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1. ABSTRACT

Cancer cell metastasis involves a series of changes in cell behaviour, driven by oncogenic transformation, that leads to local tissue invasion, migration through extracellular matrix, entry into the vascular or lymphatic system and colonisation of distant sites. It is well established that the Rho family GTPases Rho, Rac and Cdc42 orchestrate many of the processes required during metastasis. The Rho family GTPases regulate cellular behaviour through their interaction with downstream effector proteins. The p-21 activated kinases (PAKs), effector proteins for Rac and Cdc42, are known to be important regulators of cell migration and invasion. There are six mammalian PAKs which can be divided into two groups: group I PAKs (PAK1-3) and group II PAKs (PAK4-6). Although the two PAK groups are architecturally similar there are differences in their mode of regulation suggesting their cellular functions are likely to be different. This review will focus on the latest evidence relating to the role of PAK family kinases in the cell signalling pathways that drive cancer cell migration and invasion.

2. INTRODUCTION

2.1. Cancer cell invasion

A localised primary tumour can often be treated with radical surgery or radiotherapy, but once it has spread to other sites in the body it is almost impossible to eradicate. This facet of cancer progression highlights the need to improve knowledge regarding the mechanisms underlying metastasis with a view to identifying new therapeutic targets and prognostic tools. Cancer cell metastasis involves a series of changes in cell behaviour, driven by oncogenic transformation, that leads to local tissue invasion, migration through tissue, entry into the vascular or lymphatic system and colonisation of distant sites (Figure 1). Invasion of the surrounding stromal tissue requires the co-ordinated regulation of both actin cytoskeletal rearrangement and cell substratum adhesion turnover (1). To successfully migrate through the stromal microenvironment, cells must be able to extend processes (lamellipodia/filopodia/invadopodia), anchor those nascent protrusions to the underlying matrix (cell adhesions), generate the force required for forward movement and ultimately dissolve adhesions at the rear of the cell. It is well established that the Rho family GTPases Rho, Rac and

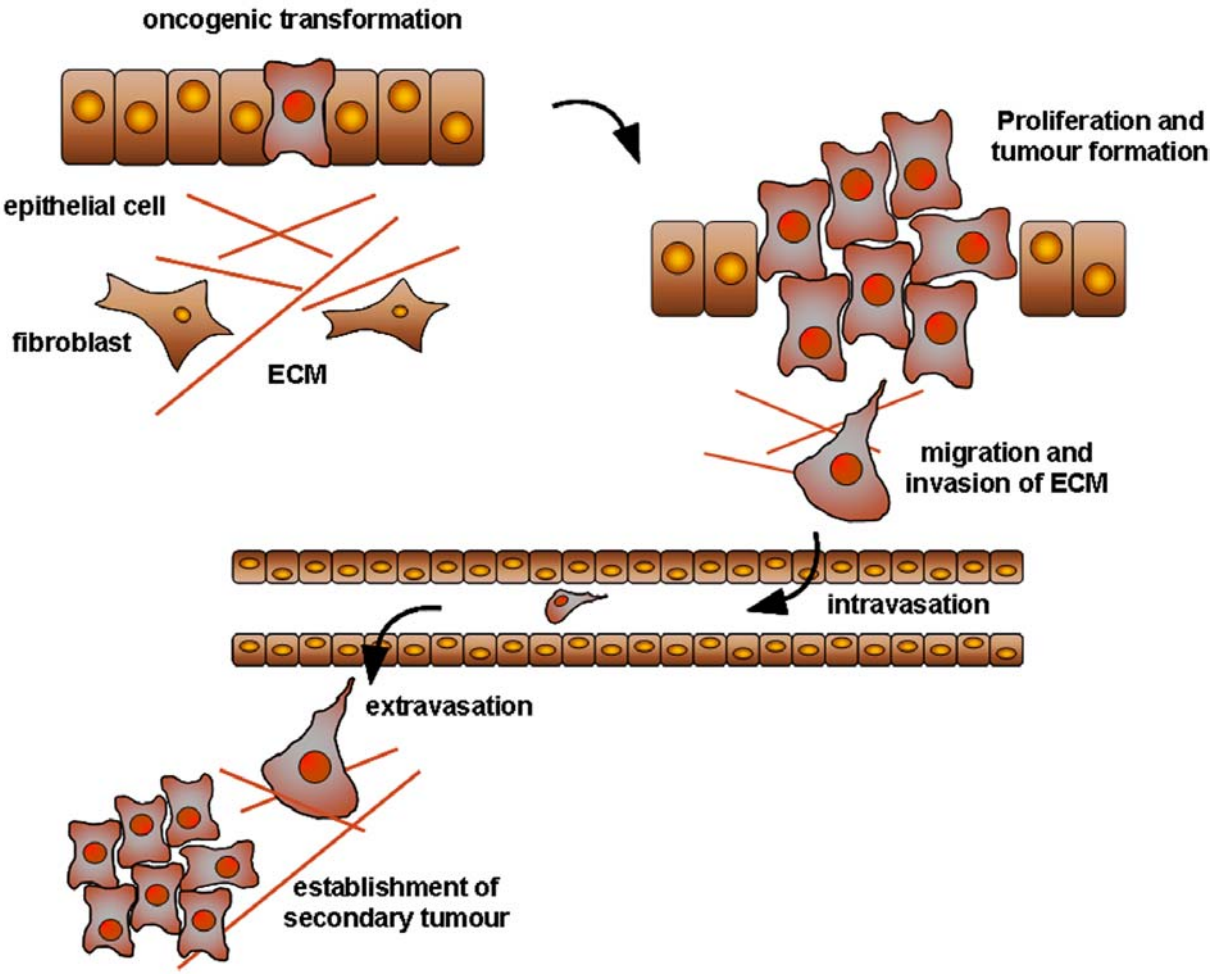

Figure 1. Progression of metastatic cancer. Schematic representation of epithelial carcinoma as a multistage process beginning with oncogenic transformation of cells, aberrant growth and proliferation to form a primary tumour. Tumour cells eventually acquire a migratory phenotype and invade their surrounding extracellular matrix (ECM) in a process possibly involving interplay with non-tumour cells such as fibroblasts. Cells metastasise, entering blood or lymphatic vessels by intravasation. At some point, metastatic cancer cells will attach and extravasate, establishing a secondary site. Non-tumour fibroblast and endothelial cells are shaded brown, and cancerous cells are depicted with red nuclei and highlighted blue.

Cdc42 orchestrate these processes (1). Rho, Rac and Cdc42 are the most studied Rho family GTPases, these proteins act as molecular switches existing in two conformational states, GDP and GTP bound. It is only in the activated GTP bound state that they interact with downstream effector molecules to elicit their cellular response. The intrinsic exchange of GDP for GTP within the Rho family is relatively slow and is accelerated by their association with guanine nucleotide exchanges factors (GEFs). Of the many effector proteins that bind to active Rac and Cdc42, the p-21 activated kinases (PAKs) are amongst the best characterised. This review will focus on the role of PAK family proteins in mediating cytoskeletal signalling events that contribute to cancer cell invasion, but will not detail PAK associated neuronal biology (recently reviewed (2)). We will address current knowledge of upstream regulation, evidence for involvement in tumour progression, contribution to cytoskeletal signalling pathways and relevance to cancer cell invasion.

2.2. PAK family kinases

p21-activated kinase 1 (PAK1) was the first PAK family member to be identified (3) as a serine threonine protein kinase activated by the small GTPases Cdc42 and Rac, followed by the closely related protein kinases, PAK2 and PAK3 (4). More recently three more family members were discovered (PAK4-6) and the six proteins are now divided into two groups (Figure 2) based upon sequence and structural homology (5). PAKs are highly conserved in evolution and have many known substrates whose phosphorylation affects numerous cellular processes, including cytoskeletal organisation, cell cycle progression, and cell survival (6, 7)as well as significant non-kinase related effects (7, 8)

2.2.1. Domain structure

Group I PAKs possess a distinctive N – terminal region that encompasses a p-21 GTPase binding domain (GBD), an overlapping autoinhibitory domain (AID) (9)

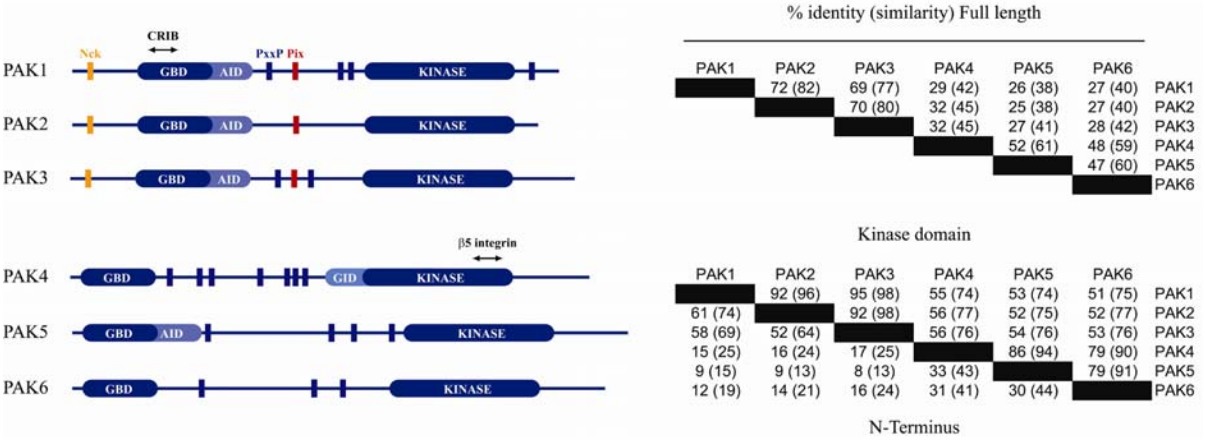

Figure 2. Domain structure of p21-activated kinases. All Pak family members share a common domain structure: an N-Terminal p21/GTPase binding domain (GBD) and a C-Terminal serine/threonine kinase domain. The GBD of Group I PAKs consists of a Cdc42/Rac interactive binding region (CRIB) and overlaps with an autoinhibitory domain (AID). PAK5 is the only member of the Group II PAKs that appear to contain an AID. All PAK proteins harbor variable numbers of core PxxP motifs, putative ligands for SH3 domains, although specific interacting partners are mostly unidentified. The N-Terminus of the Group I PAKs bind directly to the SH3 domains of Nck1/2 via a consensus binding motif (PxxPxRxxS) indicated in orange. The Group I PAKs also harbor a Pix binding site (indicated in red). Neither motif is present in any of the Group II PAKs. In addition PAK4 contains a unique GEF-H1 and Gab-1 interaction domain (GID) adjacent to the kinase domain. The kinase domain also contains a beta-5 integrin binding region. Percentage identity (similarity in parentheses) based on alignments using sequence alignment software (www.ebi.org/tools) for the full length, N-terminal and kinase domain sequences are indicated on the right.

and a C-terminal kinase domain (Figure 2). PAK3 has two alternatively spliced exons in the GBD/AID region that yield four splice variants, three of which have constitutive kinase activity (10). These splice variants have not been identified in PAK1/2. For group I PAKs the AID of one protein interacts with the kinase domain of a second, forming an autoinhibited dimer, and is important in the regulation of basal kinase activity (5, 9, 11). Active Cdc42 and Rac bind to the GBD (6, 8) releasing autoinhibition and enhancing kinase activity. Early reports suggest that binding of Cdc42 to Group II PAKs does not enhance kinase activity (12-14) and it is not clear whether these family members exist in an auto-inhibited state, are monomeric, dimeric or reside as part of a larger complex of proteins *in vivo*. However, an inhibitory region has recently been reported in PAK5 (at a region not conserved in the other group II PAKs) and this study indicated that GTP-Cdc42 was able to stimulate the autophosphorylation of purified PAK5 (15). Moreover, recombinant PAK4 proteins lacking either the N-terminal GBD (16, 17) or the ability to interact with Cdc42 (18) appear to have elevated kinase activity suggesting the GBD may interfere with kinase function. An alternative view is that interaction with active Cdc42 may influence group II PAK localisation. In support of this proposal, expression of constitutively active Cdc42 mediates PAK4 localisation to the Golgi (18). PAK4 uniquely contains an integrin binding site within the kinase domain (19), whilst both PAK5 and PAK6 possess a NLS (nuclear localisation signal) located in a region N-terminal to the GBD (20). PAK5 also possesses mitochondrial targeting signals whilst PAK6 uniquely contains a FXXMF motif which binds directly with the androgen receptor (AR) ligand-binding domain (LBD) (21).

