

p21-activated kinase family: promising new drug targets

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Abstract: The p21-activated kinase (PAK) family of serine/threonine protein kinases are downstream effectors of the Rho family of GTPases. PAKs are frequently upregulated in human diseases, including various cancers, and their overexpression correlates with disease progression. Current research findings have validated important roles for PAKs in cell proliferation, survival, gene transcription, transformation, and cytoskeletal remodeling. PAKs are shown to act as a converging node for many signaling pathways that regulate these cellular processes. Therefore, PAKs have emerged as attractive targets for treatment of disease. This review discusses the physiological and pathological roles of PAKs, validation of PAKs as new promising drug targets, and current challenges and advances in the development of PAK-targeted anticancer therapy, with a focus on PAKs and human cancers.

Keywords: p21-activated kinase, cancer, inhibitor

Introduction

The first p21-activated kinase, PAK1, was discovered in 1994 as a Cdc42/Rac-interacting protein.¹ Cdc42 and Rac proteins are members the Rho family of GTPases, which are known regulators of cell proliferation and motility.² Thereafter, a significant amount of work has revealed the connection of PAKs to a plethora of cellular processes, including proliferation, survival, motility, gene transcription, and oncogenic transformation.^{3,4} Activation of PAKs has been shown to drive oncogenic signaling in cells and contribute to the progression of cancer. This review summarizes the role of PAKs in tumorigenesis and the current status of PAK1-targeted drug development.

Functional characteristics and molecular mechanisms of action Structure and activation mechanisms of PAKs

Six mammalian PAKs are classified into two groups based on their sequence, structural homology, and activation mechanism, ie, group I (PAK1–3) and group II (PAK4–6). All PAKs have a conserved C-terminal kinase domain and an N-terminal regulatory domain.⁵ Group I PAKs are approximately 70% homologous in their sequence but with over 90% homology in their kinase domains.⁶ The N-terminal regulatory domain contains a p21-protein binding domain that overlaps with an autoinhibitory domain (AID, Figure 1). Group I PAKs normally exist as inactive homodimers, in which the AID of one PAK molecule interacts with the kinase domain of another. Binding of Cdc42/Rac to the p21-protein binding domain disrupts this interaction and initiates conformational

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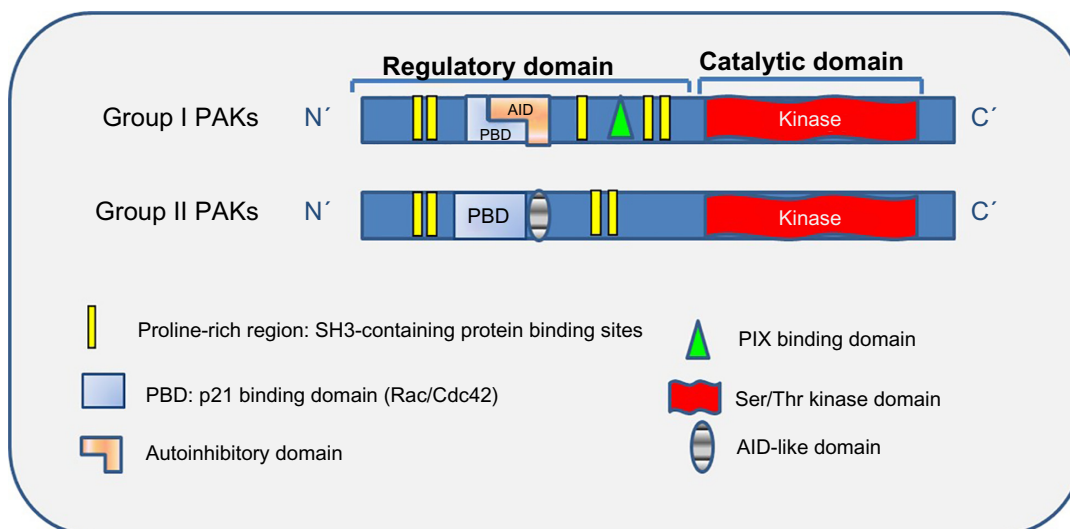


Figure 1 Structure of PAKs.

