Published in final edited form as:

Clin Cancer Res. 2012 July 15; 18(14): 3743–3749. doi:10.1158/1078-0432.CCR-11-1952.

Molecular Pathways: Targeting P21-activated Kinase 1 Signaling in Cancer: Opportunities, Challenges and Limitations

Jeyanthi Eswaran^{1,2}, Da-Qiang Li², Anil Shah¹, and Rakesh Kumar^{2,3,*}

¹McCormick Genomic and Proteomics Center, The George Washington University, Washington, DC 20037, USA

²Department of Biochemistry and Molecular Biology, The George Washington University, Washington, DC 20037, USA

³Cancer Research Program, Rajiv Gandhi Centre for Biotechnology, Thiruvananthapuram 695014, India

Abstract

The evolution of cancer cells involves deregulation of highly regulated fundamental pathways that are central to normal cellular architecture and functions. The p21-activated kinase 1 (PAK1) was initially identified as a downstream effector of the GTPases Rac and Cdc42. Subsequent studies uncovered a plethora of its new functions in growth factor and steroid receptor signaling, cytoskeleton remodeling, cell survival, oncogenic transformation, and gene transcription, largely through systematic discovery of its direct, physiologically relevant substrates. PAK1 is widely up-regulated in several human cancers including hormone-dependent cancer, and is intimately linked to tumor progression and therapeutic resistance. These exciting developments combined with the kinase-independent role of PAK1-centered phenotypic signaling in cancer cells elevated PAK1 as an attractive drug target. The structural and biochemical studies revealed the precise mechanism of PAK1 activation, offering the possibilities to develop PAK1-targeted cancer therapeutic approaches. In addition, emerging reports suggest the potential of PAK1 and its specific phosphorylated substrates as cancer prognostic markers. Here, we summarize the PAK1 molecular pathways in human cancer and discuss the current status of PAK1 targeted anti-cancer therapies.

Keywords

PAK1; Growth factor signaling; Cytoskeleton; Oncogenic transformation; Chromatin and nuclear signaling; Protein kinase inhibitor

Background

Cancer is a complex disease in which many of the characteristics of normal cell behavior are highly deregulated, including sustaining proliferation, resisting cell death, and stimulating invasion and metastasis (1). The signaling pathways behind these hallmarks of cancer cells involve multiple signaling kinases; one such family of signaling nodule is the p21-activated kinases (PAKs) (2, 3). To date, six family members of PAKs have been identified in mammalian cells and classified into group I (PAK 1–3) and group II (PAK 4–6) on the basis of the structural and functional similarities (2, 3). PAK1, the founding member of the PAK family, was first identified by Manser *et al.* in 1994 as a target for the p21ras-related but

*Correspondence: bcmrxk@gwu.edu.

The authors declare no competing financial interests

distinct proteins Cdc42 and Rac1 (4). Over the years, a large body of work has connected the PAK family of proteins to diverse cellular processes, including growth factor and steroid receptor signaling, cytoskeleton reorganization, oncogenic transformation, cell survival, and gene transcription. These emerging findings provide new clues for the development of PAK1-targeted therapeutic intervention in human cancer. Here, we summarize the current knowledge of the biological functions of PAK1 signaling in cancer and the progress in the development of PAK kinase inhibitors.

PAK1 functions as a central node in growth factor signaling

Aberrant activation of growth factor receptor signaling is a common feature in human cancer and is associated with disease progression, treatment resistance, and poor prognosis. In 1996, two groups simultaneously reported that growth factor-mediated activation of receptor tyrosine kinases recruits PAK1 to the plasma membrane through the association with the adapter protein Nck and increases its kinase activity (5, 6). Subsequent studies demonstrated that PAK1 also feed into HER2 pathway and controls the invasiveness of breast cancer cells via PI-3 kinase mediated signaling (7). Accumulating evidence further suggests a central role for PAK1 in growth factor signaling networks, thereby establishing an important mechanism for PAK1 in growth factor signaling-mediated tumorigenesis and tumor progression (2, 8). In the same vein, the Rac-Pak pathway also has been found to be critical for HER2-mediated transformation of human breast cancer cells, supporting a model in which PAK1 cooperates with other oncogenes in transforming epithelial cells (9).

