic adenoma cells the ability to invade through the ECM [23,24] and activation occurs in carcinoma cells as they invade through the ECM [25]. Src has also been implicated in the regulation of matrix metalloproteases [26]

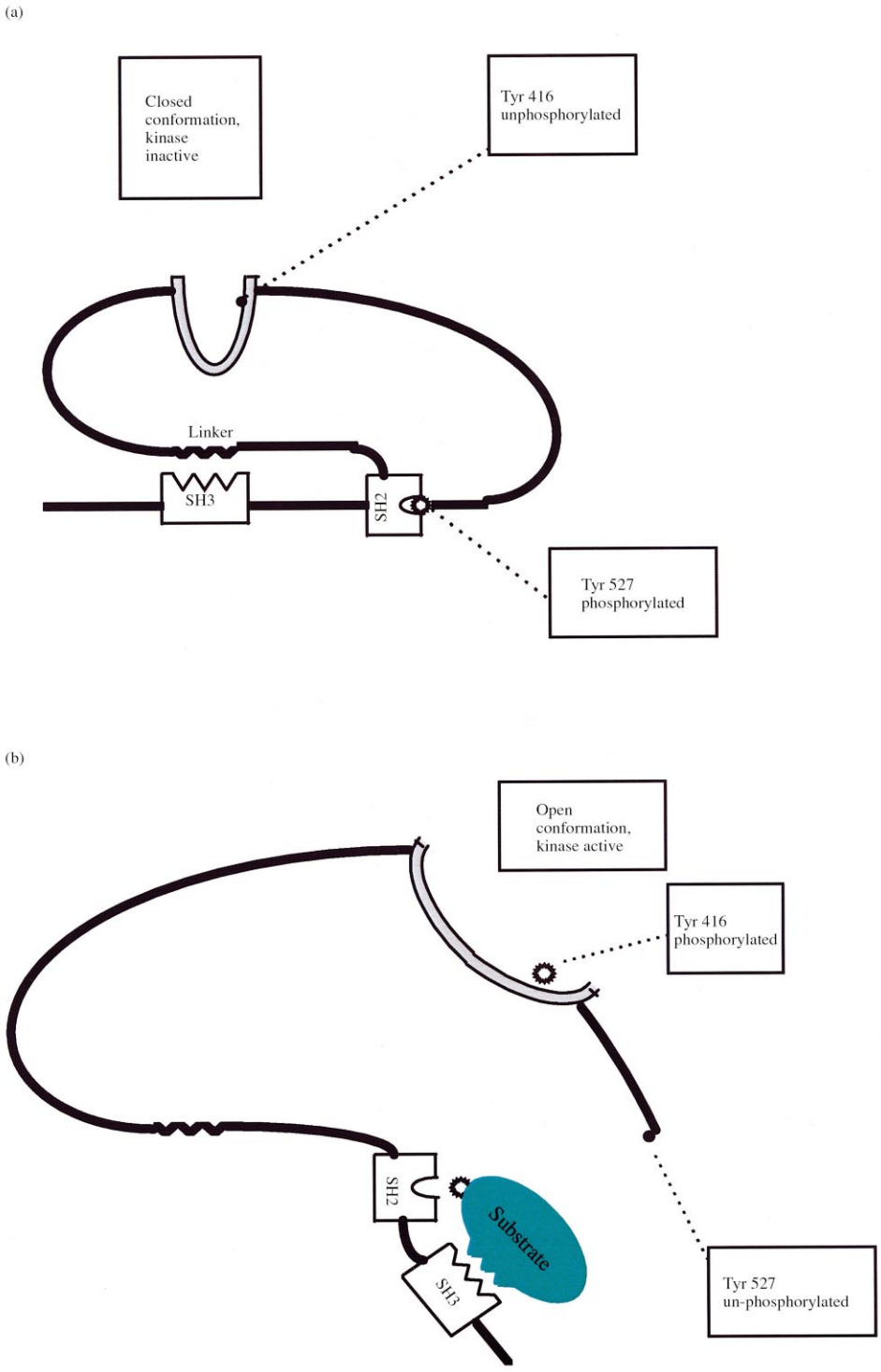

Fig. 2. Structural regulation of Src. (a) Closed conformation, kinase inactive. (b) Open conformation, kinase active.