Notes: The regulatory domain of group I PAKs contains a PBD overlapping with an AID. Group II PAKs do not have the AID, but instead an AID-like region adjacent to the PBD.

Abbreviations: PBD, p21 binding domain; AID, autoinhibitory domain; PAKs, p21-activated kinases; N, N-terminal; C, C-terminal.

changes to trigger autophosphorylation of the activation loop and several C-terminal serine residues, which are critical for full kinase activity.^{1,7} Unlike group I, the kinase domains of group II PAKs are constitutively phosphorylated. Binding to Cdc42/Rac does not result in activation but rather affects subcellular localization.⁸ It was believed that group II PAKs do not possess an AID and are constitutively catalytically active, until recent work revealed the presence of AID-like domains in group II PAKs.^{9–11} PAK5 was first identified to contain an AID and its kinase activation was found to be stimulated by Cdc42 binding similar to group I PAKs.⁹ This domain, however, was absent in PAK4 and PAK6, leading to the assumption of distinct regulation within group II PAKs. Recent studies suggest that the AID-like domain interacts with and inhibits the kinase domain. Activation does not rely on autophosphorylation of the activation loop, but rather occurs readily once this interaction is disrupted, permitting assumption of active conformation.^{10,11}

Biological functions of PAKs

PAKs play important roles in many cellular processes, including cell proliferation, motility, and survival. PAKs stimulate cell proliferation by activating the mitogen-activated protein kinase signaling pathway.^{12,13} Two components of this pathway, c-Raf and MEK1, have been reported to be direct substrates for group I PAKs.^{14–16} PAKs also promote cell proliferation via the Wnt signaling pathway. PAK1 associates and phosphorylates β -catenin to stabilize and facilitate its nuclear translocation, thus controlling transcriptional activity.^{17,18} In addition, PAKs promote cell cycle progression

by stimulating cyclin D1 expression, a key regulator of G1 cell cycle progression.¹⁹ PAK1 has also been shown to localize to centrosomes and to phosphorylate and activate centrosomal kinase Aurora A during mitosis.²⁰ PAKs regulate actin-cytoskeleton rearrangement during cell motility, division, and migration; these are processes that, when deregulated, can be exploited by cancer cells during metastatic and invasive disease progression. PAKs participate in cytoskeletal remodeling by phosphorylating many substrates that control different aspects of cytoskeletal dynamics, such as LIM kinase,²¹ p41-ARC,²² filamin A,²² and myosin light chain kinase.²³ PAK1 phosphorylates c-Raf, causing its relocalization from the cell membrane to the mitochondria, where it binds to Bcl-2 to replace Bad, thereby relieving Bcl-2 from the inhibitory complex to stimulate cell survival signals.²⁴ PAK1 and PAK5 can also phosphorylate Bad directly and dissociate it from Bcl-2,²⁵ thus enhancing cell survival.

PAKs in disease: aberrant PAK function in cancer

PAKs have been shown to affect three main areas of human health, ie, cancer, brain function, and viral infection.²⁶ However, much interest and studies accentuate the understanding of their roles in cancer. This review focuses on the functions of PAKs in different aspects of cancer.

Upregulation of PAKs in cancer

Abnormalities in expression and activity of PAKs are often reported in human cancers. Multiple PAK isoforms have been shown to contribute to tumorigenesis by stimulating

signaling pathways controlling cell proliferation, survival, motility, angiogenesis, anchorage-independent growth, and epithelial–mesenchymal transition,²⁷ processes that are conceptualized to constitute the hallmarks of cancer. PAKs are not found to be frequently mutated in cancers; yet, overexpression and/or gene amplification of PAKs are common.^{28,29} This generally results in elevated mRNA and protein levels, with subsequent accumulation of phosphorylated PAK and presumably increased PAK activity. In particular, PAK1 and PAK4 genes are located on chromosomal regions that are frequently amplified in cancer, and are most strongly associated with cancer compared with other PAK members.