2.2.2. Expression and localisation

PAK1 is expressed in muscle, spleen and basal expression has been reported in several tissues, including the mammary gland (3). All three group I PAKs are highly expressed in the brain, and PAK1 and PAK2 are both highly expressed in most cells of hematopoietic origin (Table 1). PAK1 is associated with cortical actin structures in PDGF-stimulated fibroblasts, whereas PAK2 localizes to the endoplasmic reticulum (ER) in COS-7 and 293T cells (22, 23). PAK1 localises to the leading edge of motile neutrophils (24), to pinocytic/phagocytic vesicles (22, 24) and to the mitotic spindle and centrosomes (25-28), as well as to the nucleus and nuclear membrane (29-31). PAK1 has also been localised to cell: substratum adhesions (32). PAK2 is uniquely cleaved by caspases and the catalytic fragment thus generated translocates to the nucleus or to the endoplasmic reticulum, where it is essential for the induction of growth arrest (23). In neuronal cells PAK3 is found in lamellipodia and membrane ruffles (33).

Amongst Group II PAKs, PAK4 is expressed in a wide range of tissue types (12) and is considered to be ubiquitously expressed. PAK5 is predominantly expressed in the brain (13) but has also been detected in the adrenal gland, ovary and pancreas(8). PAK6 is expressed in the prostate, testis, breast, kidney, placenta and brain (7, 34, 35). Within individual cells, PAK4 has been localised to a number of different subcellular compartments. PAK4 is predominantly found in the perinuclear region, but is re-localised to Golgi when co-expressed with active Cdc42 (12) and can also be found at the cell periphery downstream of growth factor and integrin mediated signalling (17, 19, 36). This variation in localisation suggests that PAK4 may shuttle between cytoplasmic compartments depending on

Table 1. Normal tissue distribution and alterations in expression of PAK during cancer

Pak isoform	Normal tissue expression	Alteration	Cancer	Ref
PAK1	Widespread inc. Brain, muscle, spleen	Increased pak1 phosphorylation	Glioblastoma	(132)
		Protein overexpression	Liver	(133)
			Kidney	(134)
			Colon	(135)
		Amplification of genetic locus	Ovarian	(147)
			Bladder	(148)
PAK2	Ubiquitous	Increased pak2 phosphorylation	Ovarian	(136)
PAK3	Brain, spleen, testis	Potential cancer 'driver' mutations identified		(50)
PAK4	Ubiquitous	Protein overexpression	Lung, ovarian, prostate, cns, leukaemia, renal, melanoma, breast	(18)
		Amplification of genetic locus	Pancreas	(99)
			Oral squamous cell carcinoma	(101)
		Somatic mutation	Colon	(102)
PAK5	Brain, ovary, pancreas, testis	Protein overexpression	Colon	(106)
		Likely cancer 'driver' mutations identified		(50)
PAK6	Brain, breast, kidney, prostate, placenta, testis	Protein overexpression	Prostate	(35)

the nature of the physiological input. Recent studies have confirmed that PAK5 shuttles between the mitochondria and the nucleus (20) whereas PAK6 was reported to be predominantly localised in the mitochondria of Chinese-hamster ovary cells but is present in both the cytoplasm (9) and nucleus of prostate cells (14).

2.2.3. Mouse knockout studies

Deletion of the *PAK1* gene in mice has no adverse effects on viability or fertility but there are subtle defects in neuronal function, defects in mast-cell degranulation and macrophage function. Genetic deletion of *PAK2* results in embryonic lethality at day E8 due to multiple developmental abnormalities (8) whilst deletion of the *PAK3* gene is implicated in mental retardation; *PAK3* knockout mice are viable but display cognitive impairment (37). Genetic deletion of *PAK4* in mice is embryonically lethal. *PAK4*-deficient embryos exhibit extensive and dramatic defects of heart and neuronal development and spinal cord motor neurons fail to differentiate and efficiently migrate into position (38). No abnormalities were detected in either *PAK5* or *PAK6* knockout mice, and *PAK5/PAK6*-double-knockout mice are viable and fertile (39). However these double-knockout mice do exhibit defects in learning and memory functions (39).

3. GROUP I PAKS

3.1 Group I PAKs and cancer

PAK1 kinase activity is required for the Ras-induced transformation (40) and PAK1 overexpression has been reported in colon, ovarian, bladder transitional cell carcinoma, T-cell lymphoma, and glioblastomas (41, 42) (Table 2). Indeed, glioblastoma patient survival time is significantly correlated with the presence of phosphorylated (active) PAK1 in the cytoplasm (43). More specifically, PAK1 expression is widely upregulated in human breast tumours and correlates with breast cancer invasiveness as well as tumour cyclin D1 expression (44). Furthermore, PAK1 activity has been linked to estrogen (tamoxifen) resistance in estrogen receptor-positive breast cancers (31, 45). These effects appear to involve the phosphorylation of the estrogen receptor on Ser 305 by PAK1, and correlate with PAK1 nuclear translocation. Moreover, inducible expression of a constitutively active form of PAK1 rapidly induces breast cancer cell proliferation and aggressive cell phenotypes, which included anchorage-independent growth and mitotic defects (46). PAK1 has also been shown to have a central role in the Schwann-cell tumours of neurofibromatosis type 1 (NF1), which is caused by the loss of a Ras GAP protein, through a Ras-dependent pathway (47). Both PAK1 and PAK2 have been associated with neurofibromatosis type 2 (NF2), as PAKs phosphorylate the *NF2* tumour-suppressor gene product, Merlin, on serine 518 and block its activity (48, 49). Very little is known about PAK3 function outside of neuronal cells (reviewed in(2)) however a recent screen of somatic mutations in human cancer identified PAK3 mutations as a possible driver of cancer progression (50).

3.2. Group I PAKs - Upstream regulators

Several growth factors including epidermal growth factor, heregulin, platelet-derived growth factor, and hepatocyte growth factor activate PAK1 (51-53) (Figure 3). PAK1 receptor recruitment can be mediated through binding to Grb2 (54) and localisation of PAK1 at the membrane is a critical step during PAK activation. However regulation of PAK1 activity is a complex process involving protein-protein interactions, phosphorylation/ dephosphorylation and sphingolipid binding (55, 56). The binding of Rac/Cdc42 to the PAK1 regulatory domain induces the phosphorylation of important sites throughout the protein, both by PAK1 itself (56) and/or by exogenous kinases such as JAK2, PDK1 and PKA (57-59). Indeed, phosphorylation of PAK1 serine 144 in the kinase autoinhibitory domain contributes significantly to kinase domain activation (56). PAK2 is also activated by binding to Rac/Cdc42 and is likely that the same mechanism that regulates PAK1 also regulates PAK2 catalytic activity. Like PAK1, Rac/Cdc42 interaction stimulates PAK2 autophosphorylation (Thr 402 in the activation loop (60)), a requirement for kinase activity (61).

The adapter protein Nck (62) and PIX (PAK-interacting exchange factor (63)) are key regulators of the group I PAKs, binding directly to PAK1-3 near the N-terminal GBD domain. Nck1/2 (referred to hereafter collectively as Nck) are small adapter proteins primarily

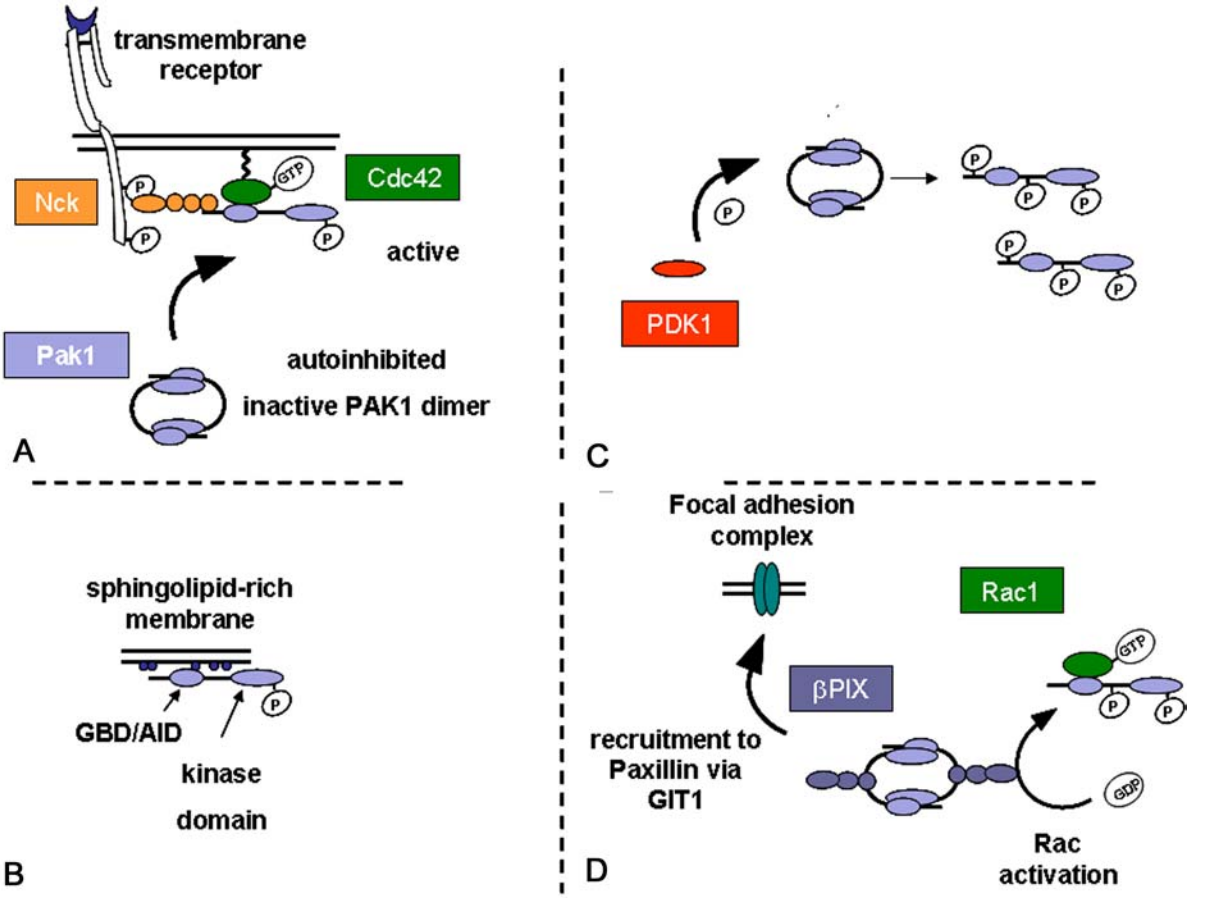

Figure 3. Models depicting activation of Group I PAKs. Group I PAKs, exemplified by PAK1, exist as autoinhibited homodimers, in which the kinase domain of one PAK molecule is inhibited by interactions from the GBD/AID domain of a second PAK molecule. Upon binding to Nck bound to activated transmembrane receptor (A), sphingolipids (C) or Rho family GTPases Cdc42 or Rac (A and D) autoinhibitory interactions are relieved enabling the kinase domain to undergo conformational change and autophosphorylation to become active. PAK1, 2 and 3 are also activated by phosphorylation by additional kinases, for example PDK1 (B), which inhibit AID-kinase and PIX interaction. Note that, despite their depiction, these mechanisms may not be mutually exclusive.