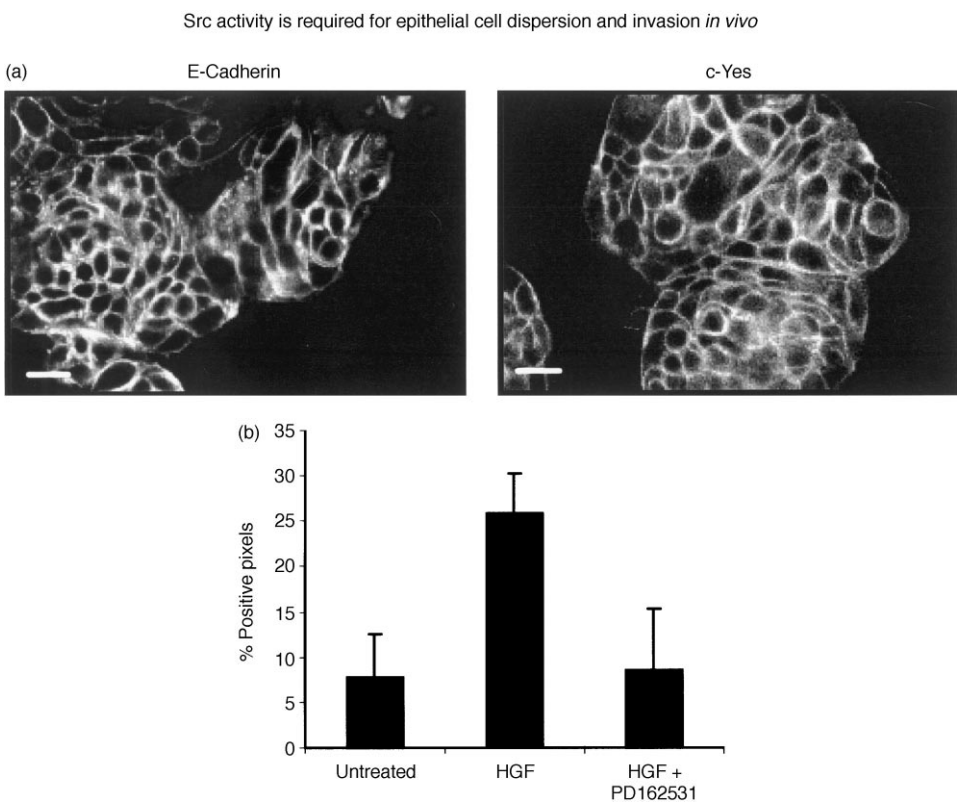

Fig. 3. (a) ALT-G colon carcinoma cells were grown on a reconstituted basement membrane preparation (Matrigel). Cells were fixed and stained with antibodies to E-cadherin and the Src family member c-Yes. c-Yes was present at sites of cell–cell contact where it co-localised with E-cadherin. Scale bar 25 μm. (b) ALT-G cells were able to invade into Matrigel gels in the presence of HGF, which was quantitated as previously described [25]. HGF-dependent invasion was inhibited by the Src kinase inhibitor PD162531 (provided by Alan Kraker, Parke-Davis, Ann Arbor, MI, USA and described in [14]).

and hypoxia-induced vascular endothelial growth factor (VEGF) expression [27].

Src probably also plays a role in signalling pathways leading to cell survival, most likely mediated by the PI 3-kinase/Akt pathway discussed below [28,29]. In particular, Src might couple lymphokine receptor activation with inhibition of apoptosis via activation of Akt/protein kinase B (PKB) [29] suggesting another way in which Src might influence angiogenesis (for review see [30]) and, consequently, tumour progression.