Aberrant function of PAKs, particularly PAK1, is well documented in breast cancer. PAK1 gene amplification at 11q13 is reported as prevalent in luminal breast cancer.³⁰ The frequency of PAK1 amplification is 17% in the tumor panel examined and well correlated with mRNA expression. Immunohistochemistry analysis of breast tumor tissues of different stages revealed that PAK1 expression was upregulated and positively correlated with disease progression³⁰ as well as recurrence rate and mortality.^{31,32} PAK1 small interfering RNA induces robust cell apoptosis associated with caspase activation and attenuated phosphorylation of MEK1 and ERK1/2. Amplification of PAK1 is also reported in ovarian cancer, and more importantly, elevated PAK1 levels are used as an independent prognostic predictor of poor survival in ovarian cancer.³³ Overexpression of PAK1 is observed in over 70% of colorectal cancers (CRCs),³⁴ where its expression increases with disease progression from adenoma to carcinoma, with significant increases in invasive and metastatic CRC.³⁵ In addition, expression of PAK4 and PAK5 is also elevated in CRC.³⁶ PAK4 gene amplification at 19q13.2 has been shown in 22% and 11% of pancreatic cancers and CRCs, respectively.^{37,38} Increased expression of PAK4 is positively correlated with disease aggressiveness and a poor prognosis.³⁹

Loss-of-function mutations of the tumor suppressor genes NF1 and NF2 predispose mutation carriers to development of the dominantly inherited autosomal disease neurofibromatosis type 1 or type 2 (NF1 and NF2), respectively, which is characterized by formation of tumors of the central and peripheral nervous systems. PAK1 promotes the malignant growth of both NF1 and NF2 through different mechanisms. Neurofibromin, encoded by NF1, is a GTPase-activating protein that stimulates Ras GTPase activity, leading to its inactivation and downstream inhibition of PAK1 through the effector pathways. Merlin, the tumor suppressor gene encoded by NF2, directly interacts with the Cdc42/Rac

binding domain of PAK1 to prevent its activation.^{40,41} Merlin is a negative regulator of PAK1 and exerts growth suppressive activity. PAK1 can phosphorylate Merlin on S518, causing conformational changes and consequently disrupting interaction with PAK1, liberating PAK1 for activation.⁴¹ Although mutations of PAKs are not frequently described in cancer, a gain-of-function point mutation of PAK5 was recently reported in 5%–10% of lung cancers.⁴² Inhibition of PAK5 by small interfering RNA decreased cell survival and ERK activity.⁴² Hyperactivated PAK functions can also result from mutations in upstream regulators such as Ras/Rac. The discovery of the role of PAKs in carcinogenesis warrants the potential of targeting PAKs in cancer therapy.

PAK signaling in cancer

Many human cancers are driven by the oncogenic protein Ras, with more than 30% showing somatic gain-of-function mutations of this oncogene.⁴³ Over 90% of pancreatic adenocarcinomas⁴⁴ and 30%–50% of CRCs^{45,46} carry Ras mutations. Of the three human Ras isoforms, NRas, HRas, and KRas, KRas mutations comprise 86%⁴⁷ of all Ras mutations and occur in 17%–25% of all cancers.⁴⁶ KRas mutations are probably best described in CRC, and are clinically associated with a poorer prognosis, increased tumor aggressiveness, and treatment resistance.^{45,46,48} Mutations of Kras cause deregulated cell growth, migration, apoptosis, and differentiation via activation of its downstream effectors. The two canonical Ras effectors are the Raf serine/threonine protein kinases and the phosphoinositide 3-kinases (PI3Ks). The development of anti-Ras therapy has not proved successful due to the complex nature of Ras signaling. The next most sensible approach therefore has been focused on targeting the two mentioned Ras effector signaling pathways. Over 30 and 50 inhibitors targeting the RAF/MEK/MAPK/ERK and the PI3K/AKT cascade, respectively, are currently under evaluation.²⁸ However, the efficacy of targeting components in these two pathways is compromised by the cross-talk and inhibitory effects that exist between the two pathways, and ablating signal from one pathway may result in compensatory signal in the other. Consequently, combination targeting of both pathways may prove helpful in anti-Ras therapy.

