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PAK signaling in cancer

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Abbreviations: Abl1, Abelson murine leukemia viral oncogene homolog 1; BAD, Bcl-2 antagonist of cell death; CPI17, 17-kDa PKC-potentiated inhibitory protein of PP1; CtBP1, C-terminal-binding protein 1; DLC1, dynein light chain 1; ER, estrogen receptor; ESE1, epithelium-specific Ets transcription factor 1; FKHR, Forkhead box protein O1; G α z, guanine nucleotide binding protein (G protein), alpha z; GEF-H1, guanine nucleotide exchange factor H1; GIT1, G protein-coupled receptor kinase-interactor 1; MBS, myosin binding subunit of type 1 protein phosphatase; MEK1, mitogen-activated protein kinase kinase 1; MEKK1, mitogen-activated protein kinase kinase kinase 1; MLCK, myosin light chain kinase; MNK1, MAP kinase interacting kinase 1; NET1, neuroepithelial cell transforming gene 1 (RhoA-specific guanine nucleotide exchange factor); P41-ARC, actin-related protein 2/3 complex 41kDa subunit; p47 phox, neutrophil NADPH oxidase activator 1; p67 phox, neutrophil NADPH oxidase factor 2; PGAM-B, phosphoglyceratemutase-B; PGM, phosphoglucomutase; PIX, PAK-interacting exchange factor; Plk1, Polo-like kinase 1; Rho GDI, Rho GDP dissociation inhibitor; R-MLC, regulatory myosin light chain; SHARP, SMART/HDAC1 associated repressor protein; SNAIL1, snail 1 zinc finger protein; STAT5A, signal transducer and activator of transcription 5A; Syk, spleen tyrosine kinase; TCoB, tubulin cofactor B

Transformation of a normal cell to a cancer cell is caused by mutations in genes that regulate proliferation, apoptosis, and invasion. Small GTPases such as Ras, Rho, Rac and Cdc42 orchestrate many of the signals that are required for malignant transformation. The p21-activated kinases (PAKs) are effectors of Rac and Cdc42. PAKs are a family of serine/threonine protein kinases comprised of six isoforms (PAK1–6), and they play important roles in cytoskeletal dynamics, cell survival and proliferation. They act as key signal transducers in several cancer signaling pathways, including Ras, Raf, NFκB, Akt, Bad and p53. Although PAKs are not mutated in cancers, they are overexpressed, hyperactivated or amplified in several human tumors and their role in cell transformation make them attractive therapeutic targets. This review discusses the evidence that PAK is important for cell transformation and some key signaling pathways it regulates. This review primarily discusses Group I PAKs (PAK1, PAK2 and PAK3) as Group II PAKs (PAK4, PAK5 and PAK6) are discussed elsewhere in this issue (by Minden).

Introduction

Douglas Hanahan and Robert Weinberg developed a set of "hallmarks of cancer," which serve as defining principles for understanding the complex series of changes in tissues that give rise to malignant tumors. The hallmarks include sustaining proliferative signaling, evading growth suppressors, resisting cell death, enabling replicative immortality, inducing angiogenesis and activating invasion and metastasis. Two emerging hallmarks from the last decade of research are working their way into general acceptance, reprogramming of energy metabolism and evading immune destruction.[1] Cancer cells acquire their hallmarks through mutations in oncogenes, some 200 or so, which have been identified. Despite this large number of genes, the mutations cluster in only about a dozen processes and cell signaling pathways in each tumor.[2] The dissection of these processes and signaling pathways has identified a wealth of targets for therapeutic intervention and several drugs are already on the market to treat tumors. Protein kinases are often mutated themselves and even when not mutated, often regulate key steps in hallmark processes. Biological studies suggest that PAKs play a key role in some of these hallmarks, including proliferative signaling, resisting cell death, activating invasion, metastasis and inducing angiogenesis.

The small GTPases Ras, Rho, Rac and Cdc42 orchestrate many of the hallmarks of cancer. These proteins act as molecular switches existing in two conformational states, GDP and GTP bound. The exchange of GDP for GTP is accelerated by the association of guanine nucleotide exchange factors (GEFs). Mutations in Ras that disrupt the subsequent hydrolysis of GTP and cause Ras to remain its activated GTP-bound state, are found in about 20% of tumors. Upon activation, small GTPases interact with downstream effectors to elicit their responses. The p21-activated kinases (PAKs) are among the best characterized effectors of Rac and Cdc42. They are a family of serine/threonine protein kinases comprised of six isoforms (PAK1–6). PAKs are overexpressed and/or hyperactivated in several human tumors such as breast cancer, neurofibromatosis, colon cancer and lung cancer. They maintain cell transformation by promoting a number of hallmark processes including cell proliferation, survival, motility and angiogenesis (**Fig. 1**).

