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

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## Abstract

The evolution of cancer cells involves deregulation of highly regulated fundamental pathways that are central to normal cellular architecture and functions. p21-activated kinase 1 (PAK1) was initially identified as a downstream effector of the GTPases Rac and Cdc42. Subsequent studies uncovered a variety of new functions for this kinase 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 upregulated in several human cancers, such as 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. Structural and biochemical studies revealed the precise mechanism of PAK1 activation, offering the possibility 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 recent findings about the PAK1 molecular pathways in human cancer and discuss the current status of PAK1-targeted anticancer therapies. *Clin Cancer Res; 18(14); 3743–9. ©2012 AACR.*

## Background

Cancer is a complex disease in which many of the characteristics, such as sustaining proliferation, resisting cell death, and stimulating invasion and metastasis, are highly deregulated (1). The signaling pathways behind these hallmarks of cancer cells involve multiple signaling kinases. One such family of signaling nodules is composed of p21-activated kinases [PAK (2, 3)]. To date, 6 PAK family members have been identified in mammalian cells and classified into group I (PAK1–3) and group II (PAK4–6) on the basis of their structural and functional similarities (2, 3). PAK1, the founding member of the PAK family, was first identified by Manser and colleagues (4) in 1994 as a target for the p21ras-related but distinct proteins Cdc42 and Rac1. 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 findings have provided new clues for the development of PAK1-targeted therapeutic interventions in human cancer. Here, we summarize recent findings regarding the biologic functions of PAK1 signaling in cancer, and we discuss the progress that has been made in the development of PAK kinase inhibitors.

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## 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, 2 groups simultaneously reported that growth factor–mediated activation of receptor tyrosine kinases (RTK) recruits PAK1 to the plasma membrane through association with the adapter protein Nck and increases its kinase activity (5, 6). Subsequent studies showed that PAK1 also feeds into the 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

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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 (e.g., actin filaments, intermediate fibers, and microtubules) that control the internal organization, shape, motility, and life cycle of eukaryotic cells (10). Although it is well established that the GTPases Cdc42 and Rac1 regulate the reorganization of the actin cytoskeleton, the involvement of PAK1 kinase in regulation of the cytoskeleton was not reported until 1997. Studies showed 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 showed 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 [LIMK (14)] and filamin A [FLNA (15)], and the microtubule-destabilizing proteins stathmin [STMN (16)] and tubulin cofactor B [TCoB (17)]. In addition, PAK1 kinase is stimulated by the direct interaction of proteins other than Cdc42 and Rac1, such as deleted in liver cancer 1 (DLC1) and FLNA (15, 18). Of interest, a kinase-independent mechanism of cytoskeletal regulation by PAK1 has been also proposed (19, 20), raising the possibility that PAK1 may elicit signaling through protein–protein interaction rather than through kinase activity only (20). These findings suggest that PAK1 serves as an effector for Cdc42 and/or Rac1 in promoting cell motility. Indeed, overexpression of PAK1 has been shown to enhance 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. Because PAK1 functions as a central node in a complex network of oncogenic signaling pathways (2), its overexpression could potentially influence oncogenic transformation and survival in multiple ways. In 1997, Tang and colleagues (21) showed that expression of a catalytically inactive PAK1 kinase inhibited Ras transformation in Rat-1 fibroblast cells. Of interest, 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 showed that the 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 by phosphorylating the dynein light chain LC8 on Ser88, rescuing the programmed cell death anoikis, and regulating NF-κB–dependent survival pathways (18, 25). In support of these findings, using a transgenic mouse model, Wang and colleagues (26) provided the first direct evidence that PAK1 deregulation may be sufficient for the formation of mammary gland tumors *in vivo*. These observations establish PAK1 as a breast cancer oncogene that coordinately regulates multiple signaling pathways leading to malignant transformation. Consistent with this notion, therapeutic agents that suppress PAK1-mediated survival signals were shown to 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 the signaling network, accumulating evidence is highlighting the nuclear functions of PAK1 in biologic processes that are relevant for endocrine resistance and gene transcription. Further, the lessons learned from work on PAK1 have been extended to other family members, such as PAK4, in terms of its role in cancer as well as its nuclear localization and functions (27). The first clue to the nuclear functions of PAK1 in cancer cells came from a study in 2000, in which Vadlamudi and colleagues (28) showed that overexpression of PAK1 in breast cancer cells led to multiple spindle poles and abnormal mitosis, 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 shown to depend on its kinase activity (30). These results provided distinct clues about the participation of other molecules, such as Aurora A (31), TCoB (17), and Arpc1b, a newly discovered resident of the spindle pole that is directly phosphorylated on Thr21 by PAK1 or Aurora A (32) in mitosis. It is now accepted that the nuclear functions of PAK1 are important not only in cancer cells but also in normal cells to ensure completion of mitosis (30). Recent studies showed that interaction with dynein light chain, LC8, is required for PAK1 nuclear import (33).

PAK1 influences gene transcription in several ways. Singh and colleagues (34) showed that PAK1 is directly associated with chromatin and actively participates in the process of gene expression. In addition to a direct association with chromatin, PAK1 affects gene transcription by posttranslational modification of transcriptional coregulators. In this context, PAK1 phosphorylates transcriptional corepressor 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 it regulates SHARP-mediated repression of Notch target reporter gene activation, thereby modulating Notch signaling in human cancer cells (36). In addition, PAK1 phosphorylates Snail on Ser246,

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which in turn modulates the process of epithelial-to-mesenchymal transition by affecting the expression of E-cadherin (37).