recruited via an SH2 domain to the cytoplasmic tail of activated tyrosine phosphorylated cell surface receptors and/or sites of cell: substratum adhesion. Initially it was thought that Nck recruitment alone was sufficient to induce PAK kinase activity (64), though it later emerged that activation of PAK by membrane clustered Nck is dependent on Rho family GTPases (65). Thus Nck serves to recruit PAK to areas of the cell where active Rac/Cdc42 are likely to be localised. Nck is also able to recruit a PAK1:PIX complex to sites of cell adhesion (66). PIX is a GEF for Rac/Cdc42 (67) which can complex with paxillin (a major component of cell: substratum adhesions) and the interaction between PIX and PAK1 is thought to mediate adhesion dynamics by localising both active Rac/Cdc42 and PAK1 at sites of cell adhesion (68)(Figure 3). Autophosphorylation of PAK1, an early event in PAK1 activation, drives the dissociation of PIX and Nck (69) suggesting that there is a complex feedback mechanism; moreover the interaction between Nck and PAK can also be disrupted by phosphorylation of PAK on serine 21 by kinases such as Akt (70) . In neuronal cells, PAK2 interacts with beta-PIX (71) leading to the formation of a PAK2-beta-PIX-Erk1/2 complex, which is essential for neurite outgrowth (72). Intriguingly, in this instance PAK2 inhibition blocks Rac activation, suggesting that PAK2 may also function upstream of Rac by regulating beta-PIX activity (73).

PAK2 is unique among the PAK isoforms because it can also be activated through proteolytic cleavage by caspases or caspase-like proteases to release an amino (N)-terminal fragment (PAK2p27) and a pro-apoptotic catalytic fragment (PAK2p34). Activation of full length PAK2 stimulates cell survival, whereas proteolytic activation of PAK2p34 is involved in programmed cell death. PAK2p34 exerts its pro-apoptotic effects via the activation of Jun N-terminal kinase (JNK) (74, 75).

Much less is known about the inactivation of PAKs; however the protein phosphatases POPX1 and

POPX2 can bind to the PIX/PAK complex and contribute to the deactivation of PAK. In addition to dephosphorylation, group I PAKs are also subject to inhibition by interaction with various proteins, including hPIP1, CRIPak, Nischarin, p110C, and Merlin (8), as well as down regulation by ubiquitin-mediated proteosomal degradation following binding to the small GTPase Chp (Cdc42 homologous protein) (2).

3.3. Group I PAKs signalling to the cytoskeleton

Although the substrate preferences among group I PAKs have never been directly or systematically compared, PAK1, PAK2 and PAK3 share 92-95% identity within their kinase domains (Figure 2), suggesting that they may phosphorylate common substrates (8). Indeed, PAK1 and PAK2 have been reported to have many identical substrate *in vitro* (76). It is therefore likely that isoform-specific functions of the group 1 PAKs are mediated by their participation in distinct molecular complexes and their localization to distinct subcellular structures. To date, more than 30 direct substrates of group I PAKs have been identified, proteins involved in the regulation of cytoskeletal dynamics, cell motility, cell death and survival signalling pathways (6). This review will focus on those interactions most closely related to cell migration and invasion (Table 1).

It has been known for some time that changing the activity level of PAK1 in cells leads to membrane ruffling as a result of actin cytoskeletal rearrangement (77) and that inhibition of PAK activity can block cell migration (78). We are now beginning to understand how PAKs orchestrate these effects on the actin cytoskeleton and cell migration.

Both PAK1 and PAK2 are thought to modulate the activity of myosin II (an actin interacting motor protein that can drive cell contractility) during cell migration. Myosin II is activated by myosin light chain kinase (MLCK) phosphorylation. Whilst PAK1 phosphorylates MLCK leading to a reduction in its catalytic activity (79) PAK2 can directly phosphorylate myosin II regulatory light chain inducing an activation of myosin II and increased cell contractility (80). PAK1 is also known to form a complex with, and phosphorylate, LIM-kinase (LIMK). LIMK is involved in reorganization of actin cytoskeleton through inactivating phosphorylation of ADF/ cofilin family proteins (81). ADF/cofilins are actin binding proteins that can promote actin polymerization by severing actin filaments to increase the concentration of free barbed ends (reviewed by(82). LIMK1 inactivates ADF/cofilin by phosphorylating cofilin at Ser3, inhibiting its ability to bind to F-actin (81). PAK1 regulation of actin dynamics at the leading edge of motile cells may also be mediated by phosphorylation of filamin A, a large actin binding protein which activates PAK1 and is required for PAK1 induced membrane ruffling (83). Evidence has now emerged that PAK1 may also regulate cross talk between Rac/Cdc42 signalling pathways and RhoA. GEF-H1 (guanine-nucleotide-exchange factor H1) is an exchange factor for RhoA whose activity is regulated through a cycle of microtubule binding and release. PAK1 phosphorylation of GEF-H1 induces microtubule binding resulting in suppression of RhoA activation (84).

PAK1 also binds to and phosphorylates p41-Arc, a subunit of the Arp2/3 complex. Arp2/3 drives the de novo nucleation of actin filaments during cell migration. Phosphorylation of p41-Arc by PAK1 promotes the formation of the Arp2/3 complex and PAK1 mediated phosphorylation of p41-Arc is required for breast cancer cell migration (85). These studies are the first to identify kinase regulation of Arp2/3 function and may place PAK activity at the centre of actin cytoskeletal dynamics.

In addition to actin cytoskeletal regulation PAK1 has also been implicated in the regulation of cell adhesion through its interaction with the PIX: paxillin complex (66). PAK1 activation has been shown to promote the interaction between PIX and Rac1 (86) at sites of nascent cell adhesion and a PAK/PIX/GIT complex has been implicated in adhesion regulation during migration (87). It should be noted however, that PAK1 kinase activity is not always required for cytoskeletal remodelling. Overexpression of PAK1 kinase dead mutants have been shown to induce the formation of lamellipodia, cell spreading and increased cell substratum adhesions (88, 89).

3.4. Group I PAKs and cancer cell invasion

Group 1 PAKs have been implicated in cell migration through their ability to phosphorylate multiple cytoskeletal regulators. In fibroblasts, PAK1 regulates lamellipodial extension and directionality (90, 91) and the formation and disassembly of focal adhesions (32, 87). In contrast, in endothelial cells both kinase-dead and constitutively active PAK1 inhibited migration (78), indicating that the role of PAKs in cell migration is likely to be cell-type specific. In prostate cancer cells, knockdown of PAK1 inhibits hepatocyte growth factor (HGF) induced loss of cell-cell junctions and subsequent migration whilst knockdown of PAK2 increases lamellipodium extension but does not affect migration speed (92). However, expression of either kinase-dead or constitutively active PAK1 has been shown to increase migration towards HGF in Boyden chambers (93). siRNA-mediated knockdown of PAK1 in breast epithelial cells leads to decreased myosin light chain phosphorylation and smaller focal adhesions whilst dominant negative PAK1 blocks the invasiveness of breast tumour cells (88) . In contrast, knockdown of PAK2 has the opposite effects (94). Interestingly, PAK1 has also been shown to co-ordinate extracellular matrix proteolysis in a three-dimensional (3D) breast cancer model (95). Moreover, a recent study reported that PAK1 and PAK2 are involved in promoting cell migration and invasion in ovarian cancer cells (96). Cancer cell dissemination may require a loss of cell: cell contact and PAK1 kinase mutants can induce a loss of cell-cell junctions (93). Moreover, active Rac acts via PAK1 to induce disassembly of E-cadherin-based adhesions (97). A process that may depend on an interaction between PAK1 and E-cadherin associated protein beta-catenin (98).

Table 2. PAK kinase substrates implicated in (metastatic) cell migration

Substrate	Cellular function	PAK	Ref
Caldesmon	Inhibitor of myosin ATPase activity	PAK1 & 3	(137)
CPI17	Inhibitor of myosin phosphatase	PAK1	(138)
Desmin	Intermediate filament protein	PAK1	(139)
Filamin A	Actin cross linking and adhesion protein	PAK1	(83)
GIT1	GTPase regulation Arf GAP	PAK1	(140)
GEF-H1	Rho GTPase regulation, RhoA GEF	PAK1 & 4	(84, 119)
LIMK1	Actin cytoskeleton dynamics; cofilin kinase	PAK1, 2 & 4	(113, 125)
MLCK	Regulation of myosin activity and actin cytoskeleton dynamics	PAK1 & 2	(79, 80)
Merlin	ERM binding protein	PAK2	(49)
p41-ARC	Subunit of Arp2/3 complex, actin nucleation	PAK1	(85)
Paxillin	Focal adhesion scaffold	PAK4	(36)
PDZ-RhoGEF	Rho GTPase regulation, RhoA GEF	PAK4	(141)
αPIX	Rho GTPase regulation, Rac GEF	PAK1 & 2	(56)
□PIX	Rho GTPase regulation, Rac GEF	PAK1 & 2	(72, 86)
Raf-1	MEK kinase	PAK1 & 3	(142, 143)
Rho-GDI	Inhibitor of Rho GTPase activity	PAK1	(144)
R-MLC	Regulatory chain of myosin motor	PAK2	(145)
SSH-1	Actin cytoskeleton dynamics; cofilin phosphatase	PAK4	(126)
Vimentin	Intermediate filament protein	PAK1	(146)

4. GROUP II PAKS

4.1. Group II PAKs and cancer

PAK4 has been found to be overexpressed or genetically amplified in numerous cancer cell lines and tumours including those derived from breast, lung and prostate (18) as well as pancreas (99, 100) squamous cell carcinoma (101) and colon cancer (102) (Table 2). Overexpression of PAK4 in a range of cell lines has revealed several phenotypes suggestive of a role in cancer. Overexpression of constitutively active PAK4 confers anchorage independent growth to cultured fibroblasts in soft agar assays independently of Ras transformation (18, 103) and kinase inactive PAK4 can inhibit either Dbl (103) or Ras (18) mediated oncogenic transformation of fibroblasts. Further, expression of kinase-dead PAK4 inhibits anchorage independent growth of a human colon cancer cell line (18). In addition to the role PAK4 plays in oncogenic transformation, overexpressed PAK4 is associated with protection from apoptosis (104). Finally, overexpression of both wild-type and constitutively active PAK4 in a nude mice model leads to an increased incidence of tumours, strongly implicating PAK4 as a driving force in cancer (105).