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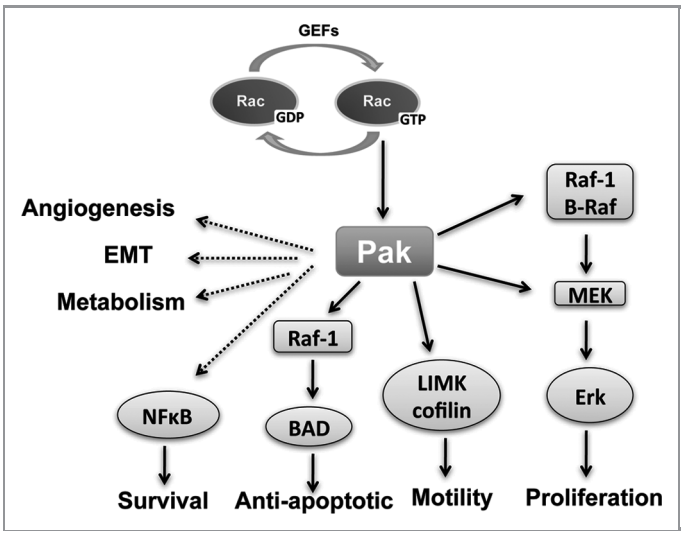

Figure 1. PAKs and cancer hallmarks. PAKs are effectors of Rac/Cdc42 and play a key role in some of cancer hallmarks, including proliferative signaling, resisting cell death, activating invasion and metastasis and inducing angiogenesis. PAKs can regulate cell proliferation through the Raf/Mek pathway. Cell motility can be affected by PAKs phosphorylation of cytoskeletal targets, such as LIMK, which phosphorylates cofilin. PAK1 also phosphorylates Bad directly and indirectly via Raf-1, thus promoting cell survival by anti-apoptosis. NFκB is regulated by PAK indirectly to promote cell survival. Other cancer hallmarks are also affected indirectly by PAKs.

PAK Activation and Amplification in Cancer

There is little evidence for cancer cells having activating mutations in PAK genes although a mutation was found in the kinase domain of PAK4 (E329K) in a colorectal tumor sample. It is not known if the mutation affects kinase activity.[3] However, PAK family members are amplified, overexpressed or hyperactivated in a number of human tumors. PAK1 is the isoform most commonly overexpressed but other family members, most often PAK4 is overexpressed in specific cancers (**Table 1**). PAK4, for example, is overexpressed in 75% of the NCI 60 cell line panel and a dominant negative mutant will block cell transformation of a colon cancer cell line.[4]

Several distinct molecular mechanisms cause aberrant PAK signaling in cancer, including gene amplification and alteration of upstream regulators. Both PAK1 and PAK4 are localized to genomic regions, which are frequently amplified in cancer cells. The PAK1 gene is localized within the 11q13 region, and 11q13.5-q14 amplifications involving the PAK1 locus are found in bladder, ovary and breast cancer.[5-8] PAK4 localizes to another amplicon, 19q13.2, and PAK4 gene amplification has been found in colorectal and pancreatic cancers.[3,9]

PAK gene amplifications are not frequent enough to be the only molecular mechanism leading to PAK overexpression in cancer. A 2008 report identified a novel mechanism for the overexpression of PAK1 through microRNA downregulation. Reddy et al. found that the levels of endogenous microRNA miR-7 inversely correlated with PAK1 expression in a variety of cancer cell lines.[10] Moreover, transfection of miR-7 downregulated PAK1 expression in breast cancer cells, and suppressed motility and

Table 1. Cancers with amplified, overexpressed or activated PAK family members

Cancer type	PAK isoform	Type of alterations	References
Brain	PAK1	Increased phospho-PAK1 in cytoplasm	89
Esophagus	PAK4	Protein overexpression	56
Breast	PAK1, PAK4	Protein overexpression and increased nuclear localization; Gene amplification (11q13→q14 amplicon)	5, 7, 8, 23, 56 and 90
Liver	PAK1	Protein and gene overexpression	91
Kidney	PAK1	Protein overexpression and increased activity	92
Pancreas	PAK4	Gene amplification (19q13 amplicon), protein overexpression	93
Colon	PAK1, PAK4	Protein overexpression. PAK4 gene amplification (19q13 amplicon) and 2 somatic mutations	3, 9, 54 and 56
Bladder	PAK1	Gene amplification (11q13→q14 amplicon)	94
Lung	PAK1	Protein overexpression	8
Ovarian	PAK1	Protein overexpression and gene amplification (11q13→q14 amplicon)	6, 95 and 96
Prostate	PAK6	Protein overexpression	97
T-cell lymphoma	PAK1	Gene amplification	98
NF1	PAK1	Deletion of NF1	42
NF2	PAK1	Deletion of NF2	47 and 48
Neuroendocrine	PAK3	Protein overexpression	99

In some cases PAKs are amplified, while in other cases, the mechanism of overexpression is not known.[100,101]

invasiveness of these cells.[10] PAKs are also overexpressed in lung cancer but the mechanism is not known, although gene amplification is not likely.[8]