PAK1 regulates cytoskeletal signaling

The cytoskeleton is a complex network of polymeric filaments including, actin filaments, intermediate fibers and microtubules, which controls the internal organization, shape, motility and life cycle of eukaryotic cells (10). Although it has been well established that the GTPases Cdc42 and Rac1 regulate the reorganization of the actin cytoskeleton, involvement of PAK1 kinase in regulation of the cytoskeleton was not reported until 1997, wherein it was found that PAK1 regulates the dissolution of stress fibers and reorganization of focal complexes (11) and the structure of the actin cytoskeleton in mammalian cells (12). Later studies further demonstrated that PAK1 regulates cytoskeleton signaling through phosphorylation of distinct cytoskeletal substrates including the p41-Arc subunit of human Arp2/3 complex (13), the actin-binding proteins LIM-kinase (14), filamin A (FLNA) (15), the microtubule-destabilizing proteins stathmin (16) and tubulin cofactor B (17). In addition, PAK1 kinase is stimulated by direct interaction of proteins other than Cdc42 and Rac1 such as deleted in liver cancer 1 (DLC1) and FLNA (15, 18). Interestingly, a kinase-independent mechanism of cytoskeletal regulation by PAK1 has been also proposed (19, 20), thus opening up the possibility that PAK1 might elicit signaling through protein-protein interaction rather than through kinase activity (20). These findings suggest that PAK1 serves as an effector for Cdc42 and/or Rac1 in promoting cell motility. Indeed, overexpression of PAK1 enhances cancer cell migration and metastasis in various tumor model systems (2).

PAK1 promotes oncogenic transformation and survival

Oncogenic transformation requires a coordinated regulation of signals from oncogenes to stimulate anchorage-independent proliferation, rearrange the actin cytoskeleton, and promote cell survival. As PAK1 functions as a central node in a complex net of oncogenic signaling pathways (2), its overexpression could potentially influence oncogenic transformation and survival in multiple ways. In 1997, Tang *et al.* demonstrated that the expression of a catalytically inactive PAK1 kinase inhibits Ras transformation in Rat-1 fibroblast cells (21). Interestingly, a functional p21-binding domain (PBD) was not required for Ras inhibition, indicating that this inhibition was not due to sequestration of Cdc42 and

Rac (21). Further studies demonstrated that Akt proto-oncogene is a key intermediate between Ras and PAK1 (22). In this context, Akt stimulates PAK1 through a GTPase-independent mechanism, and then PAK1 phosphorylates the death agonist BAD and protects cells from apoptosis (22, 23). Consistently, inhibition of PAK1 reduces Bad phosphorylation and increases apoptosis (24). PAK1 also promotes cell survival through phosphorylating the dynein light chain LC8 on Ser88, rescuing the programmed cell death anoikis, and regulating NF- κ B-dependent survival pathways (18, 25). In support of these findings, using a transgenic mouse model Wang et al. provided the first direct evidence that PAK1 deregulation may be sufficient for the formation of mammary gland tumors *in vivo* (26). These observations establish PAK1 as a breast cancer oncogene that coordinately regulates multiple signaling pathways, the cooperation of which leads to malignant transformation. Consistent with this notion, therapeutic agents that suppress PAK1-mediated survival signals improve the efficacy of current cancer chemotherapies by inducing cell death (24).

PAK1 interjects into chromatin and nuclear signaling

In addition to the significance of PAK1 in cytoskeleton remodeling and signaling network, accumulating evidence highlights the nuclear functions of PAK1 in biologic processes relevant for endocrine resistance and gene transcription. Further, the lessons from PAK1 work have allowed the scientists to expend to other family members such as PAK4, both in terms of its role in cancer as well as nuclear localization and functions (27). The first clue of nuclear functions of PAK1 in cancer cells was from an earlier study in 2000, wherein Vadlamudi *et al.* demonstrated that over-expression of PAK1 in breast cancer cells led to multiple spindle poles and abnormal mitosis (28), a process known as aneuploidy in cancer cells. Soon after, PAK1 was found to be localized in the nucleus of breast tumors (29) and its localization to the spindle poles was dependent on its kinase activity (30), providing distinct clues about the participation of other molecules such as Aurora A (31), tubulin cofactor B (17), and Arpc1b, a newly discovered resident of the spindle pole which is directly phosphorylated on Thr21 by PAK1 or Aurora A (32) in mitosis. It is now accepted that the nuclear functions of PAK1 are not only important in cancer cells but also in the normal cells for a faithful completion of mitosis (30). Recent studies demonstrated that interaction with dynein light chain LC8 is required for PAK1 nuclear import (33).

An important nuclear function of PAK1 is involved in gene transcription, which occurs in the context of chromatin. Singh et al demonstrated that PAK1 is directly associated with chromatin and actively participates in the process of gene expression (34). In addition to a direct association with chromatin, PAK1 affects gene transcription by post-translational modification of transcriptional co-regulators. In this context, PAK1 phosphorylates transcriptional co-repressor C-terminal binding protein 1 (CtBP) selectively on Ser158 within a putative regulatory loop, triggering CtBP cellular redistribution and blocking CtBP corepressor functions (35). Similarly, PAK1 phosphorylates SMRT/HDAC1 associated repressor protein (SHARP) at Ser3486 and Thr3568 within the SHARP repression domain, and regulates SHARP-mediated repression of Notch target reporter gene activation, thereby modulating Notch signaling in human cancer cells (36). In addition, PAK1 also phosphorylates Snail on Serine 246, which, in-turn, modulates the process of epithelial-to-mesenchymal transition (EMT) via affecting the expression of E-cadherin (37).