Little is known about PAK5 function outside of neuronal cells but PAK5 overexpression was recently detected in numerous colorectal carcinoma cell lines where increased expression correlated with cancer progression and invasive potential (106). Furthermore, PAK5 somatic mutations were also identified in the same cancer genetic screen as PAK3 (50).

PAK6 was identified in a screen to identify proteins that interact with the Androgen Receptor (AR) which mediates the development and differentiation of androgen-sensitive tissues and it is also important in the manifestation of prostate cancer (107). PAK6 binds to the ligand binding domain (LBD) of the AR (also to the estrogen receptor) and leads to the suppression of AR signalling (107). The aptitude of PAK6 to bind to steroid hormone receptors suggests that it may contribute to the hormonal independence that is characteristic of many aggressive tumours (76, 107). In support of this hypothesis, increased PAK6 expression has been detected in both prostate cancer cells and breast tumours (7, 35). Moreover, a recent study demonstrated that reduced PAK6 expression, combined with irradiation, decreased prostate cancer cell viability (108). In contrast, the *PAK6* gene is hypermethylated in some prostate cancer cells; hypermethylated genes are often linked with tumour growth inhibition (109).

4.2. Group II PAKs - Upstream regulators

PAK4 kinase activity is specifically stimulated in response to hepatocyte growth factor (HGF) in a phosphatidylinositol 3-kinase (PI3K) dependent manner (17). HGF is a multifunctional cytokine and signals via it's oncogenic receptor c-Met/HGF receptor. HGF signalling plays a critical role in chemotaxis, cell growth, morphogenesis and metastatic migration and invasion (recently reviewed (110, 111). PAK4 recruitment to activated c-Met /HGFR is mediated by the large adaptor protein Gab-1. PAK4 binds directly to Gab-1 via a GEF-H1 interaction domain (GID) adjacent to the kinase domain(112) (Figure 4). PAK4: Gab-1 interaction is required for HGF-dependent scattering of MDCK cells and PAK4 and Gab-1 act synergistically to enhance cell scattering in 2D and invasion into matrigel (112). Unlike the Group I PAKs, PAK4 does not contain a prototypical Nck SH3 binding site, and reportedly does not bind Nck (113). In mice, PAK4 interacts with Grb2 downstream of KGFR (114), suggesting Grb2 and Gab-1 rather than Nck may regulate PAK4 recruitment to transmembrane receptors. PAK4 has also been implicated in signal transduction downstream of a number of transmembrane receptors, for example in C2C12 muscle precursor cells, PAK4 is activated downstream of BMP2, which induces cell migration (115).

Upstream regulators of PAK5/PAK6 have not be clearly identified however kinase activity is elevated by co-expression with the active form of MKK 6 (MAPK kinase 6)(116). At least for PAK6, MKK6 stimulates activity by interacting with serine – 165 (a p38 MAP kinase site) and tyrosine 566 located in the activation loop within the PAK6 kinase domain (116). Although the activity of group I PAKs can be regulated by the binding of Rac/Cdc42, it has

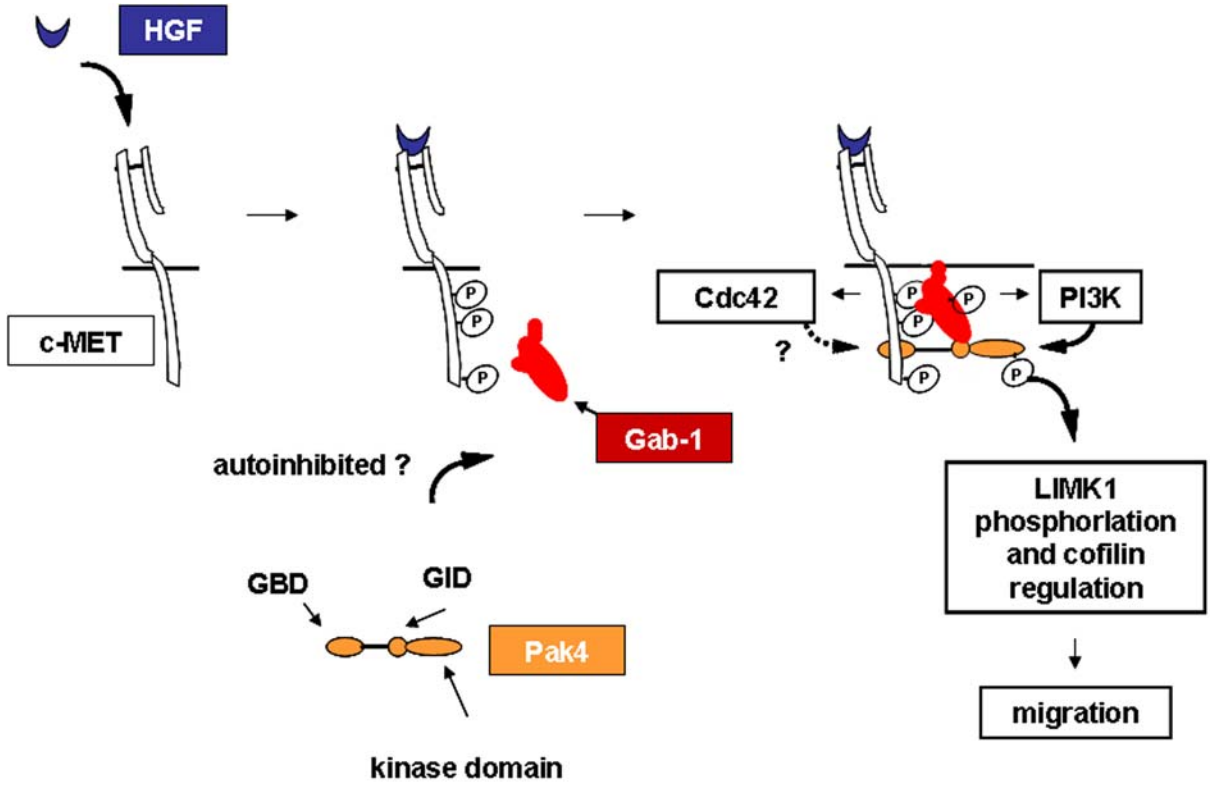

Figure 4. Activation of PAK4 in response to hepatocyte growth factor (HGF) signaling. HGF-induced activation of c-MET/HGFR results in activation of the intracellular portion of c-Met that consists of a kinase domain and tyrosine residues. Once phosphorylated, these tyrosines serve as docking sites for the recruitment of a number of adaptor/scaffold proteins (not shown for clarity), including Gab-1. Subsequently, Gab1 itself becomes phosphorylated recruiting a host of signalling molecules, including PAK4, which in turn is activated possible from an inactive inhibited state. HGF signalling involves activation of both Rho family GTPases including Cdc42 and PI3K. PAK4 activation in response to HGF is PI3K-dependent; though the mechanism remains illusive as does the role of Cdc42. Active PAK4 regulates the activity of cofilin via LIMK1, promoting cytoskeletal rearrangements and cell migration.

not yet been resolved whether Cdc42 is also an upstream regulator of Group II PAK activity even though these family members bind specifically to Cdc42 (see section 1.2.1).

4.3. Group II signalling to the cytoskeleton

To date, no kinase independent functions of PAK4 have been identified, thus the primary mechanism by which PAK4 regulates the activity of downstream effectors is via phosphorylation. PAK4 phosphorylates two RhoA GEFs, PDZ RhoGEF and GEF-H1, the adhesion associated protein paxillin, LIMK1 and slingshot homologue (SSH-1) both regulators of actin dynamics mediated through cofilin (117). Many of these substrates, at least *in vitro*, are shared with PAK1 (Table 2). GEF-H1, a RhoA exchange factor, is a key orchestrator of cell migration, mediating localised regulation of RhoA activity at the cell leading edge during migration; depletion of GEF-H1 leads to decreased cell migration due to the loss of the Rho exchange activity of GEF-H1 (118). GEF-H1 binds directly to PAK4 via a GEF-H1 interaction domain (GID) and PAK4 phosphorylates GEF-H1 on serine 885 (119), which inactivates RhoA exchange activity (120). The PAK4-GEF-H1 complex subsequently interacts with microtubules (MT) and the release of this MT bound GEF-H1 into the cytoplasm results in a dissolution of stress fibres and the formation of actin-rich lamellipodia in murine NIH3T3 fibroblast cells (119). In DU145 prostate carcinoma cells, co-expression of active PAK4 and GEF-H1 significantly reduces GEF-H1-mediated increases in active GTP-bound RhoA. Conversely, knockdown of PAK4 by RNAi increases the level of active RhoA, suggesting that PAK4 regulates RhoA via regulation of GEFH1 activity (36). The loss of GEF-H1 is also associated with decreased rates of focal adhesion turnover (118). Interestingly, reduction of PAK4 expression in DU145 prostate cancer cells not only triggers the formation of prominent actin stress fibres but also leads to an increase in the size and number of focal adhesions that exhibit reduced turnover rates (36). Paxillin is a central scaffold protein of focal-adhesion complexes, coordinating both complex assembly and disassembly (121). It has recently been reported that paxillin is serine phosphorylated on serine 272 which leads to increased turnover of cell adhesions (87). Initially PAK1 was identified as the kinase but subsequent reports have disputed this finding (122). The kinase domain of PAK4 binds paxillin and phosphorylates paxillin on serine 272 *in vitro*, suggesting a mechanism by which PAK4 regulates focal adhesion

turnover and therefore cell migration (36). In a separate study PAK4 has also been shown to interact directly with alpha-v beta-5 Integrins (19). A beta-5 Integrin Binding Domain (IBD) within the C-terminus of the PAK4 kinase domain binds directly to a SERS motif in the cytoplasmic tail of β5 and phosphorylates both serine residues of this motif *in vitro* (123). Overexpression of either full length PAK4 or the kinase domain alone in MCF-7 breast cancer cells is sufficient to induce haptotactic cell migration towards the alpha-v beta-5 ligand vitronectin (19, 123). Although initially reported to be a kinase-independent process, *in vitro* binding of PAK4 to beta-5 integrin and concomitant haptotactic migration appears to require PAK4 kinase activity (19, 123). Interestingly, the IBD is well conserved amongst other PAK family members and mutation of several key residues within the IBD abolishes both kinase activity and beta-5 interaction(123). The SERS sequence motif is only partially conserved between integrin isoforms, so it will be interesting to see if PAK4 can bind other integrins (for example beta-6, which is highly upregulated during cancer progression), likewise it will be interesting to see if other PAK family members can interact with any of the integrin cytoplasmic tails .

In some cells PAK4 acts predominantly via LIMK1 and cofilin downstream of HGF rather than via GEF-H1 and paxillin (36, 124). Like PAK 1/2, PAK4 influences actin polymerisation by activating LIMK1 via phosphorylation of the LIMK1 active site threonine 508 (125) and (113) leading to serine 3 phosphorylation of cofilin as described above. PAK4 further regulates cofilin activity by phosphorylating and inactivating the cofilin phosphatase slingshot homologue 1 (SSH-1L) (126). SSH-1L both dephosphorylates and inactivates LIMK1 through dephosphorylation of Thr508 and dephosphorylates and activates ADF/cofilin on Ser3, resulting in a net increase in ADF/cofilin activity and actin filament turnover. PAK4 also has recently been shown to bind to GDCR6L, the product of a gene deleted in the rare genetic disorder Digeorge syndrome. DGCR6L colocalises with PAK4 in human gastric cancer cells and enhances the phosphorylation level of both LIMK1 and cofilin (127). Both LIMK1 and SSH have been implicated in co-ordinated chemotactic cell migration (Nishita *et al.*, 2005) and deregulation of the PAK4-LIMK1 pathway by co-overexpression of PAK4 and LIMK1 or knockdown of PAK4 in PC3 cells leads to increased chemotaxis towards HGF and decreased cell motility, respectively (124).