1.4. Animal models for addressing Src's involvement in cancer

The *in vitro* evidence discussed above supports a role for Src in tumour growth, invasion and metastasis.

Table 1
Roles of Src, FAK and PI 3-kinase in cancer

Role of Src in cancer	Role of FAK in cancer	Role of PI 3-kinase in cancer
Increase in cell motility	Involved in cell adhesion	Regulation of cell survival
Increase in cell invasion	Increase in cell spreading	Role in control of motility
Increase in cell adhesion	Involved in cell migration	Role in control of invasion
Increase in cell spreading		
Transforming mutants	Involved in cell survival pathways	Closely linked to *PTEN* tumour suppressor gene
Regulation of gene expression	Involved in transforming pathways	
Cellular transformation		Transformation of fibroblasts
Cell cycle progression	Involved in mitogenic pathways	
Regulation of cell survival		
Implicated in metastasis	Implicated in regulation of matrix proteases	
Implicated in tumour growth		
Implicated in regulation of matrix proteases		
Implicated in angiogenesis		

PTEN, phosphate and tensin homolog; FAK, focal adhesion kinase; PI 3-kinase, phosphatidylinositol 3-kinase.

Clearly it is an important step to try and establish whether this is the case in animal models of cancer. In this context, expression of antisense *src* reduces the tumour growth rate in experimental colonic adenocarcinoma [31] and ovarian carcinoma [32] in mice. Likewise, overexpression of wild-type *c-src* in colorectal cancer cells can increase the growth rate of experimental primary tumours [33]. With regard to metastasis, endogenous Src expression is increased in secondary tumours compared with their primary counterparts in an animal model of colorectal cancer metastasis [34]. In addition, expression of *v-src*, or activated *c-src*, can confer on cells the ability to colonise the mouse lung when injected into the tail vein [35,36]. However, overexpression of wild type *c-src* in a colon tumour cell line that has low metastatic potential can increase the primary tumour growth rate but apparently does not increase the likelihood of metastasis [33].

1.5. Evidence for a role for Src in human cancer

A recent report has suggested that an activating mutation of tyrosine-527 in the carboxy-terminal regulatory region of Src (which would have similar functional consequences to deletion of the carboxy-terminal region that conferred transforming properties on *v-src*) is found in a subset of highly aggressive metastasising bowel tumours [36]; however, another study could not substantiate these findings [37]. Notwithstanding the failure to conclusively demonstrate a primary genetic change in human tumorigenesis, the expression and activity of Src is increased in a variety of human cancers, including ovary [32], breast [38], head and neck [39] and pancreas [40]. This is probably best characterised in colorectal cancer where there seems to be a stepwise increase in expression from normal epithelium through the premalignant stages to metastatic tumours [41–43]. In addition, there is evidence of increased activity of Src-terminal tyrosine phosphatase in human breast cancer cell lines [44]. Thus, there is a body of evidence that a primary abnormality in the molecular events upstream of Src expression and activation has occurred in human epithelial cancers. Fig. 4 summarises the potential functions of Src during epithelial tumour progression.

2. Focal adhesion kinase

FAK was discovered in the early 1990s as a tyrosine phosphorylated protein in Src transformed cells [45], and in a screen designed to identify novel tyrosine kinases [46]. Subsequently, FAK was found to reside at cellular focal adhesion sites where integrins cluster [45–47]. The primary sequence of FAK is well conserved between species, with greater than 90% identity between the amino acid sequences of chicken, mouse and human [45,46,48,49]. Its structure differs from other non-receptor tyrosine kinases in that it contains no SH domains in its non-catalytic regions (Fig. 5). FAK contains a central catalytic domain that is flanked by a large amino-terminal region containing a putative β1-integrin binding site [50], and a carboxy-terminal region that contains a number of potential protein interacting sites, including two proline-rich domains that can bind $p130^{CAS}$ or the Rho GTPase activating protein Graf