PAK1, acting downstream of Ras, can enhance the RAF/MEK/ERK signaling pathway and increase cell proliferation by phosphorylating Raf1 (S338)⁴⁹ and MEK1 (S298).⁵⁰ PAK3 can also phosphorylate Raf1 on S338 to enhance its activation both *in vitro* and *in vivo*.¹⁵ PAK1 regulates the PI3K/AKT pathway in a kinase-independent manner by facilitating the PDK1-mediated recruitment of AKT to the plasma

membrane where it is activated.⁵¹ We have recently reported that PAK1 is required for proliferation, survival, migration, and vascular endothelial growth factor secretion of CRC cells harboring mutations in Ras, PI3K, and Apc¹³ and that PAK1 knockdown inhibited these cellular processes by inactivation of ERK and AKT.¹³ Moreover, PAK1 knockdown suppressed growth and metastasis of CRC cell lines in xenograft and liver metastasis models in mice.¹⁷ These findings are supported by the work of Li et al showing that PAK1 regulates CRC cell metastasis via ERK-dependent phosphorylation of FAK in both cell lines and clinical samples.⁵² Inhibition of PAK1, both genetically and pharmacologically, causes inhibition of ERK and AKT activity, and tumor regression in a KRas-driven skin cancer model.⁵³ These results imply that instead of combination therapies targeting both RAF/MEK/ERK and PI3K/AKT pathways, targeting PAK1 alone could be an alternative approach in cancer treatment, specifically Kras-driven cancers.

However, there was a report that knockdown of PAK1 or PAK4 inhibited the proliferation of mutant KRas colon cancer cells via RAF/MEK/ERK-independent and/or PI3K/AKT-independent pathway(s),⁵⁴ suggesting an alternative signaling pathway is involved. Indeed, the interactions of PAKs with the Wnt pathway have also been reported by several studies.^{17,34,55,56} In breast cancer and CRC cell lines, PAK1 associates with and phosphorylates β -catenin on

S663 and S675,^{34,56} and promotes its nuclear translocation and subsequent transcriptional upregulation of T-cell factor-responsive genes including myc and cyclin D1, a key driver of cell cycle progression. Consistent with this, we have reported that PAK1 binds to β -catenin in CRC cells and that PAK1 knockdown inhibited β -catenin expression and β -catenin/TCF4 transcriptional activity.¹⁷ Taken together, PAK1 facilitates cross-talk between the Ras effector pathways and the Wnt signaling pathway, which is important in tumorigenesis, and thus becomes an appealing target in cancer therapy (Figure 2).

Accumulating evidence indicates that micro (mi)RNAs play a key role in a wide range of biological functions, including cellular proliferation, differentiation, and apoptosis in cancer. Emerging evidence shows that PAKs are regulated by a number of miRNAs that are also recognized to promote hyperactivation of oncogenic Kras signaling. MiRNA-433 inhibits proliferation of hepatocellular carcinoma cells by downregulation of PAK4.⁵⁷ miRNA-145 inhibits P-ERK expression by targeting PAK4 and leads to inhibition of colon cancer growth.⁵⁷ Similarly, miRNA-133 inhibits gastric cancer growth by downregulation of CDC42-PAK1 pathway.⁵⁸ Further investigation of the mechanism(s) of regulation by aberrantly expressed miRNAs of PAKs in cancer and its implications for oncogenic signaling would advance the development of successful therapies targeting PAKs.