PAK5 is a key component in the signaling pathway by which Rho GTPases regulate cytoskeletal changes required for promoting neurite outgrowth (128), PAK5 is yet to be extensively studied, however it is known that PAK5 triggers neurite outgrowth in a mouse neuroblastoma cell line via down regulation of RhoA activity (128). Still less is known about signalling between PAK6 and the actin cytoskeleton. PAK6 interacts with IQ-domain GTPase-activating protein 1 (IQGAP1) (129). IQGAP1 overexpression has been observed in a number of tumours (129), although the precise role of IQGAP in cancer progression remains unresolved and is the focus of much current research.

4.4. Group II PAKs and cancer cell invasion

Several reports implicate PAK4 in regulation of cancer cell migration and metastasis. PAK4-null fibroblasts migrate slower than wild-type fibroblasts in response to an electric field (galvanotatic migration) (130), and knockdown of PAK4 by RNAi reduces both the mean speed of migration of prostate carcinoma cell migration (124) the ability of pancreatic ductal adenocarcinoma cells to invade matrigel (100) and inhibits HGF-induced cell scattering responses (36, 112) . Reciprocally, overexpression of PAK4 enhances the migration speed of fibroblasts during galvanotaxis (130) and promotes the invasiveness of pancreatic cancer cells (100).

Very little is known about how PAK5 might contribute to tumour progression but colorectal carcinoma cells overexpressing PAK5 exhibited decreased cell adhesion concomitant with increased cell motility on collagen I substratum whilst siRNA knockdown of PAK5 expression in the same cells lead to enhanced cell adhesion and reduced cell migration. This study at least implies that PAK5 may play a role in colorectal carcinoma cell migration (106). The role of PAK6 in cancer cell invasion has not been extensively studied however it has been recently reported that a loss of PAK6 expression significantly reduces the invasive ability of prostate cancer cells (131).

5. PERSPECTIVE

PAKs are pluripotent kinases involved in many cellular functions including cell motility, regulation of neuronal outgrowth, hormone signalling, gene transcription and cell survival. Their role in these processes makes this family of kinases attractive therapeutic targets. Since the discovery of p-21 activated kinases in the early 1990s we have learnt much about the regulation and activity of Group I PAKs. Much less is known about group II PAKs, particularly PAK5 and PAK6. It is currently the case that much of our knowledge of their biology (as reviewed here) is derived from one or two publications. The future challenge is to validate these data and further elucidate their biological function *in vivo*.

Many cell types express multiple PAK family members and it will be important to understand how PAK activity is coordinated at the subcellular level to mediate the cytoskeletal events that orchestrate cell migration and invasion. Indeed, many of the original PAK1 studies were conducted before Group II PAKs were even discovered and recent work suggests that PAK1 and PAK4, at least *in vitro*, share many common substrates. There is also evidence to suggest that different PAK family members can play antagonistic roles in the same cell (94) . Moreover, substrate specificity *in vivo* remains to be elucidated. Evidence from knockout mice studies points to both unique and overlapping functions and it is likely that spatial and temporal regulation of activity is required to elicit specific cellular responses. It is also likely that the activity of individual family members may differ between cells types.

Overexpression and amplification of PAK family members is reported in many tumour types and it is

important to note that PAKs originally thought to be neuronally restricted in expression (PAK3 and PAK5) are both implicated in tumour progression. It will be interesting to establish whether these family members also play a role in cancer cell migration and invasion.

6. REFERENCES

1. F. M. Vega and A. J. Ridley: Rho GTPases in cancer cell biology. *FEBS Lett*, 582(14), 2093-101 (2008)

2. P. Kreis and J. V. Barnier: PAK signalling in neuronal physiology. *Cellular Signalling*, 21(3), 384-393 (2009)

3. E. Manser, T. Leung, H. Salihuddin, Z. S. Zhao and L. Lim: A brain serine threonine protein kinase activated by Cdc42 and Rac1. *Nature*, 367(6458), 40-46 (1994)

4. S. Bagrodia, S. J. Taylor, C. L. Creasy, J. Chernoff and R. A. Cerione: Identification of a mouse p21Cdc42/Rac activated kinase. *J Biol Chem*, 270(39), 22731-7 (1995)

5. Z. M. Jaffer and J. Chernoff: p21-activated kinases: three more join the Pak. *International Journal of Biochemistry & Cell Biology*, 34(7), 713-717 (2002)

6. G. M. Bokoch: Biology of the p21-activated kinases. *Annu Rev Biochem*, 72, 743-81 (2003)

7. B. Dummler, K. Ohshiro, R. Kumar and J. Field: Pak protein kinases and their role in cancer. *Cancer and Metastasis Reviews*, 28(1-2), 51-63 (2009)

8. L. E. Arias-Romero and J. Chernoff: A tale of two Paks. *Biology of the Cell*, 100(2), 97-108 (2008)

9. S. R. Lee, S. M. Ramos, A. Ko, D. Masiello, K. D. Swanson, M. L. Lu and S. P. Balk: AR and ER interaction with a p21-activated kinase (PAK6). *Molecular Endocrinology*, 16(1), 85-99 (2002)

10. P. Kreis, V. Rousseau, E. Thevenot, G. Combeau and J. V. Barnier: The four mammalian splice variants encoded by the p21-activated kinase 3 gene have different biological properties. *J Neurochem*, 106(3), 1184-97 (2008)

11. J. H. Carter, L. E. Douglass, J. A. Deddens, B. M. Colligan, T. R. Bhatt, J. O. Pemberton, S. Konicek, J. Hom, M. Marshall and J. R. Graff: Pak-1 expression increases with progression of colorectal carcinomas to metastasis. *Clinical Cancer Research*, 10(10), 3448-3456 (2004)

12. A. Abo, J. Qu, M. S. Cammarano, C. Dan, A. Fritsch, V. Baud, B. Belisle and A. Minden: PAK4, a novel effector for Cdc42Hs, is implicated in the reorganization of the actin cytoskeleton and in the formation of filopodia. *Embo J*, 17(22), 6527-40 (1998)

13. A. Pandey, I. Dan, T. Z. Kristiansen, N. M. Watanabe, J. Voldby, E. Kajikawa, R. Khosravi-Far, B. Blagoev and M. Mann: Cloning and characterization of PAK5, a novel member of mammalian p21-activated kinase-II subfamily that is predominantly expressed in brain. *Oncogene*, 21(24), 3939-48 (2002)

14. F. Yang, X. Li, M. Sharma, M. Zarnegar, B. Lim and Z. Sun: Androgen receptor specifically interacts with a novel p21-activated kinase, PAK6. *J Biol Chem*, 276(18), 15345-53 (2001)

15. Y. P. Ching, V. Y. L. Leong, C. M. Wong and H. F. Kung: Identification of an autoinhibitory domain of p21-activated protein kinase 5. *Journal of Biological Chemistry*, 278(36), 33621-33624 (2003)

16. A. Abo, J. Qu, M. S. Cammarano, C. Dan, A. Fritsch, V. Baud, B. Belisle and A. Minden: PAK4, a novel effector for Cdc42Hs, is implicated in the reorganization of the

actin cytoskeleton and in the formation of filopodia. *Embo J*, 17, 6527-6540 (1998)

17. C. M. Wells, A. Abo and A. J. Ridley: PAK4 is activated via PI3K in HGF-stimulated epithelial cells. *J Cell Sci*, 115, 3947-3956 (2002)

18. M. G. Callow, F. Clairvoyant, S. Zhu, B. Schryver, D. B. Whyte, J. R. Bischoff, B. Jallal and T. Smeal: Requirement for PAK4 in the anchorage-independent growth of human cancer cell lines. *J Biol Chem*, 277(550-558) (2002)

19. H. Zhang, Z. Li, E. K. Viklund and S. Stromblad: p21-activated kinase 4 interacts with integrin avb5 and regulates avb5-mediated cell migration. *J Cell Biol*, 158, 1287–1297 (2002)

20. S. Cotteret and J. Chernoff: Nucleocytoplasmic shuttling of Pak5 regulates its antiapoptotic properties. *Molecular and Cellular Biology*, 26(8), 3215-3230 (2006)

21. D. J. van de Wijngaart, M. E. van Royen, R. Hersmus, A. C. W. Pike, A. B. Houtsmuller, G. Jenster, J. Trapman and H. J. Dubbink: Novel FXXFF and FXXMF motifs in androgen receptor cofactors mediate high affinity and specific interactions with the ligand-binding domain. Journal of Biological Chemistry, 281(28), 19407-19416 (2006)

22. S. Dharmawardhane, L. C. Sanders, S. S. Martin, R. H. Daniels and G. M. Bokoch: Localization of p21-activated kinase 1 (PAK1) to pinocytic vesicles and cortical actin structures in stimulated cells. Journal of Cell Biology, 138(6), 1265-1278 (1997)

23. Z. D. Huang, J. Ling and J. A. Traugh: Localization of p21-activated protein kinase gamma-PAK/Pak2 in the endoplasmic reticulum is required for induction of cytostasis. Journal of Biological Chemistry, 278(15), 13101-13109 (2003)

24. S. Dharmawardhane, D. Brownson, M. Lennartz and G. M. Bokoch: Localization of p21-activated kinase 1 (PAK1)

to pseudopodia, membrane ruffles, and phagocytic cups in activated human neutrophils. *Journal of Leukocyte Biology*, 66(3), 521-527 (1999)

25. M. Banerjee, D. Worth, D. M. Prowse and M. Nikolic: Pak1 phosphorylation on T212 affects microtubules in cells undergoing mitosis. *Current Biology*, 12(14), 1233-1239 (2002)

26. D. A. Thiel, M. K. Reeder, A. Pfaff, T. R. Coleman, M. A. Sells and J. Chernoff: Cell cycle-regulated phosphorylation of p21-activated kinase 1. *Current Biology*, 12(14), 1227-1232 (2002)

27. Z. S. Zhao and E. Manser: PAK and other Rho-associated kinases--effectors with surprisingly diverse mechanisms of regulation. *Biochem J*, 386(Pt 2), 201-14 (2005)

28. B. Maroto, M. B. Ye, K. von Lohneysen, A. Schnelzer and U. G. Knaus: P21-activated kinase is required for mitotic progression and regulates Plk1. Oncogene, 27(36), 4900-4908 (2008)

29. F. Li, L. Adam, R. K. Vadlamudi, H. Y. Zhou, S. Sen, J. Chernoff, M. Mandal and R. Kumar: p21-activated kinase 1 interacts with and phosphorylates histone H3 in breast cancer cells. Embo Reports, 3(8), 767-773 (2002)

30. R. K. Vadlamudi, C. J. Barnes, S. Rayala, F. Li, S. Balasenthil, S. Marcus, H. V. Goodson, A. A. Sahin and R. Kumar: p21-activated kinase 1 regulates microtubule dynamics by phosphorylating tubulin cofactor B. Molecular and Cellular Biology, 25(9), 3726-3736 (2005)

31. S. K. Rayala and R. Kumar: Sliding p21-activated kinase 1 to nucleus impacts tamoxifen sensitivity. Biomedicine & Pharmacotherapy, 61(7), 408-411 (2007)