PAK1 hyperaction promotes hormone-independence

The PAK1 can directly phosphorylate estrogen receptor α (ER α) at Ser305 and promote transactivation activity of ER α (38), thereby contributing to the development of hormone-independent growth of breast cancer cells (39). Moreover, the same Ser-305 of ER-alpha was later shown to be phosphorylated by other signaling molecule PKA, which again was

associated with an expected manifestation of tamoxifen resistance (40, 41). Furthermore, PAK1 signaling-dependent activation of ER-S305 leads to an enhanced phosphorylation at Ser-118 presumably, due to an intra-molecular conformational change, and such structural modifications may participate in the development of tamoxifen resistance (42). In agreement with this notion, studies from the physiologically relevant model systems revealed a progressive nuclear accumulation of PAK1 during breast cancer progression (26). This led to a firm correlation between the increase in nuclear PAK1 and lack of tamoxifen response in breast cancer in a premenopausal breast cancer patient study whose tumors showed overexpression of PAK1 (43, 44). Thus, high PAK1 protein levels are shown to indicate tamoxifen insensitivity in postmenopausal breast cancer in clinic (45, 46); raising the possibility that tamoxifen resistance might be reversed by PAK1 inhibition. In support of this notion, histone deacetylase inhibitor FK22 is shown to significantly reduce the kinase activity of PAK1 and strongly inhibit the estrogen-dependent growth of human breast cancers (47). Since Ser-305 of ER α is now phosphorylated by more than one signaling molecule, this shifted the focus of the field to the site of modification rather than the responsible kinases that might phosphorylate ER α on Ser-335. Now it is generally accepted that Ser-305 phosphorylation plays a critical role in the ligand independent transactivation of ER α by multiple signals other than estrogen, contributing to the development of tamoxifen-resistance. Collectively, these findings opened new avenues to further the search for nuclear PAK1 functions and identify putative PAK1-interacting nuclear proteins.

Molecular mechanism of PAK1 activation

Given the crucial role of PAK1 in cancer, understanding of the molecular mechanism of PAK1 activation is of paramount importance. All PAK isoforms contain a highly conserved C-terminal catalytic domain and an N-terminal regulatory domain. The regulatory domain consists of a p21 GTPase-binding domain (PBD), an auto-inhibitory domain (AID), five SH3-domains, and one non-classical SH3-binding site for the PIX family of proteins (PXP) (2, 3) (Figure S1). Group I PAKs contain a highly conserved AID from yeasts to humans, which is not present in the group II PAK proteins (48). The AID domain in the PAK1 kinase binds in the active site cleft and sequesters the kinase activation loop in an inactive conformation (49, 50). PAK1 activators relieve this auto-inhibition and initiate conformational rearrangements and auto-phosphorylation events leading to kinase activation (49). In this context, there are seven auto phosphorylation sites in PAK1, including Ser21, Ser57, Ser144, Ser149, Ser198, Ser203 and Thr 423. Among them, the Ser21 and Ser144 have been reported to contribute towards PAK1 activation, whereas Thr 423 is proved to be the most critical since it inhibits the binding of the auto-inhibitory region and stabilizes the activation segment of PAK1 upon phosphorylation (51).

The structural studies provided snapshots of auto inhibitory, trans phosphorylated and fully active conformation of the PAK1 kinase domain providing the initial models that are needed for PAK1 inhibitor development. The most recent structural study by Wang *et al.* revealed unphosphorylated ATP analogue bound PAK1 kinase domain to adopt an asymmetric face-to-face dimeric conformation, which is different to the auto inhibited state (52). In the dimer, one monomer adopts inactive conformation as expected but surprisingly, the activation loop of the inactive monomer occupies the substrate binding region of another monomer enabling the active conformation albeit without Thr 423 phosphorylation. Once the substrate interacts with the kinase domain it appears to adopt an active conformation (52). Thus, it is evident that the activation process of PAK1 is a dynamic versatile process that has immense adaptability and flexibility. Taken together, these studies offer several stages from inactive to active state of PAK1 that could be exploited for inhibitor development.

Clinical-Translational Advances

PAK1 has been shown to be highly up-regulated in several human cancers, including hormone-dependent breast and ovarian cancers, digestive system cancers, and central nervous system cancers, by increased copy number of the *Pak1* gene, hyperactivation of GTPases or other upstream signaling adaptors, down-regulation or inhibition of endogenous negative regulators of PAK1 (Table S1) (2, 8). Interestingly, PAK1 is not itself activated by mutation. Given its functional importance in tumorigenesis and metastasis (2), there are considerable efforts to develop highly selective and potent PAK1 inhibitors, which will serve as a basis for further development of PAK1-based therapeutic implication in targeting cancer.