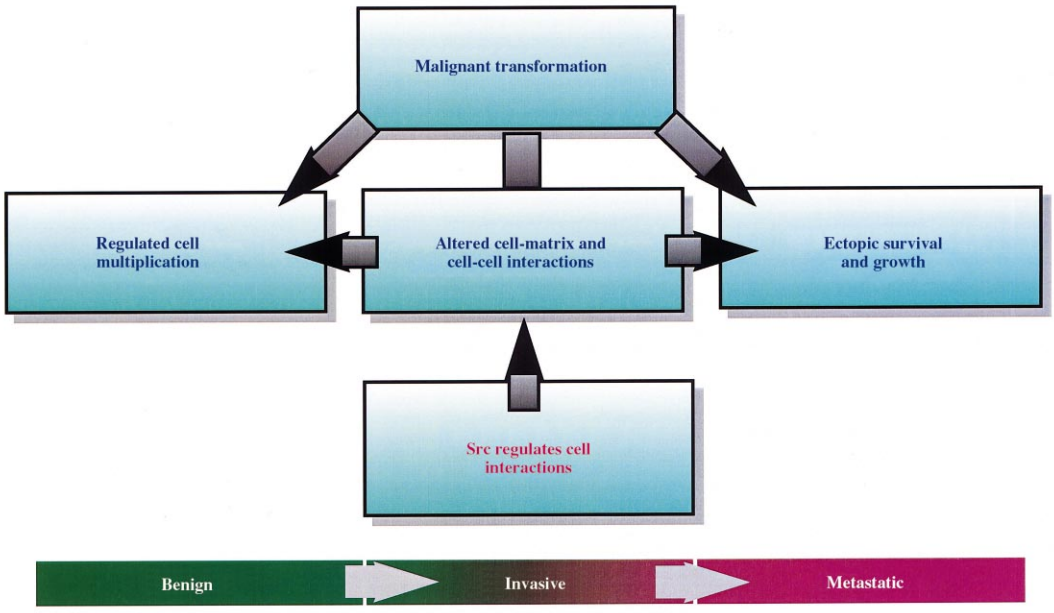

Fig. 4. Src functions might contribute to epithelial tumour progression at various stages. Contributed by JA Wyke, Beatson Institute, Glasgow, UK.

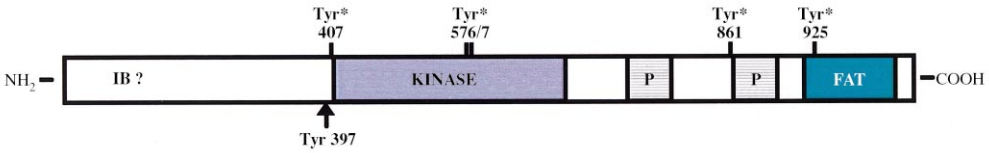

Fig. 5. FAK domain structure. P, proline-rich sequences; Tyr*, Src-specific phosphoacceptor sites; Tyr 397, putative autophosphorylation site; FAT, focal adhesion targeting sequences; KINASE, catalyic domain; IB, putative BI-integrin-binding domain.

[51,52]. In addition, the carboxy-terminal region of FAK contains sequences responsible for its focal adhesion targeting of FAK (FAT domain; [53]), which overlap with the sequences that bind the multi-adaptor protein paxillin [54]. Further regulation of FAK is achieved through alternative splicing, which can result in autonomous expression of a carboxy-terminal portion of FAK, termed FAK-related non-kinase (FRNK) that acts as a molecular inhibitor when overexpressed in cells [55].