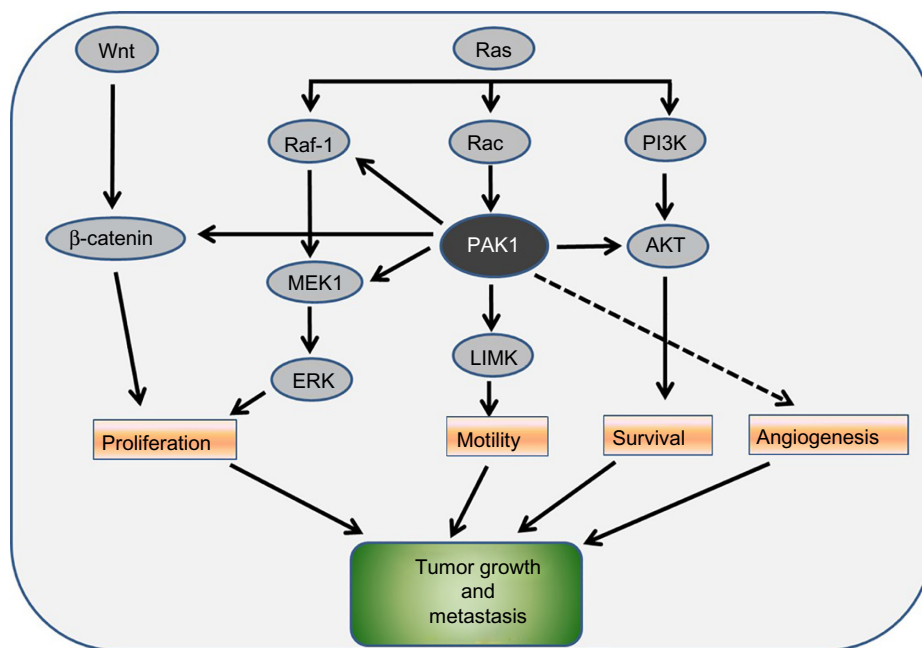


Figure 2 PAK1 functions as a node for multiple signaling pathways.

Notes: PAK1 facilitates cross-talk between the two important effectors of Ras signaling, ie, the Raf/MEK/ERK and PI3K/AKT pathways. PAK1 connects Ras to Wnt signaling pathways, via phosphorylation of β -catenin, modulating downstream cellular processes that favor tumor growth and metastasis.

Abbreviations: PAK, p21-activated kinase; LIMK, LIM kinase; PI3K, phosphatidylinositol 3-kinase.

PAKs can stimulate cell survival through interaction with the proteins of the Bcl-2 family. PAK1,^{25,59} PAK4,⁶⁰ and PAK5^{61,62} can phosphorylate Bad on S112 and/or S136, disabling it from interacting with and inhibiting the pro-survival Bcl-2 and Bcl-xL.⁶² PAK1 and PAK5 can phosphorylate and translocate Raf-1 to the mitochondria, where it can in turn phosphorylate Bad on S112 to inhibit its proapoptotic activity.^{24,25,63} In addition, PAK1 can phosphorylate BimL, another proapoptotic protein, dissociating it from Bcl-2, thereby enhancing cell survival.⁶⁴ Another mechanism employed by PAKs to protect cells from apoptosis involves stimulating the transcription factor nuclear factor kappa B, which regulates genes important for cell survival and proliferation.³ Also, PAK1 can directly phosphorylate and inhibit the proapoptotic transcription factor FKHR by sequestering it in the cytosol, preventing it from activating transcription of proapoptotic genes.⁶⁵ Unlike PAK1, PAK4, or PAK5, PAK2 is unique in containing a caspase site that is cleaved by caspase 3 upon apoptotic signaling, generating a constitutively active fragment known as PAK-2p34,⁶⁶ which can regulate morphological changes during late apoptosis⁶⁷ and stimulate cell death in Jurkat cells.⁶⁸ The full-length PAK2 localizes in the cytoplasm and promotes cell survival in a manner similar to PAK1 phosphorylation of Bad. PAK2 is reported to phosphorylate and inhibit caspase-7 in breast cancer cells, hence reducing cell apoptosis.⁶⁹ The dual role of PAK2 in promoting cell survival or apoptosis is due to differential regulation of subcellular localization of PAK2 and PAK-2p34, where the latter is localized to the nucleus to activate a proapoptotic substrate.⁶⁶

Cell migration requires formation of filopodia and lamellipodia at the leading edge, establishing new adhesions at the forefront, detaching adhesions at the trailing edge, and contracting to propel the cell forward. PAKs coordinate the formation of new adhesions at the leading edge with contraction and detachment at the trailing edge of human microvascular endothelial cells.⁷⁰ PAK1 was observed to localize to focal adhesions in fibroblasts, and when stimulated by platelet-derived growth factor, PAK1 redistributed into the dorsal and membrane ruffles and into the edges of lamellipodia, where it colocalized with polymerized actin.^{71,72} Constitutively active PAK1 induced rapid formation of filopodia and membrane ruffles in mammalian cells⁷³ and enhanced cell motility, invasiveness, and anchorage-independent growth.^{74,75} PAKs appear to control actin filaments turnover and assembly via its downstream target LIM kinase. PAK1²¹ phosphorylates LIM kinase on T508, which in turn phosphorylates cofilin to prevent actin depolymerization.^{76,77} LIM kinase is also