Endogenous PAK1 inhibitors

Given that PAK1 influences a variety of cellular functions, the identification of factors that control the level of PAK1 protein and activity is of great importance. Recent studies revealed that the Rho-family GTPases Cdc42 and Chp target their downstream effector protein PAK1 for proteasomal degradation (53). The NF2 tumor suppressor gene product merlin inhibits the activation of PAK1 through binding to the PBD domain of PAK1 (54). Similarly, the serine/threonine protein kinase LKB1, a tumor suppressor gene that is implicated in suppression of cell growth and metastasis, suppresses PAK1 by phosphorylation of Thr109 in the PBD (55). By performing a yeast two-hybrid screen of a mammary gland library, Talukder et al. found that cysteine-rich inhibitor of PAK1 (CRIPAK) is an endogenous PAK1 inhibitor that has a role in the modulation of PAK1-mediated ER transactivation in breast cancer cells (56). Nischarin, a binding partner for the $\alpha 5 \beta 1$ integrin, selectively inhibits PAK1 kinase activity and PAK1-mediated cell migration through direct interaction with the C-terminal domain of PAK1 (57). Similarly, another PAK1 interacting protein, hPIP1, which is homologous to fission yeast PAK1 regulator Skb15, also blocks PAK1 auto-activation. Similarly, two phosphatases, POPX1 and POPX2 dephosphorylate and inactivate PAK1.

MicroRNAs are noncoding RNAs that inhibit the expression of their targets in a sequence-specific manner and play crucial roles during oncogenesis. It was first reported that microRNA-7 (miR-7) inhibits PAK1 expression by targeting the 3'-untranslated region (UTR) of PAK1 mRNA, and consequently, modulates the function of PAK1 in human cancer cells (58). In agreement with the above finding, miR-7 attenuation in schwannoma tumors stimulates growth partially by up-regulating PAK1-mediated oncogenic signaling pathways (59). Recently, it has also been shown that two functional endothelial cell-specific miRNAs, miR-126a and miR-126b, repress PAK1 expression to regulate vascular integrity in zebrafish (60).

PAK1 small molecule inhibitors

Initial studies by Nheu et al. showed that CEP-1347, a synthetic derivative of the ATP antagonist K252a, directly inhibits PAK1 activity and selectively block the growth of Ras transformants (61). Subsequent screening efforts resulted in identification of a derivative of a cyclooxygenase inhibitor, OSU-03012, which inhibited PAK1 with an IC₅₀ of 1 micromolar (62). Another more recent effort to screen a small library of 48 ruthenium complexes yielded highly specific tetrahedral organoruthenium based PAK1 inhibitor. Further optimization of the initial hit compound from the above screen by Maksimoska et al. (63) recently reported a highly selective and potent inhibitor FL172, which shows better selectivity in a panel of 264 screened kinases and little potency against group II PAKs. The co-crystallization of PAK1-FL172 indicates possibilities for further optimization that might yield more selective and potent compound (63).

Apart from the PAK1 targeted inhibitors, a recent work that employs high-throughput screening and structure-based drug design reports PF-3758309, a potent ($K_D = 2.7$ nM), ATP-competitive, pyrrolopyrazole inhibitor of group II PAKs, PAK4. However, PF-3758309 is shown to inhibit both group I and group II PAKs perhaps due to the similarities in the kinases domains and their active sites (8). This ATP-mimetic compound is orally administrable and shows potent binding and inhibition of PAKs. When this compound was screened against 92 tumor cell lines, many of them showed good IC₅₀ values (~10 nM). Apart from the small molecule inhibitor developments, there has also been a recent report on “de novo” computational design based PAK1 interacting protein, which can bind PAK1 auto inhibitory domain interaction interface in its active conformation and inhibit the activation by Cdc42 (64). However, the newly reengineered proteins bind to active PAK1 with micromolar affinities, which is a good starting point but further optimizations is needed to be therapeutically useful.

Allosteric inhibitors that target the PAK1 activation transition states

The allosteric inhibitors that can interact with non-ATP binding sites such as the interface of the regulatory and catalytic domain or other regulatory regions is also pursued and led to the identification 2,2'-dihydroxy-1,1'-dinaphthyl disulfide (IPA-3), which binds covalently to the PAK1 regulatory domain and prevents binding to upstream activator Cdc42 (49, 65). While IPA-3 showed high potency and slightly more selectivity for group I in controlled experimental conditions, IPA-3 is not effective against pre activated group I kinases. Unfortunately, the disulfide bond of the compound is likely to be reduced under physiological conditions and therefore IPA-3 could be an effective laboratory tool to explore group I PAK functions but not useful towards therapy (66).

Taken together, a handful of endogenous or chemically synthesized PAK1 inhibitors have been identified to date. Among them, the most potent and selective PAK1 inhibitor developed so far is the organometallic FL172 compound, which exhibits a favorable selectivity profile as well as the potent ability to inhibit PAK1 activity (66). Based on these findings, it is expected that these inhibitors could be used in the treatment of cancer as a monotherapy or in combination with the commonly used chemotherapy or radiotherapy. Given that PAK1 is widely expressed in a variety of normal tissues and exhibits a large structural similarity with other members of the PAK family, identifying highly selective PAK1 inhibitors that can distinguish between the group I and II PAKs and selectively kill cancer cells without harming normal cells are the huge challenge we are facing. We also should take into account the potential drug toxicity of PAK1 inhibitors due to their off-targeting effects and the development of acquired resistance to PAK1 inhibitor therapy.