PAK Target Recognition

To date, over 40 proteins have been identified as substrates for PAKs (see **Table 2**). As for most protein kinases, there is some flexibility in the recognition sequences phosphorylated by PAK. Shown in **Figure 2** are the examples of phosphorylation sites for several PAK substrates. One study used PAK2 and compared a limited number of peptides derived from the substrate KKRKSGL. This yielded a recognition sequence for PAK2 that is characterized by two basic amino acids in the -2 and -3 positions. For example, the peptide (K/R)RXS, in which the -2 position is an arginine and the -3 position is an arginine or a lysine, is efficiently phosphorylated at the serine residue (X can be an acidic, basic or neutral amino acid).[11] A more comprehensive study used a wider array of peptides and found that PAK1 and PAK2 preferred large hydrophobic residues in positions from +1 to +3, in addition to their preference for basic amino acids at the -2 and -3 positions.[12] PAK1 and PAK2 have nearly identical substrate specificities, but the substrate specificity of PAK4 is significantly different. PAK4 has strong preference and for alanine at the +2 and serine at the +3 position. It should be noted that although there are differences in the preferred consensus sequences for Group I (PAK1, PAK2 and PAK3) and Group II PAKs (PAK4, PAK5 and PAK6), most known substrates are phosphorylated by both groups. Additionally, both groups strongly prefer serine over threonine as a phospho-acceptor site and do not phosphorylate tyrosine at all. Although the Rennefahrt study was able to identify a new PAK substrate by scanning databases, there are limitations to identifying substrates by sequence searches. The study found that none of the known PAK substrates fell into the top 2% of the predicted substrates, suggesting that other factors such as protein-protein interactions facilitate phosphorylation of what are otherwise less-ideal substrates.[12]

PAK Regulation of Cancer Cell Hallmarks

The primary hallmark of cancer is the ability to form tumors. There are several ways to measure tumor cell growth. The most common way is to inject tumor cells into immune compromised or nude mice, where they will grow into tumors. A simpler assay is to grow cells suspended in soft agar. Tumor cells will grow into colonies, a property called anchorage independence, while untransformed cells will not grow. PAKs were first shown to be important for transformation in experiments where a kinase dead mutant of PAK was expressed in fibroblasts together with an oncogenic Ras mutant. The mutant behaved as a dominant negative mutant and prevented Ras from inducing anchorage independent growth in soft agar assays.[13] Kinase dead mutants of PAK4 also inhibit cell transformation.[4,14] The kinase dead mutants do not act by sequestering Rac and Cdc42 because the p21 binding sites can be deleted and the inactive kinase domain by itself will inhibit transformation.[13] Although the use of these dominant negative mutants, and other technologies based on expressing fragments of PAK have since been replaced with siRNAs and small molecule drugs, they were invaluable in establishing the function of PAK in cancer.

To establish if PAK activation could cause tumors, studies were performed expressing activated mutants. Since PAK is not mutated in tumors, activated mutants were constructed. In most studies, activation of only PAK4 caused anchorage independent growth, although some studies found that activated PAK1 induced tumors when expressed with a weakly activated Raf-1 mutant.[14-17] Additionally, transgenic mice that overexpresses a constitutively active PAK1 under a β-lactoglobulin promoter develops malignant mammary gland tumors, although with a relatively long latency period and low penetrance.[18] These studies established that activation primarily of PAK4 is sufficient for tumorigenesis, although in many tumors, PAK1 and PAK4 are necessary for transformation. The precise relationship between PAK1 and PAK4, and indeed other PAK isoforms are not understood. This may be important clinically if isoform specific inhibitors are eventually used therapeutically.

The most prominent hallmarks of cancer for which a role of PAK has been established are stimulation of cell proliferation (including anchorage-independent growth), stimulation of cell survival (e.g., inhibition of apoptosis), and stimulation of cell motility. PAK activation will stimulate each of these hallmarks, while PAK inhibition inhibits the hallmark. Each of these three hallmarks has at least one known target in a well-established signaling pathway, which is a direct PAK target (**Fig. 1**). For cell proliferation, PAK contributes to the canonical MAP kinase cascade of Ras/Raf/MeK/ERK. In anti-apoptotic signaling PAK contributes to the BAD/Bcl-2 pathway. To regulate cell motility, PAK targets LIM kinase, which phosphorylates cofilin. PAKs have also been implicated in other cellular processes that are relevant in tumorigenesis, including angiogenesis,[19] epithelial-mesenchymal transition[20] and metabolism,[21,22] although the signaling pathways are not as well established as for other processes. The molecular targets of PAK and the effects on their signaling pathways will be discussed later, but first we will address PAK in several specific tumors for which a role has been established including breast, neurofibromatosis 1, neurofibromatosis 2, colon and lung.