reported to be activated in a PAK2-dependent manner.⁷⁸ In addition, PAK1 phosphorylates the p41-ARC subunit of the Apr2/3 complex, which is important for branching of the network of actin filaments in the cell cortex, contributing to extension of the leading edge.²² PAKs can enhance cell motility by controlling microtubule stability, mediated by the microtubule-destabilizing protein Op18/Stathmin. PAK1 mediates the phosphorylation of Stathmin by Rac1 and Cdc42, blocking its ability to destabilize microtubule, resulting in net growth at the cell's leading edge.^{79,80} PAK1 can directly phosphorylate the tubulin cofactor B, and this is essential for polymerization of new microtubules.⁸¹ PAK-mediated phosphorylation of myosin light chain kinase inhibits phosphorylation of myosin light chain, leading to reduced stress fiber formation.^{23,70,74} For a tumor cell to invade and metastasize, elevated cell motility must be parallel to destruction and reorganization of the extracellular matrix.²⁶ Matrix metalloproteinases (MMPs) control the restructure of the extracellular matrix and mediate human tumor metastasis.⁸² Increased activity of PAK1 in breast cancer cells induces expression of MMP1 and MMP3 and increased cell invasion.^{83,84} PAK5 regulates breast cancer and glioma cell migration and invasion, possibly through Egr1-MMP2 signaling pathway.^{85,86} PAK4 associated with MMP2, and inhibition of PAK4 by small interfering RNA decreased tumor growth by inhibiting MMP-2, β 3-integrin, and phosphorylated epidermal growth factor levels in tumors.⁸⁷ In addition, PAKs are speculated to further facilitate this process by downregulation of adhesion junctions to increase cell permeability and by stimulation of angiogenesis.^{27,88-91}

PAKs as therapeutic targets

The unique central position of PAKs in many intersecting signaling pathways controlling cell proliferation, survival, transformation, and mobility that are frequently deregulated in tumorigenesis and the observation of highly upregulated expression of PAKs in various cancers have validated the rationale for targeting PAKs in treatment of disease, including cancers.

Functional inhibition of PAK1 has been achieved experimentally using a few forms of dominant-negative mutants, aiming to exploit the protein-protein interaction properties of PAKs, including kinase-dead mutant (K299A) and expressing the AID alone. Either approach had been successful to some degree in suppressing kinase-dependent functions of PAKs both in vitro and in vivo.^{55,92} For example, PAK1 transgenic mice expressing a kinase-dead form of PAK1 ameliorate some symptoms in a mouse model of fragile X syndrome.⁹³

However, PAKs do have kinase-independent activities, most of which are implicated in the scaffolding and cytoskeletal reorganization. Attempts to block their functions in this respect include a PAK1-specific cell-permeable small peptide called WR-PAK18, which contains a PIX-binding site. This peptide was demonstrated to block Ras-induced malignant transformation in fibroblasts.⁹⁴ Another small peptide containing a Nck-binding motif effectively disrupted PAK1-Nck interaction and consequentially, blocked cell migration, contractility, and tube formation in endothelial cells.⁹¹ Despite this, the use of dominant-negative forms or peptides to inhibit PAK1 still faces the question of specificity and functional redundancy as it is difficult to delineate the functions of PAK1 from PAK2 and PAK3.

The next approach is using RNA interference. The advantage is that it overcomes the issue of non-specificity presented by the dominant-negative forms, providing improved discrimination between the different PAK isoforms. However, the difficulties associated with RNA interference are unoptimized delivery method, stability, and off-target effects.⁹⁵