32. E. Manser, H. Y. Huang, T. H. Loo, X. Q. Chen, J. M. Dong, T. Leung and L. Lim: Expression of constitutively active alpha-PAK reveals effects of the kinase on actin and focal complexes. *Molecular and Cellular Biology*, 17(3), 1129-1143 (1997)

33. K. J. Marler, R. Kozma, S. Ahmed, J. M. Dong, C. Hall and L. Lim: Outgrowth of neurites from NIE-115 neuroblastoma cells is prevented on repulsive substrates through the action of PAK. *Mol Cell Biol*, 25(12), 5226-41 (2005)

34. J. Eswaran, M. Soundararajan, R. Kumar and S. Knapp: UnPAKing the class differences among p21-activated kinases. *Trends in Biochemical Sciences*, 33(8), 394-403 (2008)

35. R. Kaur, X. Yuan, M. L. Lu and S. P. Balk: Increased PAK6 expression in prostate cancer and identification of PAK6 associated proteins. *Prostate*, 68(14), 1510-1516 (2008)

36. C. M. Wells, A. D. Whale, M. Parsons, J. R. Masters and G. E. Jones: PAK4: a pluripotent kinase that regulates prostate cancer cell adhesion. *J Cell Sci* 123 (10) 1663-73 (2010)

37. J. Meng, Y. Meng, A. Hanna, C. Janus and Z. Jia: Abnormal long-lasting synaptic plasticity and cognition in mice lacking the mental retardation gene Pak3. *J Neurosci*, 25(28), 6641-50 (2005)

38. J. Qu, X. Li, B. G. Novitch, Y. Zheng, M. Kohn, J. Xie, S. Kozinn, R. Bronson, A. A. Beg and A. Minden: PAK4 Kinase Is Essential for Embryonic Viability and for Proper Neuronal Development. *Mol Cell Biol*, 23, 7122–7133 (2003)

39. T. Nekrasova, M. L. Jobes, J. H. Ting, G. C. Wagner and A. Minden: Targeted disruption of the Pak5 and Pak6 genes in mice leads to deficits in learning and locomotion. *Developmental Biology*, 322(1), 95-108 (2008)

40. Y. Tang, Z. X. Chen, D. Ambrose, J. H. Liu, J. B. Gibbs, J. Chernoff and J. Field: Kinase-deficient Pak1 mutants inhibit Ras transformation of Rat-1 fibroblasts. *Molecular and Cellular Biology*, 17(8), 4454-4464 (1997)

41. R. Kumar and R. K. Vadlamudi: Emerging functions of p21-activated kinases in human cancer cells. *J Cell Physiol*, 193(2), 133-44 (2002)

42. R. Kumar, A. E. Gururaj and C. J. Barnes: p21-activated kinases in cancer. *Nature Reviews Cancer*, 6(6), 459-471 (2006)

43. H. Aoki, T. Yokoyama, K. Fujiwara, A. M. Tari, R. Sawaya, D. Suki, K. R. Hess, K. D. Aldape, S. Kondo, R. Kumar and Y. Kondo: Phosphorylated Pak1 level in the cytoplasm correlates with shorter survival time in patients with glioblastoma. *Clinical Cancer Research*, 13, 6603-6609 (2007)

44. S. Balasenthil, A. A. Sahin, C. J. Barnes, R. A. Wang, R. G. Pestell, R. K. Vadlamudi and R. Kumar: p21-activated kinase-1 signaling mediates cyclin D1 expression in mammary epithelial and cancer cells. *Journal of Biological Chemistry*, 279(2), 1422-1428 (2004)

45. C. Holm, S. Rayala, K. Jirstrom, O. Stal, R. Kumar and G. Landberg: Association between Pak1 expression and subcellular localization and tamoxifen resistance in breast cancer patients. *Journal of the National Cancer Institute*, 98(10), 671-680 (2006)

46. R. K. Vadlamudi, L. Adam, R. A. Wang, M. Mandal, D. Nguyen, A. Sahin, J. Chernoff, M. C. Hung and R. Kumar: Regulatable expression of p21-activated kinase-1 promotes anchorage-independent growth and abnormal organization of mitotic spindles in human epithelial breast cancer cells. *Journal of Biological Chemistry*, 275(46), 36238-36244 (2000)

47. Y. Tang, S. Marwaha, J. L. Rutkowski, G. I. Tennekoon, P. C. Phillips and J. Field: A role for Pak

protein kinases in Schwann cell transformation. *Proceedings of the National Academy of Sciences of the United States of America*, 95(9), 5139-5144 (1998)

48. G. H. Xiao, A. Beeser, J. Chernoff and J. R. Testa: p21-activated kinase links Rac/Cdc42 signaling to merlin. *Journal of Biological Chemistry*, 277(2), 883-886 (2002)

49. J. L. Kissil, K. C. Johnson, M. S. Eckman and T. Jacks: Merlin phosphorylation by p21-activated kinase 2 and effects of phosphorylation on merlin localization. *Journal of Biological Chemistry*, 277(12), 10394-10399 (2002)

50. C. Greenman, P. Stephens, R. Smith, G. L. Dalgliesh, C. Hunter, G. Bignell, H. Davies, J. Teague, A. Butler, C. Stevens, S. Edkins, S. O'Meara, I. Vastrik, E. E. Schmidt, T. Avis, S. Barthorpe, G. Bhamra, G. Buck, B. Choudhury, J. Clements, J. Cole, E. Dicks, S. Forbes, K. Gray, K. Halliday, R. Harrison, K. Hills, J. Hinton, A. Jenkinson, D. Jones, A. Menzies, T. Mironenko, J. Perry, K. Raine, D. Richardson, R. Shepherd, A. Small, C. Tofts, J. Varian, T. Webb, S. West, S. Widaa, A. Yates, D. P. Cahill, D. N. Louis, P. Goldstraw, A. G. Nicholson, F. Brasseur, L. Looijenga, B. L. Weber, Y. E. Chiew, A. DeFazio, M. F. Greaves, A. R. Green, P. Campbell, E. Birney, D. F. Easton, G. Chenevix-Trench, M. H. Tan, S. K. Khoo, B. T. Teh, S. T. Yuen, S. Y. Leung, R. Wooster, P. A. Futreal and M. R. Stratton: Patterns of somatic mutation in human cancer genomes. *Nature*, 446(7132), 153-8 (2007)

51. L. Adam, R. Vadlamudi, S. B. Kondapaka, J. Chernoff, J. Mendelsohn and R. Kumar: Heregulin regulates cytoskeletal reorganization and cell migration through the p21-activated kinase-1 via phosphatidylinositol-3 kinase. *Journal of Biological Chemistry*, 273(43), 28238-28246 (1998)

52. H. He, A. Levitzki, H. J. Zhu, F. Walker, A. Burgess and H. Maruta: Platelet-derived growth factor requires epidermal growth factor receptor to activate p21-activated kinase family kinases. *J Biol Chem*, 276(29), 26741-4 (2001)

53. I. Royal, N. Lamarche-Vane, L. Lamorte, K. Kaibuchi and M. Park: Activation of cdc42, rac, PAK, and rho-kinase in response to hepatocyte growth factor differentially regulates epithelial cell colony spreading and dissociation. *Mol Biol Cell*, 11(5), 1709-25 (2000)

54. L. A. Puto, K. Pestonjamasp, C. C. King and G. M. Bokoch: p21-activated kinase 1 (PAK1) interacts with the Grb2 adapter protein to couple to growth factor signaling. *J Biol Chem*, 278(11), 9388-93 (2003)

55. G. M. Bokoch, A. M. Reilly, R. H. Daniels, C. C. King, A. Olivera, S. Spiegel and U. G. Knaus: A GTPase-independent mechanism of p21-activated kinase activation. Regulation by sphingosine and other biologically active lipids. *J Biol Chem*, 273(14), 8137-44 (1998)

56. C. Chong, L. Tan, L. Lim and E. Manser: The mechanism of PAK activation. Autophosphorylation events in both regulatory and kinase domains control activity. *J Biol Chem*, 276(20), 17347-53 (2001)

57. L. Rider, A. Shatrova, E. P. Feener, L. Webb and M. Diakonova: JAK2 tyrosine kinase phosphorylates PAK1 and regulates PAK1 activity and functions. *Journal of Biological Chemistry*, 282, 30985-30996 (2007)

58. C. C. King, E. M. Gardiner, F. T. Zenke, B. P. Bohl, A. C. Newton, B. A. Hemmings and G. M. Bokoch: p21-activated kinase (PAK1) is phosphorylated and activated by 3-phosphoinositide-dependent kinase-1 (PDK1). *J Biol Chem*, 275(52), 41201-9 (2000)

59. A. K. Howe and R. L. Juliano: Regulation of anchorage-dependent signal transduction by protein kinase A and p21-activated kinase. *Nature Cell Biology*, 2(9), 593-600 (2000)

60. B. N. Walter, Z. D. Huang, R. Jakobi, P. T. Tuazon, E. S. Alnemri, G. Litwack and J. A. Traugh: Cleavage and activation of pal-activated protein kinase gamma-PAK by CPP32 (Caspase 3) - Effects of autophosphorylation on activity. *Journal of Biological Chemistry*, 273(44), 28733-28739 (1998)

61. A. Gatti, Z. D. Huang, P. T. Tuazon and J. A. Traugh: Multisite autophosphorylation of p21-activated protein kinase gamma-PAK as a function of activation. *Journal of Biological Chemistry*, 274(12), 8022-8028 (1999)

62. G. M. Bokoch, Y. Wang, B. P. Bohl, M. A. Sells, L. A. Quilliam and U. G. Knaus: Interaction of the Nck adapter protein with p21-activated kinase (PAK1). *Journal of Biological Chemistry*, 271(42), 25746-25749 (1996)

63. S. Bagrodia, S. J. Taylor, K. A. Jordon, L. Van Aelst and R. A. Cerione: A novel regulator of p21-activated kinases. *J Biol Chem*, 273(37), 23633-6 (1998)

64. W. Lu, S. Katz, R. Gupta and B. J. Mayer: Activation of Pak by membrane localization mediated by an SH3 domain from the adaptor protein Nck. *Curr Biol*, 7(2), 85-94 (1997)

65. W. Lu and B. J. Mayer: Mechanism of activation of Pak1 kinase by membrane localization. *Oncogene*, 18(3), 797-806 (1999)

66. C. E. Turner, M. C. Brown, J. A. Perrotta, M. C. Riedy, S. N. Nikolopoulos, A. R. McDonald, S. Bagrodia, S. Thomas and P. S. Leventhal: Paxillin LD4 motif binds PAK and PIX through a novel 95-kD ankyrin repeat, ARF-GAP protein: A role in cytoskeletal remodeling. *J Cell Biol*, 145(4), 851-63 (1999)

67. G. Rosenberger and K. Kutsche: AlphaPIX and betaPIX and their role in focal adhesion formation. *Eur J Cell Biol*, 85(3-4), 265-74 (2006)

68. M. C. Brown, K. A. West and C. E. Turner: Paxillin-dependent paxillin kinase linker and p21-activated kinase

localization to focal adhesions involves a multistep activation pathway. *Mol Biol Cell*, 13(5), 1550-65 (2002)

69. Z. S. Zhao, E. Manser and L. Lim: Interaction between PAK and nck: a template for Nck targets and role of PAK autophosphorylation. *Mol Cell Biol*, 20(11), 3906-17 (2000)