In addition to the modes of regulation described, FAK is itself subject to regulatory phosphorylation. Specifically, FAK is autophosphorylated on tyrosine-397, which results in increased kinase activity after integrin engagement [56–58]. In cells transformed by activated mutants of Src, FAK is phosphorylated on a number of other tyrosine residues that are carboxy-terminal to tyrosine-397 [57,59–61]. Furthermore, in the case of v-Src there is evidence that the integrin- and Src-specific phosphoacceptor sites might be distinct, providing evidence that different stimuli can induce phosphorylation of FAK on distinct tyrosine residues, linking specific phosphorylation events to ensuing biological responses [62]. A model has been proposed whereby integrin-induced autophosphorylation of tyrosine-397 creates a high-affinity binding site for the Src family kinases via their SH2 domains [46,57,58,60], and also for the p85 regulatory subunit of PI 3-kinase [63]. Furthermore, the process of Src binding, which requires displacement of the intramolecular Src SH2–tyrosine-527 interaction [64]; see Fig. 2), might contribute to Src activation and enhance phosphorylation of FAK on its carboxy-terminal sites. Consequently, downstream signalling is stimulated by the creation of additional phosphotyrosine binding sites for SH2 domain-containing proteins, including Grb2 that link integrin engagement to the Ras/MAP kinase pathway [59,65]. FAK also mediates the integrin signalling requirement for growth factor activation of MAP kinase [66]. However, there is also evidence for FAK-independent mechanisms of integrin-induced MAP kinase activity [67].

2.1. FAK function

Early experiments indicated that FAK was at a point of convergence of a number of signalling pathways associated with cell adhesion, mitogenesis and oncogenic transformation (for review see Ref. [68]), suggesting that FAK might contribute to tumour development. Support for this has come from more recent reports of an important role for FAK in fundamental aspects of cell behaviour and cellular responses, including the dynamic regulation of integrin focal adhesions, cell motility and cell survival.

2.2. FAK regulates cell adhesion and migration

FAK's role in cell adhesion is complex. A dominant-negative approach implicated FAK in the process of cell spreading [55,69], whilst v-Src-induced FAK phosphorylation is visibly linked to focal adhesion disruption during transformation [70]. Furthermore, although FAK-deficient mice die during early embryonic development as a result of gastrulation abnormalities similar to those found in fibronectin-deficient animals, cells derived from FAK null embryos exhibit a migration defect as a result of impaired focal adhesion turnover [71]. The role of FAK in cell migration was confirmed by introducing a mutant of FAK that contained the carboxy-terminal focal adhesion targeting sequences, but was deficient in kinase activity [72]. Taken together, these findings imply that FAK is likely to influence both focal adhesion assembly and disassembly, and is required for the dynamic regulation of integrin focal adhesions during cell migration. In keeping with this, enforced expression of FAK can stimulate cell migration [52,73], an effect that is mediated by the binding of $p130^{CAS}$ [52]. In epithelial cells *in vivo*, there is evidence that FAK is rate limiting for cell motility since FAK expression in skin is limited to keratinocytes that are actively migrating after wounding [74].

2.3. FAK has a role in cell survival signalling

Several lines of evidence now implicate FAK in mediating an integrin-induced survival signal. Introduction of a β1-integrin peptide that contains the putative FAK binding site, or a FAK-specific antibody reactive with a region proximal to the focal adhesion targeting domain, results in apoptosis [75]. Conversely, expression of a constitutively activated FAK protein prevents MDCK cells from undergoing anoikis after detachment from the ECM [76]. Also, reduced FAK expression in tumour cells achieved by antisense oligonucleotides, or expression

of a dominant-negative FAK protein, leads to cell detachment and apoptosis [77,78]. In addition, FAK is cleaved by caspase proteases during apoptosis induced by a variety of stimuli in different cell types [79,80]. These reports indicate FAK's involvement in life or death decisions cells make in response to environmental cues. Thus, deregulated FAK could affect the ability of cancer cells to die in response to stimuli that would kill their normal counterparts.