Early generations of PAK inhibitors have focused on ATP-competitive compounds, a typical approach to inhibit protein kinases. Extensive structural studies have revealed the ATP binding and substrate catalysis pocket deeply tucked in the cleft between the N-terminal and C-terminal lobes of PAK1.⁴³ This catalytic pocket is unusually large and highly flexible, and in addition to the highly mobile N lobe, presents a challenge in developing a PAK inhibitor.⁴³ Several broad-range kinase inhibitors demonstrate potent PAK inhibition; however, with poor selection due to the strong similarity between the ATP binding pocket of kinases.⁴³ Such non-selective compounds have limited use clinically because of undesired side effects. Attempts to achieve higher selectivity identified the bulky ATP antagonist, CEP-1347. Although CEP-1347 inhibited both PAK1 and MLKs and suppressed PAK-dependent growth of Ras-transformed cells,⁹⁶ disappointingly, it was later demonstrated to have more selectivity for MLK3 and poor potency for PAK1, with an IC_{50} (half maximal inhibitory concentration) value of more than 1 μ M, which is too low to be of clinical importance.⁹⁷ Further attempts to exploit the capacious ATP binding pocket of PAKs identified an organoruthenium compound F172, which showed improved selectivity and potency for PAK1, with an IC_{50} value of around 100 nM.⁹⁸ Nevertheless, compounds based on organometal conjugates like this usually display poor solubility and high toxicity, so their use in clinical settings is questionable.^{27,43}

An ATP-competitive pyrrolopyrazole pan-PAK inhibitor, PF-3758309, was the first PAK inhibitor to enter clinical trials and was developed by Pfizer.⁹⁹ Although designed as a PAK4 inhibitor, it effectively inhibits all PAK members in addition to other off-target protein kinases. PF-3758309 has highest affinity for PAK4 (K_d 2.7 nM), followed by PAK1, PAK5, and PAK6 (K_d 14–18 nM), and lowest affinity for PAK2 and PAK3 (K_d 100–190 nM). Preclinical evaluation demonstrated great potency of PF-3758309 in suppressing proliferation of a panel of tumor cell lines, with an impressive IC_{50} of less than 10 nM and high antitumor effects in human xenograft tumor models.¹⁰⁰ However, a clinical trial in patients with advanced solid tumors was prematurely terminated in Phase I due to low bioavailability, lack of responses in some instances, and in particular adverse effects⁴³ (<http://clinicaltrials.gov/show/NCT00932126>).

Recently, Licciulli et al discovered a group I-specific ATP-competitive PAK inhibitor, FRAX597,¹⁰¹ and illustrated selectivity and potency of FRAX597 against group I PAKs, with a biochemical IC_{50} value of 7–20 nM, while group II PAKs were spared from inhibition even at a concentration higher than 10 μ M. Despite this, FRAX597 also exhibits potency against other kinases, especially the receptor tyrosine kinases. FRAX597 effectively blocked in vitro growth of NF2-deficient Schwann cells as well as tumor formation involving these cells in a xenograft model. In a Kras-driven skin cancer model, treatment with FRAX597 inhibited tumorigenesis associated with near-abolished PAK activation, the reduction of total PAK1 and PAK2 levels and the reduced activity of both ERK and AKT to a level comparable with that of PAK1 knockout mice.⁵³ These data highlight the importance of PAK1 signaling cross-talk with the two Ras effector pathways. Interestingly, the decreased PAK1 and PAK2 expression but not their kinase activity is abolished in cells treated with the proteasome inhibitor, MG132, suggesting that FRAX597 has a dual inhibitory effect on group I PAKs, ie, acting as an ATP-competitive inhibitor and a destabilizing agent.^{27,53} It is worth noting that the inhibitory effects observed in the two models here are consistent with the pan-PAK inhibitor, PF-3758309, yielding similar results. Although both FRAX597 and PF-3758309 have off-target effects on other kinases, these targets are largely non-overlapping. This suggests that the similar antitumor effects exerted by both compounds can be, at least in part, attributed to inhibition of PAKs.

In addition, Zhang et al identified LCH-7749944 as a potent inhibitor of PAK4.¹⁰² LCH-7749944 inhibited PAK4 kinase activity with an IC_{50} of 14.93 μ M, and had less of an

inhibitory effect against PAK1, PAK5, and PAK6. They predicted a binding model of LCH-7749944 in the PAK4 ATP binding pocket, suggesting that LCH-7749944 acts as an ATP-competitive inhibitor. They further reported that LCH-7749944 inhibited gastric cancer growth and migration/invasion via suppression of PAK4-mediated signaling pathways.