Conclusions

Since the discovery of PAK1 in 1990s and its well established cancer connection with cancer in 2000, there has been an incredible progress made in defining the signaling functions of PAK1 in oncogenic pathways as well as the mechanism of activation (Figure 1). Several recent studies have identified the role of PAK1 in human cancer and PAK1 modulators such as the allosteric inhibitors, microRNA, organometallic compounds, pyrrolopyrazole inhibitor and synthetic proteins that reflect the intensive focus in the area of PAK1-targeted anti-cancer therapy. However, understanding the role of PAK1 in individual cancer types and its crucial nuclear function will provide an opportunity to develop new therapeutic interventions. In this context, a particularly useful approach will be to employ the lessons learnt from the history of RTK targeting into the development of PAK-directed anti-cancer therapies.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

Grant support: The work in the Kumar laboratory is supported by National Institute of Health grant CA90970 (to RK).

We would like to acknowledge Liana Adam, Ratna K. Vadlamudi, Sudhir Babu Kondapaka, and all the past members of the Kumar laboratory for their contributions in the PAK1 signaling field since its inception in 1997. We apologize to several of our colleagues for not citing their primary references due to space restrictions.

References

1. Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. *Cell*. 2011; 144:646–74. [PubMed: 21376230]
2. Kumar R, Gururaj AE, Barnes CJ. p21-activated kinases in cancer. *Nat Rev Cancer*. 2006; 6:459–71. [PubMed: 16723992]
3. Bokoch GM. Biology of the p21-activated kinases. *Annu Rev Biochem*. 2003; 72:743–81. [PubMed: 12676796]
4. Manser E, Leung T, Salihuddin H, Zhao ZS, Lim L. A brain serine/threonine protein kinase activated by Cdc42 and Rac1. *Nature*. 1994; 367:40–6. [PubMed: 8107774]
5. Bokoch GM, Wang Y, Bohl BP, Sells MA, Quilliam LA, Knaus UG. Interaction of the Nck adapter protein with p21-activated kinase (PAK1). *J Biol Chem*. 1996; 271:25746–9. [PubMed: 8824201]
6. Galisteo ML, Chernoff J, Su YC, Skolnik EY, Schlessinger J. The adaptor protein Nck links receptor tyrosine kinases with the serine-threonine kinase Pak1. *J Biol Chem*. 1996; 271:20997–1000. [PubMed: 8798379]
7. Adam L, Vadlamudi R, Kondapaka SB, Chernoff J, Mendelsohn J, Kumar R. Heregulin regulates cytoskeletal reorganization and cell migration through the p21-activated kinase-1 via phosphatidylinositol-3 kinase. *The Journal of biological chemistry*. 1998; 273:28238–46. [PubMed: 9774445]
8. Ong CC, Jubb AM, Haverty PM, Zhou W, Tran V, Truong T, et al. Targeting p21-activated kinase 1 (PAK1) to induce apoptosis of tumor cells. *Proc Natl Acad Sci U S A*. 2011; 108:7177–82. [PubMed: 21482786]
9. Arias-Romero LE, Villamar-Cruz O, Pacheco A, Kosoff R, Huang M, Muthuswamy SK, et al. A Rac-Pak signaling pathway is essential for ErbB2-mediated transformation of human breast epithelial cancer cells. *Oncogene*. 2010; 29:5839–49. [PubMed: 20711231]
10. Janke C, Bulinski JC. Post-translational regulation of the microtubule cytoskeleton: mechanisms and functions. *Nat Rev Mol Cell Biol*. 2011; 12:773–86. [PubMed: 22086369]
11. Manser E, Huang HY, Loo TH, Chen XQ, Dong JM, Leung T, et al. Expression of constitutively active alpha-PAK reveals effects of the kinase on actin and focal complexes. *Mol Cell Biol*. 1997; 17:1129–43. [PubMed: 9032240]
12. Sells MA, Knaus UG, Bagrodia S, Ambrose DM, Bokoch GM, Chernoff J. Human p21-activated kinase (Pak1) regulates actin organization in mammalian cells. *Curr Biol*. 1997; 7:202–10. [PubMed: 9395435]
13. Vadlamudi RK, Li F, Barnes CJ, Bagheri-Yarmand R, Kumar R. p41-Arc subunit of human Arp2/3 complex is a p21-activated kinase-1-interacting substrate. *EMBO Rep*. 2004; 5:154–60. [PubMed: 14749719]
14. Edwards DC, Sanders LC, Bokoch GM, Gill GN. Activation of LIM-kinase by Pak1 couples Rac/Cdc42 GTPase signalling to actin cytoskeletal dynamics. *Nat Cell Biol*. 1999; 1:253–9. [PubMed: 10559936]
15. Vadlamudi RK, Li F, Adam L, Nguyen D, Ohta Y, Stossel TP, et al. Filamin is essential in actin cytoskeletal assembly mediated by p21-activated kinase 1. *Nature cell biology*. 2002; 4:681–90.