Breast cancer. The cancer for which PAK is most extensively documented is breast cancer. More than 50% of human breast cancers display overexpression and/or hyperactivation of PAK1 and PAK1 is found on a chromosomal region amplified in 17% of breast cancers.[8,23] In addition, transgenic expression of an activated PAK1 mutant in mouse mammary tissue causes tumors.[18] PAK1 also promotes mammary epithelial cell transformation in 3-dimensional culture model systems. Furthermore, PAK1 expression and its nuclear accumulation increased progressively during the transition from ductal hyperplasia to ductal carcinoma in situ to adenocarcinoma in widely used multistep polyoma-middle T-antigen transgenic mice.[18] PAK4 also promotes tumorigenesis in breast cancer cells.[17] Together, these studies make a strong case for an important role of PAK in breast cancer, suggesting PAK expression in the transformation

process progresses with increasing stages of tumors. Several signaling pathways such as MAPK and MET, NFκB, BAD and estrogen receptor α (ERα) are activated by PAK1 during the progression of breast cancer and these pathways will discussed below.[18,23-28]

Numerous studies have found that expression of PAK1 promotes mammary cell growth. For example, activated PAK1 causes human mammary epithelial (HMLE) cells to form anchorage-independent colonies, and its kinase activity is necessary for PAK1-induced transformation. These effects are due to PAK1 simultaneously activating of MAPK and MET signaling.[24] PAK1 overexpression in mammary tissue also increases the activation of MEK1/2 and p38-MAPK in mammary tumor epithelial cells.[18]

PAK is activated through pathways that are important for breast cancer growth. Growth factors such as prolactin and the

Table 2. Reported PAK substrates

Process	Substrate	Sites	Isoform	References
Cytoskeleton remodeling	α-PIX	S488	PAK1	12
	β-PIX	S340, S525 (transcript A), S497 and1 S682 (transcript B)	PAK1, PAK2	12 and 102
	Caldesmon	S657 and S687	PAK1, PAK3	103–105
	CPI17	T38	PAK1	106
	Desmin		PAK1	107
	Filamin A	S2152	PAK1	37
	GEF-H1	S885	PAK1	108
	GIT1	S517	PAK1	109
	LIM kinase	T508	PAK1, PAK4	83 and 110
	MBS	T641	PAK1	106
	MLCK	S439 and S991	PAK1, PAK2	111 and 112
	NET1	S152 and S153	PAK1	113
	Op18/ stathmin	S16	PAK1	114
	p41-ARC	T21	PAK1	115
	Rho GDI	S101 and S174	PAK1	116
	R-MLC	S19	PAK2	117 and 118
	SRC-3Δ4	T56, S659 and S676	PAK1	39
	TCoB	S65 and S128	PAK1	119
	Vimentin	S25, S38, S50, S56, S65 and S72	PAK1	120–124
Cell growth	Abl1	S637 and S638	PAK2	125 and 126
	Aurora A	T288 and S342	PAK1	109
	B-Raf	S446	PAK1	127
	c-Myc	T358, S373 and T400	PAK2	128
	C-Raf1	S338 and S339	PAK1, PAK2 PAK3	67, 70, 71 and 129–131
	ER α	S305	PAK1	25 and 26
	Erk 3	S189	PAK2	132
	Histone H3	S10	PAK1	133
	MEK1	S298	PAK1	67 and 134–137
	MEKK1	S67	PAK1	138
	Merlin	S518	PAK1	44 and 45
	MNK1	S39	PAK2	139
	Plk1	S49	PAK1	140
	Prolactin	S179	PAK2	141
Cell survival	BAD	S111 (indirectly at S112 and S136)	PAK1, PAK2	71, 74 and 142–144
	DLC1	S88	PAK1	35
	FKHR	S256	PAK1	36

Table 2. Reported PAK substrates (continued)

Process	Substrate	Sites	Isoform	References
Miscellaneous	CtBP1	S158	PAK1	145
	ESE1	S207	PAK1	146
	G α z	S16	PAK1	147
	p47 phox	S303, S304, S320 and S328	PAK1	148 and 149
	p67 phox	Not mapped	PAK1	150
	PGAM-B	S23 and S118	PAK1	21
	PGM	T466	PAK1	22
	SHARP	S3486 and T3568	PAK1	151
	Snail	S246	PAK1	20
	STAT5a	S779	PAK1	152
	Syk	Not mapped	PAK2	153
	Synapsin I	S603	PAK1	154
	Troponin I	S149	PAK1	155
PAK auto-phosphorylation	PAK1	S21, S57, S144, S149, S199 and S204	PAK1	156
	PAK2	S19, S20, S55, S141, S165, S192 and S197	PAK2	156 and 157
	PAK3	S50 and S139	PAK3	156

Modified from references 12 and 100.