2.4. FAK is deregulated in human cancers

The cellular processes that are influenced by FAK, including mitogenesis, control of cell adhesion, cell migration and matrix-dependent cell survival, as well as a proposed role in matrix metalloproteinase 9 expression [81], are often perturbed in cancer, suggesting that FAK could play a role in tumour development (see Table 1). This is supported by the finding that FAK is elevated in cell lines derived from human melanomas, and that FAK protein levels correlate with the rate of cell migration on fibronectin [82]. FAK is also elevated in primary human sarcomas [83], cervical carcinoma cell lines [84], prostatic carcinoma tumours and cell lines [85], and in colon and breast tumours and cell lines [86]. The latter study inferred a role for FAK in tumour invasiveness *in vivo*. More recently, FAK has also been found elevated in ovarian carcinomas [87] and has been specifically associated with liver metastases of colon cancer [88]. Furthermore, increased dosage, and occasional amplification, of the region of chromosome 8q that encodes the *FAK* gene has been found in human tumours and tumour-derived cell lines of varying origin [89].

The widely reported increases in FAK protein expression, as well as phosphotyrosine content and enzymatic activity [90], provide circumstantial evidence that FAK is associated with tumour development. As mentioned above, FAK could contribute in a number of ways since its activity is linked to many key aspects of cell regulation; however, convincing evidence that FAK is causally involved is still lacking. For this reason, 'proof of principal' experiments are required to directly address FAK's role in tumour progression *in vivo*, and determine how interfering with FAK function influences the stages of tumour development. Such studies will reveal the relevance of FAK for consideration as an anticancer target.

3. Phosphatidylinositol 3-kinase

PI 3-kinase phosphorylates inositol lipids at the 3′ position of the inositol ring, generating PI 3-phosphate (PI (3)P), PI 3,4-bisphosphate (PI (3,4)P_2) and PI 3,4,5-trisphosphate (PI (3,4,5)P_3). These lipid products are involved in a number of cellular processes including cell proliferation, survival, cytoskeletal reorganisation and membrane trafficking (reviewed in [91]).

Classical PI 3-kinase is a heterodimer consisting of a 85 kDa regulatory subunit and a 110 kDa catalytic subunit. The regulatory subunit contains two SH2 domains and an SH3 domain. These domains direct binding of a number signalling proteins including growth factor receptors and oncogenes such as Src and Abl. This association requires the activation of growth factor receptors by their ligands, or activating mutations in proteins such as Src. Indeed, PI 3-kinase was first identified as a lipid kinase associated with the oncoproteins v-Src and middle T antigen; it was subsequently shown that the transforming ability of a number of oncoproteins correlated with their ability to associate with PI 3-kinase (reviewed in [92]). The finding that an activated mutant of the 110 kDa catalytic subunit of PI 3-kinase had been transduced by a transforming avian retrovirus, ASV16 [93], confirmed the oncogenic potential of PI 3-kinase.

Here we discuss the role of PI 3-kinase-dependent signalling pathways in cell transformation and tumour progression.

3.1. PI 3-kinase and the actin cytoskeleton

Whilst early studies on the subcellular localisation of the endogenous subunits of PI 3-kinase using antibodies were inconclusive, recent evidence that PI 3-kinase is an adhesion-linked kinase was obtained by using a fusion protein between the p85 regulatory subunit and green fluorescent protein (GFP) [94]. Current information suggests that one important role for PI 3-kinase in mammalian cells is in regulation of the actin cytoskeleton. Since actin remodelling is associated with cell transformation and with matrix- and growth factor-induced cell motility and cancer cell invasion, the lipid products of PI 3-kinase might contribute to malignant conversion via its effects on these cellular processes (see Table 1). Primary regulators of actin re-modelling are the Rho family of small GTPases, which include Rho, Rac and Cdc42 [95] and there is a considerable body of evidence implicating PI 3-kinase in the control of Rho-mediated actin rearrangements. For example, during Ras transformation of fibroblasts, PI 3-kinase is essential for Ras-induced cytoskeletal rearrangements, acting upstream of Rac in this process [96], whilst in epithelial cells Cdc42 and Rac induce PI 3-kinase-dependent, integrin-mediated motility and invasiveness [97,98]. Furthermore, direct activation of PI 3-kinase itself is able to disrupt epithelial polarisation and induce cell motility and invasiveness [97]. Together with the observation that integrin-dependent invasion of breast cancer cells requires Rac downstream of PI 3-kinase [98], these data support a key role for PI 3-kinase in the acquisition