16. Daub H, Gevaert K, Vandekerckhove J, Sobel A, Hall A. Rac/Cdc42 and p65PAK regulate the microtubule-destabilizing protein stathmin through phosphorylation at serine 16. *J Biol Chem.* 2001; 276:1677–80. [PubMed: 11058583]
17. Vadlamudi RK, Barnes CJ, Rayala S, Li F, Balasenthil S, Marcus S, et al. p21-activated kinase 1 regulates microtubule dynamics by phosphorylating tubulin cofactor B. *Mol Cell Biol.* 2005; 25:3726–36. [PubMed: 15831477]
18. Vadlamudi RK, Bagheri-Yarmand R, Yang Z, Balasenthil S, Nguyen D, Sahin AA, et al. Dynein light chain 1, a p21-activated kinase 1-interacting substrate, promotes cancerous phenotypes. *Cancer Cell.* 2004; 5:575–85. [PubMed: 15193260]
19. Frost JA, Khokhlatchev A, Stippes S, White MA, Cobb MH. Differential effects of PAK1-activating mutations reveal activity-dependent and -independent effects on cytoskeletal regulation. *J Biol Chem.* 1998; 273:28191–8. [PubMed: 9774440]
20. Adam L, Vadlamudi R, Mandal M, Chernoff J, Kumar R. Regulation of microfilament reorganization and invasiveness of breast cancer cells by kinase dead p21-activated kinase-1. *J Biol Chem.* 2000; 275:12041–50. [PubMed: 10766836]
21. Tang Y, Chen Z, Ambrose D, Liu J, Gibbs JB, Chernoff J, et al. Kinase-deficient Pak1 mutants inhibit Ras transformation of Rat-1 fibroblasts. *Mol Cell Biol.* 1997; 17:4454–64. [PubMed: 9234703]
22. Tang Y, Zhou H, Chen A, Pittman RN, Field J. The Akt proto-oncogene links Ras to Pak and cell survival signals. *J Biol Chem.* 2000; 275:9106–9. [PubMed: 10734042]
23. Schurmann A, Mooney AF, Sanders LC, Sells MA, Wang HG, Reed JC, et al. p21-activated kinase 1 phosphorylates the death agonist bad and protects cells from apoptosis. *Mol Cell Biol.* 2000; 20:453–61. [PubMed: 10611223]
24. Deacon K, Mistry P, Chernoff J, Blank JL, Patel R. p38 Mitogen-activated protein kinase mediates cell death and p21-activated kinase mediates cell survival during chemotherapeutic drug-induced mitotic arrest. *Mol Biol Cell.* 2003; 14:2071–87. [PubMed: 12802076]
25. Frost JA, Swantek JL, Stippes S, Yin MJ, Gaynor R, Cobb MH. Stimulation of NFkappa B activity by multiple signaling pathways requires PAK1. *J Biol Chem.* 2000; 275:19693–9. [PubMed: 10779525]
26. Wang RA, Zhang H, Balasenthil S, Medina D, Kumar R. PAK1 hyperactivation is sufficient for mammary gland tumor formation. *Oncogene.* 2006; 25:2931–6. [PubMed: 16331248]
27. Liu Y, Xiao H, Tian Y, Nekrasova T, Hao X, Lee HJ, et al. The pak4 protein kinase plays a key role in cell survival and tumorigenesis in athymic mice. *Mol Cancer Res.* 2008; 6:1215–24. [PubMed: 18644984]
28. Vadlamudi RK, Adam L, Wang RA, Mandal M, Nguyen D, Sahin A, et al. Regulatable expression of p21-activated kinase-1 promotes anchorage-independent growth and abnormal organization of mitotic spindles in human epithelial breast cancer cells. *J Biol Chem.* 2000; 275:36238–44. [PubMed: 10945974]
29. Balasenthil S, Sahin AA, Barnes CJ, Wang RA, Pestell RG, Vadlamudi RK, et al. p21-activated kinase-1 signaling mediates cyclin D1 expression in mammary epithelial and cancer cells. *J Biol Chem.* 2004; 279:1422–8. [PubMed: 14530270]
30. Li F, Adam L, Vadlamudi RK, Zhou H, Sen S, Chernoff J, et al. p21-activated kinase 1 interacts with and phosphorylates histone H3 in breast cancer cells. *EMBO Rep.* 2002; 3:767–73. [PubMed: 12151336]
31. Zhao ZS, Lim JP, Ng YW, Lim L, Manser E. The GIT-associated kinase PAK targets to the centrosome and regulates Aurora-A. *Mol Cell.* 2005; 20:237–49. [PubMed: 16246726]
32. Molli PR, Li DQ, Bagheri-Yarmand R, Pakala SB, Katayama H, Sen S, et al. Arpc1b, a centrosomal protein, is both an activator and substrate of Aurora A. *J Cell Biol.* 2010; 190:101–14. [PubMed: 20603326]
33. Lightcap CM, Kari G, Arias-Romero LE, Chernoff J, Rodeck U, Williams JC. Interaction with LC8 is required for Pak1 nuclear import and is indispensable for zebrafish development. *PLoS One.* 2009; 4:e6025. [PubMed: 19557173]
34. Singh RR, Song C, Yang Z, Kumar R. Nuclear localization and chromatin targets of p21-activated kinase 1. *J Biol Chem.* 2005; 280:18130–7. [PubMed: 15749698]