oncogene human epidermal growth factor receptor 2 (HER2 or ErbB2) can activate MAPK signaling pathway through PAK1. The prolactin receptor (PRL-R) can initiate and sustain Erk1/2 signaling via the PI3K-dependent Rac/PAK pathway rather than the canonical ErbB2/Shc/Grb2/SOS/Ras route.[29] PRL-R signaling pathway also activates PAK1 through JAK/STAT5, leading to the induction of cyclin D1.[30] ErbB2 gene overexpression, amplification, or mutation occurs in about 25% of human breast cancer.[31] ErbB2 signaling activates a Rac-PAK signaling pathway that contributes to ErbB2 mediated transformation through the MAPK/Erk and Akt pathways.[32,33] ErbB2 expression correlates with PAK levels and enzymatic activity in ER-positive human breast cancer. ErbB2 activates Rac and PAK in a 3D breast epithelial cell culture system, and loss of Rac or PAK activity blocks the morphologic effects of ErbB2 in these cells, accompanied by loss of Erk and Akt activation.[32] Moreover, PAK is required for ErbB2 transformation in a xenograft model of breast cancer.[32]

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Raf-1 (aa 331-445):  PRGQRDSSYYWEIE
BAD   (aa 105-119):  ETRSRHSSYPAGTE
MEK   (aa 290-304):  TPGRPLSSYGMDSR
LIMK  (aa 501-515):  DRKKRYTVVGNPYW
Merlin(aa 511-526):  TDMKRLSMEIEKEK

Consensus:(K/R)RX(S/T)(hydrophobic)
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Figure 2. PAK phosphorylation sites. Activated PAK proteins phosphorylate a variety of substrates on serine/threonine residues, preferably in the context of basic residues such as K/R, R/X, X and S/T, to bring about cell survival and migration, cytoskeleton remodeling and gene regulation. Shown here are the sequences of phosphorylation sites of several PAK substrates. Consensus sequence is also shown. X can be acidic, basic or neutral amino acid.

PAK regulates survival signals in breast cancer. A study examined PAK1 activity in a pre-malignant progression series of MCF10A mammary epithelial cell variants. PAK1 expression levels increased in correlation with the progression stages in this series, indicating a role for PAK1 in the early stages of cell transformation.[34] Activation of the transcription factor NFκB appears to be a prominent mechanism by which PAK1 regulates survival of breast cancer cells. Friedland et al. showed a functional link between the resistance of mammary epithelial cells to apoptosis in 3-dimensional cultures and PAK1-mediated activation of NFκB.[28] Notably, NFκB also promoted cell proliferation via cyclin D1 transcription in breast cancer cells.[23] Phosphorylation of the pro-apoptotic proteins BAD and FKHR, and phosphorylation of DLC1 are other mechanisms by which PAK1 may promote breast cancer cell survival.[35,36]

PAK promotes cell motility signals in breast cancer. PAK substrates that control different aspects of cytoskeletal dynamics, such as LIM kinase, p41-ARC, filamin A, Op18/stathmin and TCoB, are likely to promote the invasiveness of breast cancer cells.[37] In addition, the multimodular protein Scrib positively regulates activation of PAK1 and participates in lamellipodia formation at the leading edge of migratory breast cancer cells.[38] Moreover, PAK-phosphorylated alternate-spliced isoform of the steroid receptor coactivator-3 (SRC-3Delta4) bridges EGFR and focal adhesion kinase (FAK), enhancing breast carcinoma cell migration and metastasis.[39]

PAK is also involved in estrogen receptor signals in breast cancer. Approximately 70% of all breast cancers express the estrogen receptor (ERα), and tamoxifen, a selective anti-estrogen,

is widely used to treat this group of breast cancers. PAK1 is one of many kinases that phosphorylate ERα.[25,40] Deregulated activation of PAK1 produces multiple or inappropriate phosphorylation of ERα, creating a promiscuous receptor that is resistant to tamoxifen and stimulates cell growth in the absence of estrogen.[25,40] The nuclear levels of active PAK1 increased in breast cancer patients with tamoxifen resistance.[25,41] Moreover, ER activation by PAK1 induces upregulation of cyclin D1 in breast cancer cells, as well as in the mammary epithelium.[23] Patients who were negative for PAK1 obtained more benefit from tamoxifen treatment.[41] The link between PAK1 and ERα raises the possibility that tamoxifen resistance might be prevented or reversed by PAK1 inhibition.

Neurofibromatosis. Neurofibromatosis types 1 and 2 (NF1 and NF2) are dominantly inherited autosomal diseases caused by loss-of-function mutations in the tumor suppressor genes NF1 and NF2, respectively. NF1 is a common disease, having a birth incidence of about 1 in 3,000, while NF2 is a relatively rare disorder with an incidence of about 1 in 25,000. Neurofibromatosis patients are predisposed to the development of multiple tumors of the central and peripheral nervous system. Schwann cells, the cells that comprise the myelin sheath around nerves, are predominantly affected in both tumors. Patients carry heterozygous mutations in either the NF1 or NF2 gene but their tumors typically display loss of the residual wild-type allele, conforming to the classic two hit Knudsen paradigm seen with most tumor suppressors. Although NF1 and NF2 are genetically and clinically distinct diseases, loss of each gene product leads to abnormal activation of PAK1, albeit through different mechanisms. Experimental results suggest that PAK1 is important for the malignant growth in both types of neurofibromatosis.