70. G. L. Zhou, Y. Zhuo, C. C. King, B. H. Fryer, G. M. Bokoch and J. Field: Akt phosphorylation of serine 21 on Pak1 modulates Nck binding and cell migration. *Mol Cell Biol*, 23(22), 8058-69 (2003)

71. A. Hoelz, J. M. Janz, S. D. Lawrie, B. Corwin, A. Lee and T. P. Sakmar: Crystal structure of the SH3 domain of beta PIX in complex with a high affinity peptide from PAK2. *Journal of Molecular Biology*, 358(2), 509-522 (2006)

72. E. Y. Shin, K. S. Shin, C. S. Lee, K. N. Woo, S. H. Quan, N. K. Soung, Y. G. Kim, C. I. Cha, S. R. Kim, D. Park, G. M. Bokoch and E. G. Kim: Phosphorylation of p85 beta PIX, a Rac/Cdc42-specific guanine nucleotide exchange factor, via the Ras/ERK/PAK2 pathway is required for basic fibroblast growth factor-induced neurite outgrowth. *Journal of Biological Chemistry*, 277(46), 44417-44430 (2002)

73. E. Y. Shin, K. N. Woo, C. S. Lee, S. H. Koo, Y. G. Kim, W. J. Kim, C. D. Bae, S. I. Chang and E. G. Kim: Basic fibroblast growth factor stimulates activation of Rac1 through a p85 beta PIX phosphorylation-dependent pathway. *Journal of Biological Chemistry*, 279(3), 1994-2004 (2004)

74. Y. T. Huang, C. Y. Lai, S. L. Lou, J. M. Yeh and W. H. Chan: Activation of JNK and PAK2 Is Essential for Citrinin-Induced Apoptosis in a Human Osteoblast Cell Line. *Environmental Toxicology*, 24(4), 343-356 (2009)

75. W. H. Chan, H. J. Wu and N. H. Shiao: Apoptotic signaling in methylglyoxal-treated human osteoblasts involves oxidative stress, c-jun N-terminal kinase, caspase-3, and p21-activated kinase 2. *Journal of Cellular Biochemistry*, 100(4), 1056-1069 (2007)

76. U. E. E. Rennefahrt, S. W. Deacon, S. A. Parker, K. Devarajan, A. Beeser, J. Chernoff, S. Knapp, B. E. Turk and J. R. Peterson: Specificity profiling of Pak kinases allows identification of novel phosphorylation sites. *Journal of Biological Chemistry*, 282(21), 15667-15678 (2007)

77. M. A. Sells, U. G. Knaus, S. Bagrodia, D. M. Ambrose, G. M. Bokoch and J. Chernoff: Human p21-activated kinase (Pak1) regulates actin organization in mammalian cells. *Curr Biol*, 7(3), 202-10 (1997)

78. W. B. Kiosses, R. H. Daniels, C. Otey, G. M. Bokoch and M. A. Schwartz: A role for p21-activated kinase in endothelial cell migration. *J Cell Biol*, 147(4), 831-44 (1999)

79. L. C. Sanders, F. Matsumura, G. M. Bokoch and P. de Lanerolle: Inhibition of myosin light chain kinase by p21-activated kinase. *Science*, 283(5410), 2083-5 (1999)

80. Z. M. Goeckeler, R. A. Masaracchia, Q. Zeng, T. L. Chew, P. Gallagher and R. B. Wysolmerski: Phosphorylation of myosin light chain kinase by p21-activated kinase PAK2. *J Biol Chem*, 275(24), 18366-74 (2000)

81. N. Yang, O. Higuchi, K. Ohashi, K. Nagata, A. Wada, K. Kangawa, E. Nishida and K. Mizuno: Cofilin phosphorylation by LIM-kinase 1 and its role in Rac-mediated actin reorganization. *NATURE*, 393, 809-812 (1998)

82. M. Van Troys, L. Huyck, S. Leyman, S. Dhaese, J. Vandekerkhove and C. Ampe: Ins and outs of ADF/cofilin activity and regulation. *Eur J Cell Biol*, 87, 649-67 (2008)

83. R. K. Vadlamudi, F. Li, L. Adam, D. Nguyen, Y. Ohta, T. P. Stossel and R. Kumar: Filamin is essential in actin cytoskeletal assembly mediated by p21-activated kinase 1. *Nature Cell Biology*, 4(9), 681-690 (2002)

84. F. T. Zenke, M. Krendel, C. DerMardirossian, C. C. King, B. P. Bohl and G. M. Bokoch: p21-activated kinase 1 phosphorylates and regulates 14-3-3 binding to GEF-H1, a microtubule-localized Rho exchange factor. *Journal of Biological Chemistry*, 279(18), 18392-18400 (2004)

85. R. K. Vadlamudi, F. Li, C. J. Barnes, R. Bagheri-Yarmand and R. Kumar: p41-Arc subunit of human Arp2/3 complex is a p21-activated kinase-1-interacting substrate. *EMBO Rep*, 5(2), 154-60 (2004)

86. J. P. ten Klooster, Z. M. Jaffer, J. Chernoff and P. L. Hordijk: Targeting and activation of Rac1 are mediated by the exchange factor beta-Pix. *J Cell Biol*, 172(5), 759-69 (2006)

87. A. Nayal, D. J. Webb, C. M. Brown, E. M. Schaefer, M. Vicente-Manzanares and A. R. Horwitz: Paxillin phosphorylation at ser273 localizes a GIT1-PIX-PAK complex and regulates adhesion and protrusion dynamics. *Journal of Cell Biology*, 173(4), 587-599 (2006)

88. L. Adam, R. Vadlamudi, M. Mandal, J. Chernoff and R. Kumar: Regulation of microfilament reorganization and invasiveness of breast cancer cells by kinase dead p21-activated kinase-1. *Journal of Biological Chemistry*, 275(16), 12041-12050 (2000)

89. J. A. Frost, A. Khokhlatchev, S. Stippec, M. A. White and M. H. Cobb: Differential effects of PAK1-activating mutations reveal activity-dependent and -independent effects on cytoskeletal regulation. *J Biol Chem*, 273(43), 28191-8 (1998)

90. M. A. Sells, J. T. Boyd and J. Chernoff: p21-activated kinase 1 (Pak1) regulates cell motility in mammalian

fibroblasts. *Journal of Cell Biology*, 145(4), 837-849 (1999)

91. M. A. Sells, A. Pfaff and J. Chernoff: Temporal and spatial distribution of activated Pak1 in fibroblasts. *Journal of Cell Biology*, 151(7), 1449-1457 (2000)

92. M. D. Bright, A. P. Garner and A. J. Ridley: PAK1 and PAK2 have different roles in HGF-induced morphological responses. *Cellular Signalling*, 21(12), 1738-1747 (2009)

93. M. M. Zegers, M. A. Forget, J. Chernoff, K. E. Mostov, M. B. ter Beest and S. H. Hansen: Pak1 and PIX regulate contact inhibition during epithelial wound healing. *EMBO J*, 22(16), 4155-65 (2003)

94. S. J. Coniglio, S. Zavarella and M. H. Symons: Pak1 and Pak2 mediate tumor cell invasion through distinct signaling mechanisms. *Mol Cell Biol*, 28(12), 4162-72 (2008)

95. Q. W. Li, S. R. Mullins, B. F. Sloane and R. R. Mattingly: p21-activated kinase 1 coordinates aberrant cell survival and pericellular proteolysis in a three-dimensional culture model for pre-malignant progression of human breast cancer. *Neoplasia*, 10(4), 314-U1 (2008)

96. M. K. Siu, E. S. Wong, H. Y. Chan, D. S. Kong, N. W. Woo, K. F. Tam, H. Y. Ngan, Q. K. Chan, D. C. Chan, K. Y. Chan and A. N. Cheung: Differential expression and phosphorylation of Pak1 and Pak2 in ovarian cancer: effects on prognosis and cell invasion. *Int J Cancer* (2009)

97. E. Lozano, M. A. Frasa, K. Smolarczyk, U. G. Knaus and V. M. Braga: PAK is required for the disruption of E-cadherin adhesion by the small GTPase Rac. *J Cell Sci*, 121(Pt 7), 933-8 (2008)

98. H. He, A. Shulkes and G. S. Baldwin: PAK1 interacts with beta-catenin and is required for the regulation of the beta-catenin signalling pathway by gastrins. *Biochim Biophys Acta*, 1783(10), 1943-54 (2008)

99. S. Chen, T. Auletta, O. Dovirak, C. Hutter, K. Kuntz, S. El-Ftesi, J. Kendall, H. Han, D. D. von Hoff, R. Ashfaq, A. Maitra, C. A. Iacobuzio-Donahue, R. H. Hruban and R. Lucito: Copy number alterations in pancreatic cancer identify recurrent PAK4 amplification. *Cancer Biol Ther*, 7 (2008)

100. A. C. Kimmelman, A. F. Hezel, A. J. Aguirre, H. Zheng, J. Paik, H. Ying, G. C. Chu, J. X. Zhang, E. Sahin, G. Yeo, A. Ponugoti, R. Nabioullin, S. Deroo, S. Yang, X. Wang, J. P. McGrath, M. Protopopova, E. Ivanova, J. Zhang, B. Feng, M. S. Tsao, M. Redston, A. Protopopov, Y. Xiao, P. A. Futreal, W. C. Hahn, D. S. Klimstra, L. Chin and R. A. DePinho: Genomic alterations link Rho family of GTPases to the highly invasive phenotype of pancreas cancer. *Proc Natl Acad Sci U S A*, 105, 19372–19377 (2008)

101. A. Begum, I. Imoto, K. I. Kozaki, H. Tsuda, E. Suzuki, T. Amagasa and J. Inazawa: Identification of PAK4 as a putative target gene for amplification within 19q13.12-q13.2 in oral squamous-cell carcinoma. *Cancer Sci 100, 1908-16* (2009)

102. D. W. Parsons, T. L. Wang, Y. Samuels, A. Bardelli, J. M. Cummins, L. DeLong, N. Silliman, J. Ptak, S. Szabo, J. K. Willson, S. Markowitz, K. W. Kinzler, B. Vogelstein, C. Lengauer and V. E. Velculescu: Colorectal cancer: mutations in a signalling pathway. *Nature.* 436, 792 (2005)

103. J. Qu, M. S. Cammarano, Q. Shi, K. C. Ha, P. de Lanerolle and A. Minden: Activated PAK4 regulates cell adhesion and anchorage-independent growth. *Mol. Cell. Biol.*, 21, 3523–3533 (2001)

104. N. Gnesutta, J. Qu and A. Minden: The serine/threonine kinase PAK4 prevents caspase activation and protects cells from apoptosis. *J Biol Chem*, 276, 14414-14419 (2001)

105. Y. Liu, H. Xiao, Y. Tian, T. Nekrasova, X. Hao, H. J. Lee, N. Suh, C. S. Yang and A. Minden: The pak4 protein kinase plays a key role in cell survival and tumorigenesis in athymic mice. *Mol Cancer Res*, 6, 215–24 (2008)

106. W. Gong, Z. W. An, Y. L. Wang, X. Y. Pan, W. G. Fang, B. Jiang and H. Q. Zhang: P21-activated kinase 5 is overexpressed during colorectal cancer progression and regulates colorectal carcinoma cell adhesion and migration. *International Journal of Cancer*, 125(3), 548-555 (2009)

107. N. Schrantz, J. D. Correia, B. Fowler, Q. Y. Ge, Z. J. Sun and G. M. Bokoch: Mechanism of p21-activated kinase 6-mediated inhibition of androgen receptor signaling. *Journal of Biological Chemistry*, 279(3), 1922-1931 (2004)