35. Barnes CJ, Vadlamudi RK, Mishra SK, Jacobson RH, Li F, Kumar R. Functional inactivation of a transcriptional corepressor by a signaling kinase. *Nat Struct Biol.* 2003; 10:622–8. [PubMed: 12872159]
36. Vadlamudi RK, Manavathi B, Singh RR, Nguyen D, Li F, Kumar R. An essential role of Pak1 phosphorylation of SHARP in Notch signaling. *Oncogene.* 2005; 24:4591–6. [PubMed: 15824732]
37. Yang Z, Rayala S, Nguyen D, Vadlamudi RK, Chen S, Kumar R. Pak1 phosphorylation of snail, a master regulator of epithelial-to-mesenchyme transition, modulates snail's subcellular localization and functions. *Cancer research.* 2005; 65:3179–84. [PubMed: 15833848]
38. Wang RA, Mazumdar A, Vadlamudi RK, Kumar R. P21-activated kinase-1 phosphorylates and transactivates estrogen receptor-alpha and promotes hyperplasia in mammary epithelium. *EMBO J.* 2002; 21:5437–47. [PubMed: 12374744]
39. Balasenthil S, Barnes CJ, Rayala SK, Kumar R. Estrogen receptor activation at serine 305 is sufficient to upregulate cyclin D1 in breast cancer cells. *FEBS Lett.* 2004; 567:243–7. [PubMed: 15178330]
40. Kok M, Zwart W, Holm C, Fles R, Hauptmann M, Van't Veer LJ, et al. PKA-induced phosphorylation of ERalpha at serine 305 and high PAK1 levels is associated with sensitivity to tamoxifen in ER-positive breast cancer. *Breast cancer research and treatment.* 2011; 125:1–12. [PubMed: 20213082]
41. Michalides R, Griekspoor A, Balkenende A, Verwoerd D, Janssen L, Jalink K, et al. Tamoxifen resistance by a conformational arrest of the estrogen receptor alpha after PKA activation in breast cancer. *Cancer Cell.* 2004; 5:597–605. [PubMed: 15193262]
42. Rayala SK, Talukder AH, Balasenthil S, Tharakan R, Barnes CJ, Wang RA, et al. P21-activated kinase 1 regulation of estrogen receptor-alpha activation involves serine 305 activation linked with serine 118 phosphorylation. *Cancer research.* 2006; 66:1694–701. [PubMed: 16452229]
43. Holm C, Rayala S, Jirstrom K, Stal O, Kumar R, Landberg G. Association between Pak1 expression and subcellular localization and tamoxifen resistance in breast cancer patients. *J Natl Cancer Inst.* 2006; 98:671–80. [PubMed: 16705121]
44. Rayala SK, Kumar R. Sliding p21-activated kinase 1 to nucleus impacts tamoxifen sensitivity. *Biomed Pharmacother.* 2007; 61:408–11. [PubMed: 17604944]
45. Bostner J, Ahnstrom Waltersson M, Fornander T, Skoog L, Nordenskjold B, Stal O. Amplification of CCND1 and PAK1 as predictors of recurrence and tamoxifen resistance in postmenopausal breast cancer. *Oncogene.* 2007; 26:6997–7005. [PubMed: 17486065]
46. Bostner J, Skoog L, Fornander T, Nordenskjold B, Stal O. Estrogen receptor-alpha phosphorylation at serine 305, nuclear p21-activated kinase 1 expression, and response to tamoxifen in postmenopausal breast cancer. *Clin Cancer Res.* 2010; 16:1624–33. [PubMed: 20179234]
47. Hirokawa Y, Arnold M, Nakajima H, Zalberg J, Maruta H. Signal therapy of breast cancers by the HDAC inhibitor FK228 that blocks the activation of PAK1 and abrogates the tamoxifen-resistance. *Cancer Biol Ther.* 2005; 4:956–60. [PubMed: 16082189]
48. Ching YP, Leong VY, Wong CM, Kung HF. Identification of an autoinhibitory domain of p21-activated protein kinase 5. *J Biol Chem.* 2003; 278:33621–4. [PubMed: 12860998]
49. Deacon SW, Beeser A, Fukui JA, Rennefahrt UE, Myers C, Chernoff J, et al. An isoform-selective, small-molecule inhibitor targets the autoregulatory mechanism of p21-activated kinase. *Chem Biol.* 2008; 15:322–31. [PubMed: 18420139]
50. Lei M, Lu W, Meng W, Parrini MC, Eck MJ, Mayer BJ, et al. Structure of PAK1 in an autoinhibited conformation reveals a multistage activation switch. *Cell.* 2000; 102:387–97. [PubMed: 10975528]
51. Zenke FT, King CC, Bohl BP, Bokoch GM. Identification of a central phosphorylation site in p21-activated kinase regulating autoinhibition and kinase activity. *J Biol Chem.* 1999; 274:32565–73. [PubMed: 10551809]
52. Wang J, Wu JW, Wang ZX. Structural insights into the autoactivation mechanism of p21-activated protein kinase. *Structure.* 2011; 19:1752–61. [PubMed: 22153498]