The mechanism of PAK1 activation through NF1 proceeds through the Ras pathway. The product of the NF1 gene is a cytoplasmic protein called neurofibromin. Neurofibromin is widely expressed across a range of tissues but with high concentrations in the nervous system. Neurofibromin is a GTPase activating protein (GAP) and acts by accelerating the intrinsic GTPase activity of Ras. Consequently, loss of neurofibromin is associated with increased levels of activated GTP-bound Ras, which activates oncogenic pathways, including the MAPK cascade and PI3K. Downstream signals of PI3K activate PAK via Rac and Cdc42. Dominant negative PAK mutants are potent inhibitors of Ras transformation in both rat Schwann cells and a malignant peripheral nerve sheet tumor (MPNST or neurofibrosarcoma) cell line from an NF1 patient.[42]

While NF1 activates PAK through effector pathways, NF2 interacts directly with PAK1. The NF2 gene product is a cytoskeleton-associated tumor suppressor named Merlin (also called Schwannomin). Merlin is structurally related to the moesin/ezrin/radixin proteins, which link the actin cytoskeleton to cell surface glycoproteins that control growth and cellular remodeling. Merlin is widely expressed in Schwann cells, meningeal cells, peripheral nerves and the lens. In non-neoplastic cells, Merlin mediates contact-dependent growth inhibition. The growth suppressive function of Merlin depends on its phosphorylation status at Ser518.[43] Under growth restrictive conditions, Merlin is unphosphorylated and inhibits cell proliferation, while under growth permissive conditions, Merlin is phosphorylated. Both cAMP-dependent protein kinase A (PKA) and PAK1 are able to phosphorylate Merlin at Ser518 and thereby inhibit its growth suppressive activity.[44-46] Phosphorylation of Merlin at Ser518 was also demonstrated by PAK2 and PAK6.[45]

While PAK phosphorylates and inhibits Merlin, there is also an important inhibitory feedback mechanism from Merlin to PAK. Group I PAKs are downstream targets of Merlin. Merlin associates with inactive PAK and prevents its activation, perhaps by competing with Rac.[47,48] Phosphorylation at Ser518 induces a conformation change in Merlin and consequently disrupts interaction with PAK1, allowing PAK1 to be activated. Thus, in NF2 patients, loss of Merlin is associated with abnormal PAK1 activity, which also leads to elevated levels of Rac as well as pronounced cell ruffling.[49,50] In cell culture experiments, the PAK1 inhibitors CEP-1347 and WR-PAK18 were able to inhibit the growth of Merlin-deficient tumor cells, but not Merlin-positive cells.[47] The loss of PAK activity restored normal cell growth[51] and movement to cells lacking Merlin function.[52]

Recently, PAK2 has been shown to be essential for the activation of proliferation signals Wnt/β-catenin signaling in schwannoma cells, and depletion of PAK2 suppressed active β-catenin, c-myc and cyclin D1.[53] In NF2 tumors, loss of PAK activity, however, did not reduce Erk or Akt activity, two signaling proteins that are thought to mediate PAK function in NF1.[52] Together, these studies suggest that PAK is a major player underlying Schwann cell transformation and an attractive target for therapeutics in both NF1 and NF2. There are multiple signaling pathways that PAK regulates in Schwann cells and the signals may differ between NF1 and NF2.

Colon cancer. PAK1, PAK4 and PAK5 have been implicated in colon cancer cell transformation through expression studies as well as functional studies where they regulate cell adhesion and migration.[54-56]

Overexpression of PAK1 is observed in 70% of colon cancer samples and is correlated with several signaling pathways including, Wnt, Erk and Akt pathways. Reduction of PAK1 expression decreased cell proliferation, migration/invasion, and survival. Rac1/PAK1 cascade controls β-catenin S675 phosphorylation and its activation in colon cancer cells. Downregulation of PAK1 in colon cancer cells reduces the β-catenin levels and cell proliferation. PAK1 also directly phosphorylated β-catenin at Ser675, leading to more stable and transcriptional active β-catenin.[57] Erk and Akt, downstream targets of PAK1 are involved in colon cancer progression. PAK inhibition alone is equivalent to the dual inhibition of Erk and Akt, whereas inactivation of either the Erk or Akt pathway alone partially inhibited cell migration/invasion and survival and had no effect on proliferation. Thus, in at least this one case, instead of simultaneously inhibiting both Erk and Akt, PAK1 may be a convergence point for therapy.[58]

Lung cancer. Lung cancer, although not as well established as other cancers, is emerging as a tumor depends on PAK1 signaling. A mouse model for Ras-induced lung cancers is highly sensitive to Rac inhibition, suggesting that lung cancers may be dependent on PAK.[59] PAK1 is expressed strongly in the nucleus and cytoplasm

of squamous nonsmall cell lung carcinomas (NSCLCs).[8] Finally, selective inhibition of PAK1 but not PAK2 delayed cell-cycle progression in vitro and in vivo.[8]

Melanoma and other cancers. There are several cancers in which a role for PAK is implied but has not been documented as rigorously. In melanomas, two large scale melanoma sequencing projects found a novel mutation in Rac1, P29S in about 10% of the tumor samples. The mutation caused an increase in GTP-bound Rac1 and furthermore, expression of the mutant in melanocytes increased proliferation and phosphor-ERK levels (see below for a discussion of PAK regulation of ERK).[60,61] Though neither study directly addressed PAK, it is likely that PAKs are required for some melanomas to progress.