108. M. Zhang, M. Siedow, G. Saia and A. Chakravarti: Inhibition of p21-activated kinase 6 (PAK6) increases radiosentivity of prostate cancer cells. *Proceedings of the American Association for Cancer Research Annual Meeting*, 50, 962 (2009)

109. Y. P. Wang, Q. J. Yu, A. H. Cho, G. Rondeau, J. Welsh, E. Adamson, D. Mercola and M. McClelland: Survey of differentially methylated promoters in prostate cancer cell lines. *Neoplasia*, 7(8), 748-760 (2005)

110. S. Benvenuti and P. M. Comoglio: The MET Receptor tyrosine kinase in invasion and metastasis. *J. Cell. Physiol.* , 213, 316-325 (2007)

111. A. Z. Lai, J. V. Abella and M. Park: Crosstalk in Met receptor oncogenesis. *Trends Cell Biol*, 19, 542-551 (2009)

112. G. N. Paliouras, M. A. Naujokas and M. Park: Pak4, a novel Gab1 binding partner, modulates cell migration and invasion by the Met receptor. *Mol Cell Biol* (2009)

113. C. Dan, A. Kelly, O. Bernard and A. Minden: Cytoskeletal changes regulated by the PAK4 serine/threonine kinase are mediated by LIM kinase 1 and cofilin. *J Biol Chem*, 276, 32115–32121. (2001)

114. Y. Lu, Z. Pan, Y. Devaux and P. Ray: p21-activated Protein Kinase 4 (PAK4) interacts with the Keratinocyte Growth Factor Receptor and participates in Keratinocyte Growth Factor-mediated inhibition of oxidant-induced cell death. *J Biol Chem*, 278, 10374-10380 (2003)

115. C. Gamell, N. Osses, R. Bartrons, T. Rückle, M. Camps, J. L. Rosa and F. Ventura: BMP2 induction of actin cytoskeleton reorganization and cell migration requires PI3-kinase and Cdc42 activity. *J Cell Sci*, 121, 3960-3970 (2008)

116. R. Kaur, X. Liu, O. Gjoerup, A. H. Zhang, X. Yuan, S. P. Balk, M. C. Schneider and M. L. Lu: Activation of p21-activated kinase 6 by MAP kinase kinase 6 and p38 MAP kinase. *Journal of Biological Chemistry*, 280(5), 3323-3330 (2005)

117. M. Bailly and G. E. Jones: Polarised migration: cofilin holds the front. *Curr Biol*, 13(4), R128-30 (2003)

118. P. Nalbant, Y. C. Chang, J. Birkenfeld, Z. F. Chang and G. M. Bokoch: Guanine nucleotide exchange factor-H1 regulates cell migration via localized activation of RhoA at the leading edge. . *Mol Biol Cell* 20, 4070-4082 (2009)

119. M. G. Callow, S. Zozulya, M. L. Gishizky, B. Jallal and T. Smeal: PAK4 mediates morphological changes through the regulation of GEF-H1. *J Cell Sci*, 118(1861-1872) (2005)

120. J. Birkenfeld, P. Nalbant, B. P. Bohl, O. Pertz, K. M. Hahn and G. M. Bokoch: GEF-H1 modulates localized RhoA activation during cytokinesis under the control of mitotic kinases. *Dev Cell* (12), 699-712 (2007)

121. N. O. Deakin and C. E. Turner: Paxillin comes of age. *J. Cell Sci*, 121, 2435-2444. (2008)

122. J. M. Dong, L. S. Lau, Y. W. Ng, L. Lim and E. Manser: Paxillin nuclear-cytoplasmic localization is regulated by phosphorylation of the LD4 motif: evidence that nuclear paxillin promotes cell proliferation. *Biochem J*, 418(1), 173-84 (2009)

123. Z. Li, H. Zhang, L. Lundin, M. Thullberg, Y. Liu, Y. Wang, L. Claesson-Welsh and S. Stromblad: p21-activated kinase 4 phosphorylation of integrin {beta}5 Ser 759 and Ser 762 regulates cell migration. *J Biol Chem* (2010)

124. T. Ahmed, K. Shea, J. R. Masters, G. E. Jones and C. M. Wells: A PAK4-LIMK1 pathway drives prostate cancer cell migration downstream of HGF. *Cell Signal*, 20, 1320-1328 (2008)

125. D. C. Edwards, L. C. Sanders, G. M. Bokoch and G. N. Gill: Activation of LIM-kinase by Pak1 couples Rac/Cdc42 GTPase signalling to actin cytoskeletal dynamics. *Nat Cell Biol*, 1, 253-9 (1999)

126. J. Soosairajah, S. Maiti, O. Wiggan, P. Sarmiere, N. Moussi, B. Sarcevic, R. Sampath, J. R. Bamburg and O. Bernard: Interplay between components of a novel LIM kinase-slingshot phosphatase complex regulates cofilin. *Embo J*, 24, 473-486 (2005)

127. X. Li, Q. Ke, Y. Li, F. Liu, G. Zhu and F. Li: DGCR6L, a novel PAK4 interaction protein, regulates PAK4-mediated migration of human gastric cancer cell via LIMK1. *The International Journal of Biochemistry & Cell Biology*, 42, 70-79 (2010)

128. C. Dan, N. Nath, M. Liberto and A. Minden: PAK5, a new brain-specific kinase, promotes neurite outgrowth in N1E-115 cells. *Molecular and Cellular Biology*, 22(2), 567-577 (2002)

129. M. Johnson, M. Sharma and B. R. Henderson: IQGAP1 regulation and roles in cancer. *Cellular Signalling*, 21(10), 1471-1478 (2009)

130. E. Finkelstein, W. Chang, P. H. Chao, D. Gruber, A. Minden, C. T. Hung and J. C. Bulinski: Roles of microtubules, cell polarity and adhesion in electric-field-mediated motility of 3T3 fibroblasts. *J Cell Sci*, 117, 1533-1545 (2004)

131. X. Q. Wen, X. J. Li, B. Liao, Y. Liu, J. Y. Wu, X. X. Yuan, B. Ouyang, Q. P. Sun and X. Gao: Knockdown of p21-activated Kinase 6 Inhibits Prostate Cancer Growth and Enhances Chemosensitivity to Docetaxel. *Urology*, 73(6), 1407-1411 (2009)

132. H. Aoki, T. Yokoyama, K. Fujiwara, A. M. Tari, R. Sawaya, D. Suki, K. R. Hess, K. D. Aldape, S. Kondo, R. Kumar and Y. Kondo: Phosphorylated Pak1 level in the cytoplasm correlates with shorter survival time in patients with glioblastoma. *Clin Cancer Res*, 13(22 Pt 1), 6603-9 (2007)

133. Y. P. Ching, V. Y. Leong, M. F. Lee, H. T. Xu, D. Y. Jin and I. O. Ng: P21-activated protein kinase is overexpressed in hepatocellular carcinoma and enhances cancer metastasis involving c-Jun NH2-terminal kinase activation and paxillin phosphorylation. *Cancer Res*, 67(8), 3601-8 (2007)

134. G. C. O'Sullivan, M. Tangney, G. Casey, M. Ambrose, A. Houston and O. P. Barry: Modulation of p21-activated kinase 1 alters the behavior of renal cell carcinoma. *Int J Cancer*, 121(9), 1930-40 (2007)

135. J. H. Carter, L. E. Douglass, J. A. Deddens, B. M. Colligan, T. R. Bhatt, J. O. Pemberton, S. Konicek, J. Hom, M. Marshall and J. R. Graff: Pak-1 expression increases with progression of colorectal carcinomas to metastasis. *Clin Cancer Res*, 10(10), 3448-56 (2004)

136. M. K. Siu, E. S. Wong, H. Y. Chan, D. S. Kong, N. W. Woo, K. F. Tam, H. Y. Ngan, Q. K. Chan, D. C. Chan, K. Y. Chan and A. N. Cheung: Differential expression and phosphorylation of Pak1 and Pak2 in ovarian cancer: effects on prognosis and cell invasion. *Int J Cancer*, 127(1), 21-31 (2010)

137. D. B. Foster, L. H. Shen, J. Kelly, P. Thibault, J. E. Van Eyk and A. S. Mak: Phosphorylation of caldesmon by p21-activated kinase. Implications for the Ca(2+) sensitivity of smooth muscle contraction. *J Biol Chem*, 275(3), 1959-65 (2000)

138. N. Takizawa, Y. Koga and M. Ikebe: Phosphorylation of CPI17 and myosin binding subunit of type 1 protein phosphatase by p21-activated kinase. *Biochem Biophys Res Commun*, 297(4), 773-8 (2002)

139. K. Ohtakara, H. Inada, H. Goto, W. Taki, E. Manser, L. Lim, I. Izawa and M. Inagaki: p21-activated kinase PAK phosphorylates desmin at sites different from those for Rho-associated kinase. *Biochem Biophys Res Commun*, 272(3), 712-6 (2000)

140. Z. S. Zhao, J. P. Lim, Y. W. Ng, L. Lim and E. Manser: The GIT-associated kinase PAK targets to the centrosome and regulates Aurora-A. *Mol Cell*, 20(2), 237-49 (2005)

141. A. Barac, J. Basile, J. Vazquez-Prado, Y. Gao, Y. Zheng and J. S. Gutkind: Direct interaction of p21-activated kinase 4 with PDZ-RhoGEF, a G protein-linked Rho guanine exchange factor. *J Biol Chem*, 279, 6182–6189. (2004)

142. A. J. King, H. Sun, B. Diaz, D. Barnard, W. Miao, S. Bagrodia and M. S. Marshall: The protein kinase Pak3 positively regulates Raf-1 activity through phosphorylation of serine 338. *Nature*, 396(6707), 180-3 (1998)

143. M. Zang, C. Hayne and Z. Luo: Interaction between active Pak1 and Raf-1 is necessary for phosphorylation and activation of Raf-1. *J Biol Chem*, 277(6), 4395-405 (2002)

144. C. DerMardirossian, A. Schnelzer and G. M. Bokoch: Phosphorylation of RhoGDI by Pak1 mediates dissociation of Rac GTPase. *Mol Cell*, 15(1), 117-27 (2004)

145. T. L. Chew, R. A. Masaracchia, Z. M. Goeckeler and R. B. Wysolmerski: Phosphorylation of non-muscle myosin II regulatory light chain by p21-activated kinase (gamma-PAK). *Journal of Muscle Research and Cell Motility*, 19(8), 839-854 (1998)

146. H. Goto, K. Tanabe, E. Manser, L. Lim, Y. Yasui and M. Inagaki: Phosphorylation and reorganization of vimentin by p21-activated kinase (PAK). *Genes Cells*, 7(2), 91-7 (2002)

147. P. Schraml, G. Schwerdtfeger, F. Burkhalter, A. Raggi, D. Schmidt, T. Ruffalo, W. King, K. Wilber, M. J. Mihatsch and H. Moch: Combined array comparative genomic hybridization and tissue microarray analysis suggest PAK1 at 11q13.5-q14 as a critical oncogene target in ovarian carcinoma. *Am J Pathol*, 163(3), 985-92 (2003)

148. M. Ito, H. Nishiyama, H. Kawanishi, S. Matsui, P. Guilford, A. Reeve and O. Ogawa: P21-activated kinase 1: a new molecular marker for intravesical recurrence after transurethral resection of bladder cancer. *J Urol*, 178(3 Pt 1), 1073-9 (2007)

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