53. Weisz Hubsman M, Volinsky N, Manser E, Yablonski D, Aronheim A. Autophosphorylation-dependent degradation of Pak1, triggered by the Rho-family GTPase, Chp. *Biochem J.* 2007; 404:487–97. [PubMed: 17355222]
54. Kissil JL, Wilker EW, Johnson KC, Eckman MS, Yaffe MB, Jacks T. Merlin, the product of the Nf2 tumor suppressor gene, is an inhibitor of the p21-activated kinase, Pak1. *Mol Cell.* 2003; 12:841–9. [PubMed: 14580336]
55. Deguchi A, Miyoshi H, Kojima Y, Okawa K, Aoki M, Taketo MM. LKB1 suppresses p21-activated kinase-1 (PAK1) by phosphorylation of Thr109 in the p21-binding domain. *J Biol Chem.* 2010; 285:18283–90. [PubMed: 20400510]
56. Talukder AH, Meng Q, Kumar R. CRIPak, a novel endogenous Pak1 inhibitor. *Oncogene.* 2006; 25:1311–9. [PubMed: 16278681]
57. Alahari SK, Reddig PJ, Juliano RL. The integrin-binding protein Nischarin regulates cell migration by inhibiting PAK. *EMBO J.* 2004; 23:2777–88. [PubMed: 15229651]
58. Reddy SD, Ohshiro K, Rayala SK, Kumar R. MicroRNA-7, a homeobox D10 target, inhibits p21-activated kinase 1 and regulates its functions. *Cancer Res.* 2008; 68:8195–200. [PubMed: 18922890]
59. Saydam O, Senol O, Wurdinger T, Mizrak A, Ozdener GB, Stemmer-Rachamimov AO, et al. miRNA-7 attenuation in Schwannoma tumors stimulates growth by upregulating three oncogenic signaling pathways. *Cancer Res.* 2011; 71:852–61. [PubMed: 21156648]
60. Zou J, Li WQ, Li Q, Li XQ, Zhang JT, Liu GQ, et al. Two functional microRNA-126s repress a novel target gene p21-activated kinase 1 to regulate vascular integrity in zebrafish. *Circ Res.* 2011; 108:201–9. [PubMed: 21148433]
61. Nheu TV, He H, Hirokawa Y, Tamaki K, Florin L, Schmitz ML, et al. The K252a derivatives, inhibitors for the PAK/MLK kinase family selectively block the growth of RAS transformants. *Cancer J.* 2002; 8:328–36. [PubMed: 12184411]
62. Porchia LM, Guerra M, Wang YC, Zhang Y, Espinosa AV, Shinohara M, et al. 2-amino-N-{4-[5-(2-phenanthrenyl)-3-(trifluoromethyl)-1H-pyrazol-1-yl]-phe nyl} acetamide (OSU-03012), a celecoxib derivative, directly targets p21-activated kinase. *Mol Pharmacol.* 2007; 72:1124–31. [PubMed: 17673571]
63. Maksimoska J, Feng L, Harms K, Yi C, Kissil J, Marmorstein R, et al. Targeting large kinase active site with rigid, bulky octahedral ruthenium complexes. *J Am Chem Soc.* 2008; 130:15764–5. [PubMed: 18973295]
64. Jha RK, Leaver-Fay A, Yin S, Wu Y, Butterfoss GL, Szyperski T, et al. Computational design of a PAK1 binding protein. *J Mol Biol.* 2010; 400:257–70. [PubMed: 20460129]
65. Viaud J, Peterson JR. An allosteric kinase inhibitor binds the p21-activated kinase autoregulatory domain covalently. *Mol Cancer Ther.* 2009; 8:2559–65. [PubMed: 19723886]
66. Yi C, Maksimoska J, Marmorstein R, Kissil JL. Development of small-molecule inhibitors of the group I p21-activated kinases, emerging therapeutic targets in cancer. *Biochem Pharmacol.* 2010; 80:683–9. [PubMed: 20302846]

Figure 1.

PAK1 Molecular Pathway. A, The molecular pathways influenced by PAK1. PAK1 is activated in response to extracellular stimuli and activated PAK1 in turn initiates cascades of pathways. PAK1 is established to impact the cytoskeleton remodeling and the cell survival and transformation pathways by phosphorylating various substrates and these substrates are shown. In the nucleus, PAK1 regulates cell cycle, chromatin and gene expression and the substrates involved in these pathways are also depicted. B, The current spectrum of various types of PAK1 inhibitors.