of an invasive phenotype. One possible mechanism whereby PI 3-kinase may regulate Rac-GTP and consequent cytoskeletal rearrangements is via Vav, which is a guanine exchange factor for Rac. Vav contains a pleckstrin homology (PH) domain that binds selectively to phosphoinositol lipids and can act as a potential effector of PI 3-kinase. PI (3,4,5)P_3 binds to the PH domain of Vav and enhances its activation by the tyrosine kinase Lck [99].

3.2. PI 3-kinase and cell survival

Transformation of cells by oncogenes such as Ras and Src is accompanied by the ability of cells to grow and survive in suspension. This phenomenon is also likely to play an important role in tumour progression; in particular the ability of cells to metastasise to distant sites within the body will depend, at least in part, on their ability to survive in the circulation. PI 3-kinase activity is required for growth factor-dependent survival and activated forms of PI 3-kinase can protect cells against cell death induced after detachment from the underlying ECM [100]. These effects of PI 3-kinase are mediated through activation of its effector molecule Akt (also known as protein kinase B, or PKB; reviewed in Ref. [101]). Activation of Akt occurs via binding of PI (3,4)P_2 or PI (3,4,5)P_3 to its PH domain, which results in translocation of Akt to the plasma membrane [102,103]. A second serine/threonine kinase, 3-phosphoinositide-dependent protein kinase (PDK)1, also contains a PH domain and is similarly regulated by the lipid products of PI 3-kinase [104,105]. Phosphorylation of Akt by PDK1 at the plasma membrane is required for Akt activation, although full activation requires phosphorylation of a second residue in Akt by a kinase that is, as yet, unidentified. Several downstream targets of Akt have now been identified as components of the cell's anti-apoptotic machinery, including the Bcl-2 family member Bad and the cell death enzyme caspase 9 (reviewed in [101]).

3.3. PI 3-kinase signalling pathways and tumorigenesis; the tumour suppressor protein PTEN and Akt

Compelling evidence that PI 3-kinase signalling plays a key role in tumour progression came from work on the tumour suppressor protein PTEN, a physiological antagonist of PI 3-kinase. *PTEN* (also known as *MMAC* and *TEP-1*) was first identified as a potential tumour suppressor gene located on chromosome 10q23 whose protein product had homology to the focal adhesion protein tensin [106–108]. Deletions in chromosome ten at this region occur in a number of tumour types, most notably in advanced glial tumours, but also in prostate, endometrial, renal, small cell lung cancer carcinoma and melanoma (reviewed in Ref. [109]). *PTEN* is now recognised as one of the most common targets of mutation in human cancers. Germ line mutations are found in Cowden's disease and Bannayan–Zonana syndrome that are characterised by increased susceptibility to breast and thyroid malignancies (reviewed in [109]).

PTEN was first identified as a dual specificity protein phosphatase, dephosphorylating both tyrosine and serine/threonine phosphorylated proteins [110]. However, it was a relatively poor protein phosphatase *in vitro* and its functionally important substrate *in vivo* is now recognised as PI (3,4,5)P_3, the major cellular product of PI 3-kinase [111]. This lipid phosphatase activity is essential for the tumour suppressor function of PTEN [112] and many PTEN mutations are clustered in the catalytic domain, resulting in non-functional proteins (reviewed in Ref. [109]). Furthermore, restoration of PTEN expression in tumour cells lacking PTEN causes growth suppression. Different mechanisms of PTEN-mediated growth suppression have been identified and these appear to be tumour cell type-dependent. For example, G_1 arrest occurs in glioblastoma cells, whereas induction of apoptosis is evident in prostate and breast tumours [112–115].