Considering the wide breadth of off-target effects the ATP-competitive compounds have in addition to their intended target due to the highly conserved catalytic pocket present in all kinases, the successful ATP-competitor imatinib (Gleevec®)³ achieved high kinase selectivity by binding to a less conserved region near the ATP binding pocket, which accentuated the feasibility of developing inhibitors interacting with less conserved regions of the kinase. This alternative approach seems especially appealing in the tightly regulated group I PAKs by autoinhibition, which presents an opportunity for allosteric inhibition during the multistep conformational changes accompanying kinase activation. Indeed, Deacon et al reported the discovery of a small molecule allosteric inhibitor, IPA-3,¹⁰³ which selectively inhibited group I PAKs, with greatest potency for PAK1, displaying isoform selectivity within group I PAKs. Mechanistically, IPA-3 was found to bind covalently to the AID, preventing binding of Cdc42 to PAK, thus effectively blocked Cdc42-triggered conformational changes and subsequent activation of PAKs.¹⁰⁴ In support of this finding, IPA-3 was essentially ineffective in inhibiting preactivated PAKs. As expected, group II PAKs are insensitive to IPA-3 because their activation is not regulated by AID and is therefore Cdc42-independent. In addition, IPA-3 showed a very limited inhibitory effect on a panel of diverse kinases, demonstrating high kinase specificity. Regrettably, the disulfide covalent bond that is critical for the IPA-3 inhibitory effect can be reduced in the cytoplasm by reducing agents, thereby reversing the binding of IPA-3 to and inhibition of PAKs. These limitations render IPA-3 unsuitable for clinical use. Nonetheless, allosteric inhibition provided an approach from a different angle, ie, targeting the PAK activation mechanism rather than its kinase activity.

Future prospects

A substantial amount of research has been conducted to decipher the roles of PAKs in a range of diseases, with a focus on cancer. Increasing experimental evidence affirm PAKs as potential drug targets due to these observations: PAKs are frequently upregulated in various forms of human cancers, with their overexpression being correlated with

disease progression. PAKs mediate the effects of major signaling pathways, which are often deregulated during cell transformation, such as the Ras/Raf/MEK/ERK, PI3K/AKT/mammalian target of rapamycin and Wnt/ β -catenin pathways. PAKs occupy a unique central position where many oncogenic signaling pathways intersect, enabling cross-talk between them. PAKs also have an established role in many cellular processes that are the hallmarks of cancer initiation, growth, and metastasis. Thus, inhibition of PAKs is attracting increasing interest. The search for PAK inhibitors started in the late 1990s and has intensified over the years. Despite this, only a handful of inhibitors have been developed to date due to the challenges mentioned in the previous section. The currently available inhibitors still face the issue of kinase selectivity and isoform specificity. Such limitations preclude the advancement of these compounds to clinical trials. In cancers, the ideal choice of target should have a critical role in disease progression and persistence while having a more dispensable role for the survival of the whole organism as to enable a more cancer-targeted effect that is tolerable by the subject undergoing treatment. In the case of PAK1, PAK1 knockout mice develop an overall normal phenotype with normal life expectancy and health,¹⁰⁵ suggesting that PAK1-targeted therapy may be well tolerated by the patient. However, given the highly conserved features among the PAKs, especially group I PAKs, isoform specificity in targeting PAKs may trigger compensatory effects by other isoforms due to functional redundancy. This has a more serious implication in the treatment of cancer, ie, acquired resistance to therapy. As PAKs also play important roles physiologically, targeting a single isoform may cause less side effects than targeting all of them. After all, combined knockout of all PAK isoforms has not been achieved and therefore the consequences are still unknown. On the other hand, multiple isoform inhibition of PAK may be necessary to circumvent therapeutic resistance caused by functional redundancy, thereby increasing the risk of side effects. The current understanding of the functions of PAKs has advanced and continues to improve. Nonetheless, there are still gaps in our knowledge that need to be filled, such as the precise underlying mechanism for PAK-targeted therapeutic resistance and identification of substrates unique to specific isoforms. Such knowledge will help to advance the development and improvement of PAK inhibitors, which can conquer the current obstacles of isoform specificity in parallel with functional redundancy, off-target effects, and acquired resistance to anti-PAK therapies.

Disclosure

The authors report no conflicts of interest in this work.

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