In some cases such as pancreatic tumors and ovarian cancers, PAKs are amplified, but functional data are not available. In other cases, the reagents used to test the involvement of PAK were not that specific. For example, a new PAK inhibitor OSU-03012 inhibited migration in thyroid tumor cells, but since this compound also inhibits PDK1, albeit at higher doses, it is premature to conclude that PAK is required in thyroid tumors.[62]

PAK Regulation of Cell Signals

PAKs regulate several cell signaling pathways controlling tumor cell growth and survival including MAPK/Erks,[13] p53,[63] NFκB,[64] Smad[65] and STAT3.[66] In some cases the relationship between PAK with these signaling pathways has been established, while in other cases the direct connection with PAK has yet to be determined. The Erk, NFκB and more recently p53 pathways are the best documented examples of PAK regulation of cancer signaling pathways, and they will be discussed in this section.

MAPK. The canonical MAPK cascade is widely associated with cell proliferation and consists of Ras/Raf/MEK/(MAPK)Erk. Historically, this was the first cancer relevant signal shown to be regulated by PAK pathway. PAK phosphorylates two mediators of the MAP kinase pathway, MEK1 and Raf1, at Ser298 and at Ser338, respectively.[13,67-70] While phosphorylation of these sites by PAK is not sufficient to activate Raf1 or MEK1, it significantly facilitates the activation of these kinases by their upstream activators Ras and Raf1, respectively. The ability of PAK to regulate the MAP kinase pathway is likely to contribute to cell proliferation.

Akt and BAD. Apoptosis, or programmed cell death, is a fundamental process in the development of multicellular organisms. Apoptosis enables an organism to eliminate unwanted or defective cells through an organized process of cellular disintegration. It is a prominent tumor-suppression mechanism and cancer cells require inactivation of pro-apoptotic pathways for tumor formation and progression. PAK activity has been shown to downregulate several important pro-apoptotic pathways.

PAK1 protects cells from intrinsic apoptotic signals via a PAK-Raf1-BAD pathway. PAK1 and PAK5 phosphorylate Raf1 at Ser338 and stimulate translocation of a subpopulation of Raf1 to the mitochondria.[71-74] At the mitochondria, Raf-1 forms a protective complex with Bcl-2 and phosphorylates the pro-apoptotic protein BAD at Ser112. Bcl-2 is a proto-oncogene that maintains the integrity of the mitochondrial barrier if bound in protective complexes, whereas binding of Bcl-2 to the pro-apoptotic protein BAD induces release of pro-apoptotic factors from the mitochondria and leads to apoptosis. Phosphorylation of BAD at specific sites, including Ser112, renders it unable to bind Bcl-2. The phenotype of Raf-1 knock out cells supports a protective role of Raf-1 in apoptosis, as these cells have high rates of apoptosis while exhibiting normal proliferative rates and Erk activation.[75]

NFκB. PAK activates nuclear factor-κB (NFκB) a transcription factor, which is important for cell transformation through its effects on cell survival and proliferation, and it is essential for oncogenes such as Ras and Raf to transform cells. Inactive NFκB is retained in the cytoplasm due to a heterodimeric interaction with its inhibitory protein known as the inhibitor of κB (IκB). Phosphorylation and degradation of IκB is required for the activation and nuclear translocation of NFκB and the subsequent transactivation of NFκB target genes. Phosphorylation of IκB on serine 32 and serine 36 by the IκB kinases inhibitor of IκB kinase IKKα and IKKβ is an important initiation signal for IκB degradation and NFκB release.

Several studies showed that PAK1 can activate NFκB.[64,76-81] It has been shown that PAK1 activates NFκB through the phosphorylation and degradation of IκB,[64,76] however, there is no evidence that PAK1 phosphorylates IκB. Moreover, PAK1 stimulation of the nuclear translocation of the p65 subunit of NFκB is independent of the phosphorylation of IKKα/β.[64,76] In a report of *Helicobacter pylori*-induced NFκB activation, the PAK1 autoregulatory domain was shown to be required for interaction with NFκB-interacting kinase (NIK), which controls the activities of IKKα/β.[82] Therefore, PAK1 may affect the association of NIK, the IKKs, IκB, or NFκB with the scaffolding proteins IKK complex-associated protein or IKKγ. Indeed, it has been shown that the expression of active PAK1 reduces the coprecipitation of IKKβ with NIK from cells and dominant negative forms of IKKα/β block the PAK1 activation of NFκB.[64] However, despite numerous studies showing that PAK regulates NFκB, the direct target of PAK in this pathway has not been determined.