Although deregulation of PI 3-kinase in fibroblasts leads to transformation [93], there is very little evidence indicating that PI 3-kinase activity *per se* is upregulated in tumours. In one study, PI 3-kinase activity in colon tumours was found to be higher than normal adjacent mucosa from the same patients, although a relatively small sample size was examined. These authors observed that 86% of the tumour samples had elevated PI 3-kinase activity when compared with the adjacent normal mucosal tissue [116]. However, the finding that PTEN can downregulate the cellular lipid products of PI 3-kinase *in vivo* supports the notion that stimulation of PI 3-kinase effector pathways contributes significantly to tumorigenesis. Indeed, increased levels of PI (3,4,5)P_3 have been found in PTEN-deficient tumour cell lines, as well as in immortalised fibroblasts from PTEN-deficient mice [117,118].

Several studies have now established a link between the PI 3-kinase/Akt pathway and PTEN defects in tumours. The apoptotic action of PTEN in prostate tumour cells [110], and G1 arrest in renal carcinoma cells [119], is overcome by expression of constitutively active forms of Akt. Furthermore, Akt activity is enhanced in PTEN-deficient tumour cell lines [117,120]. Consistent with this, PTEN-deficient fibroblasts are resistant to multiple pro-apoptotic stimuli and have elevated endogenous Akt activity; re-expression of PTEN restores normal Akt regulation and sensitivity to apoptotic stimuli [118]. The reported effects of PTEN on cell cycle progression are also attributed to the activity of Akt, via phosphorylation of glycogen synthase 3 and regulation of cyclin D [121]. As well as

PTEN alterations, amplification of *Akt2* is found in ovarian and pancreatic tumours [122,123]. In addition, the fact that Akt was itself first identified as the product of a transforming retrovirus [124], indicates that deregulation of PI 3-kinase/Akt signalling occurring by other mechanisms can also contribute to malignant behaviour.

PTEN might also play a role in cell migration and invasion. One report suggests that re-expression of PTEN in a glioma cell line that is PTEN-deficient, results in dephosphorylation of FAK and inhibition of integrin-mediated cell migration and invasion [125]. This is apparently dependent on the protein phosphatase activity of PTEN, and not the lipid phosphatase activity, established by using a discriminating mutant PTEN protein containing a G129E mutation found in patients with Cowden's disease. However, other reports indicate that there is no altered FAK phosphorylation in cells from PTEN-deficient mice [126], and no observable dephosphorylation of FAK after re-expression of PTEN in the same glioma cell line, although there is agreement that invasion is inhibited [127]. Thus, although the tumour suppressor functions of PTEN clearly depend on its lipid phosphatase activity, it remains to be established whether the loss of protein tyrosine phosphorylation contributes to this effect. Notwithstanding the discrepancy in proposed mechanisms of action, it seems likely that loss of PTEN's normal function in mediating both cell survival and cell migration (and invasion) is contributing to malignancy in a clinically important way since PTEN mutations are often associated with an aggressive metastatic phenotype.

4. Therapeutic perspectives

Although there are a number of specific inhibitors of Src kinase, PI 3-kinase and, possibly, also FAK under development, there are as yet no data to indicate how these agents might best be used in the clinical setting. If selective inhibitors of these kinases become available it seems unlikely that they would, by themselves, be powerful inhibitors of proliferation. A more attractive postulate is that inhibitors of the adhesion-linked kinases could suppress cancer cell invasion or metastasis, and might be most effectively used as an adjunct to conventional surgical or oncological intervention.

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