LIMK. There are several established PAK substrates that control cytoskeletal dynamics. The most well established target is LIM kinase. PAK1 and PAK4 both phosphorylate LIM-kinase at threonine residue 508 within LIM-kinase's activation loop, which stimulates LIM-kinase activity. LIM-kinase phosphorylates and inhibits the actin-regulatory protein cofilin. Cofilin depolymerizes actin filaments, thus by phosphorylating cofilin, PAK1 stimulates filaments accumulation by preventing their depolymerization.[83]

p53. The tumor suppressor p53 is mutated in over 50% of human tumors, where it cooperates with Ras to transform cells and acts as a DNA damage checkpoint in the cell cycle.[84] p53 was identified in screen of 113 cell based reporter assays with the pan PAK inhibitor PF-3758309, an ATP-competitive inhibitor.[63] Induction of p53 by a DNA-damaging agent is reduced in cells treated with PF-3758309.[63] Conversely, activating p53 with the p53 degradation inhibitor, Nutlin-3, has no effect on PAK4 activation, consistent with PAK acting upstream of p53.[63] Moreover, other reports also showed activated PAK4 induces

p53 and p21,[85] and PAK-family kinases and p53 expression have been reported to be co-regulated.[86,87] Together these studies suggest that PAK is upstream of p53, although the mechanisms by which PAK regulates p53 are not well understood. The physiological significance of PAK regulation in cancer cells remains to be worked out. In one study, there was no correlation between p53 status and cancer cell sensitivity to PAK inhibition,[63] while another study found that loss of p53 was a synthetic lethal with PAK3. That is, loss of either p53 or PAK3 did not affect cells, but loss of both p53 and PAK3 together prevented cell growth.[88]

Therapeutic Prospects

Because of their central position in cancer hallmarks, protein kinases currently constitute a major focus for drug discovery and most major pharmaceutical companies have kinase programs to develop inhibitors. Small molecular weight inhibitors typically target the highly conserved ATP-binding pockets of the kinase domain and compete with ATP binding. Because of similarities in the active sites of many kinases, specificity issues are common for inhibitors targeting the ATP-binding pocket, and cross-reactivity may cause unwanted toxicities. However, this approach has been successful and in recent years a number of protein kinase inhibitors have successfully been taken through clinical trials to enter clinical practice. Sorafenib (Nexavar®), imatinib mesylate (Gleevec®), temsirolimus (Torisel®), erlotinib (Tarceva®), sunitinib (Sutent®) and gefitinib (Iressa®) are examples of such small molecule kinase inhibitors. The targets for these drugs include Raf-1, Abl, mTOR and the receptor tyrosine kinases EGFR and VEGFR. PAKs have roles in several cellular processes, including cell cycle, cell motility, angiogenesis and evasion from apoptosis. PAK has been shown to be upregulated or hyperactive in several cancers such as breast, glioma, colorectal, prostate, lung (NSCLC) and MPNST. The importance of PAK in cell and animal models of tumorigenesis and metastasis provides the rationale for developing PAK inhibitors as anti-cancer therapeutics. The current status of inhibitor development is discussed in this issue by Coleman and Kissil.

One of the pressing issues with the use of drugs is identifying the tumors and subpopulations of patients who will respond to a given treatment. With most kinase inhibitors, the patients who respond the best have mutations in the targeted kinase. Patients with mutations in Ras fail to respond to any kinase inhibitor, which is unfortunate because Ras is mutated in about 20% of tumors, far more frequently than any of the kinases. Since many of the signals that are regulated by PAK are intrinsic to the Ras pathway, tumors with mutations in Ras may respond to PAK inhibitors in addition to those in which PAK itself is amplified. A survey with the PAK inhibitor PF-3758309 of 92 tumor cell lines derived from colorectal, non-small-cell lung cancer, pancreatic, and breast tumors, found that 46% exhibited IC_{50} values less than 10 nM.[63] In another study, a strong synergy was found with inhibiting PAK and drugs that act in cell signaling pathways that have been discussed in this review. Among the tested compounds, antagonists of inhibitor of apoptosis proteins (IAP; 12- and 57-fold), epidermal growth factor receptor (EGFR; 2.9-, 7.4-, 12.8- and 15-fold), MEK1/2 (8.5-fold), and Src family kinases (5.4-fold) displayed dramatically enhanced efficacy when tested in cells with PAK1 knocked down.[8] It is encouraging that so many tumors respond to PAK inhibitors. However, the mutations and amplifications in tumors that respond to PAK inhibitors have yet to be determined. Additionally, the synergies observed with PAK inhibitors and other drugs suggest that PAK inhibitors are likely to be most effective in combination with other treatments.

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