## PAK1 Hyperaction Promotes Hormone Independence

PAK1 can directly phosphorylate estrogen receptor α (ERα) at Ser305 and promote the transactivation activity of ERα (38), thereby contributing to the development of hormone-independent growth of breast cancer cells (39). Moreover, the same Ser305 of ERα was shown to be phosphorylated by another signaling molecule, PKA, which 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 Ser118, presumably due to an intramolecular conformational change, and such structural modifications may participate in the development of tamoxifen resistance (42). In agreement with this notion, studies of 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 a study of premenopausal patients with breast cancer whose tumors showed overexpression of PAK1 (43, 44). Thus, high PAK1 protein levels may indicate tamoxifen insensitivity in postmenopausal patients with breast cancer in the clinical setting (45, 46), raising the possibility that tamoxifen resistance can be reversed by PAK1 inhibition. In support of this notion, histone deacetylase inhibitor FK22 was shown to significantly reduce the kinase activity of PAK1 and strongly inhibit the estrogen-dependent growth of human breast cancers (47). Because Ser305 of ERα is phosphorylated by more than one signaling molecule, this shifted the focus of the field to the site of modification rather than the kinases that may be responsible for phosphorylating ERα on Ser305. It is now generally accepted that Ser305 phosphorylation plays a critical role in the ligand-independent transactivation of ERα through multiple signals other than estrogen, contributing to the development of tamoxifen resistance. Collectively, these findings open 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 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 PBD, an autoinhibitory domain (AID), 5 SH3 domains, and 1 nonclassical SH3-binding site for the PIX family of proteins (Supplementary Fig. S1; refs. 2, 3). Group I PAKs contain an AID that is highly conserved in organisms ranging from yeasts to humans but 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 autoinhibition and initiate conformational rearrangements and autophosphorylation events leading to kinase activation (49). In this context, PAK1 has 7 autophosphorylation sites: Ser21, Ser57, Ser144, Ser149, Ser198, Ser203, and Thr423. Of these, Ser21 and Ser144 have been reported to contribute toward PAK1 activation, and Thr423 has proved to be the most critical because it inhibits the binding of the autoinhibitory region and stabilizes the activation segment of PAK1 upon phosphorylation (51).

Structural studies have presented snapshots of autoinhibitory, transphosphorylated, and fully active conformations of the PAK1 kinase domain, providing the initial models that are needed for PAK1 inhibitor development. The most recent structural study by Wang and colleagues (52) revealed that the unphosphorylated ATP analogue-bound PAK1 kinase domain adopts an asymmetric face-to-face dimeric conformation, which is different from the autoinhibited state. In the dimer, one monomer adopts an 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 Thr423 phosphorylation. Once the substrate interacts with the kinase domain, it seems to adopt an active conformation (52). Thus, it is evident that the activation of PAK1 is a dynamic, versatile process that has immense adaptability and flexibility. Taken together, these studies highlight several stages, ranging from the inactive to the active state of PAK1, that could be exploited for inhibitor development.

## Clinical–Translational Advances

PAK1 has been shown to be highly upregulated 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, or downregulation or inhibition of endogenous negative regulators of PAK1 (Supplementary Table S1; refs. 2, 8). Of interest, PAK1 is not itself activated by mutation. Given its functional importance in tumorigenesis and metastasis (2), investigators are making considerable efforts to develop highly selective and potent PAK1 inhibitors, which will serve as a basis for further development of PAK1-based therapeutics 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 of PAK1 (54). Similarly, the serine/threonine

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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 2-hybrid screen of a mammary gland library, Talukder and colleagues (56) 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. Nischarin, a binding partner for the α5β1 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 the fission yeast PAK1 regulator Skb15, also blocks PAK1 autoactivation. Similarly, 2 phosphatases, POPX1 and POPX2, dephosphorylate and inactivate PAK1.

microRNAs (miRNA) 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 miRNA-7 (miR-7) inhibits PAK1 expression by targeting the 3′-untranslated region 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 was shown to stimulate growth partially by upregulating PAK1-mediated oncogenic signaling pathways (59). Recently, it was also shown that 2 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 and colleagues (61) showed that CEP-1347, a synthetic derivative of the ATP antagonist K252a, directly inhibits PAK1 activity and selectively blocks the growth of Ras transformants. Subsequent screening efforts resulted in the identification of a derivative of a cyclooxygenase inhibitor, OSU-03012, that inhibited PAK1 with an $IC_{50}$ of 1 μmol/L (62). Another, more recent effort to screen a small library of 48 ruthenium complexes yielded a highly specific tetrahedral organoruthenium-based PAK1 inhibitor. Further optimization of the initial hit compound from the above screen by Maksimoska and colleagues (63) revealed a highly selective and potent inhibitor, FL172, which showed better selectivity in a panel of 264 screened kinases but little potency against group II PAKs. The cocrystallization of PAK1-FL172 indicated that further optimization might yield more selective and potent compounds (63).

Apart from the PAK1-targeted inhibitors, a recent work that employed high-throughput screening and structure-based drug design reported the development of PF-3758309, a potent (dissociation constant = 2.7 nmol/L) ATP-competitive, pyrrolopyrazole inhibitor of PAK4, a group II PAK.However, PF-3758309 has been shown to inhibit both group I and group II PAKs, perhaps due to the similarities in the kinases' domains and 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 $IC_{50}$ values (~10 nm). In addition to small-molecule inhibitor developments, there has been a recent report on *de novo* computational design-based PAK1 interacting protein, which can bind the PAK1 autoinhibitory domain interaction interface in its active conformation and inhibit activation by Cdc42 (64). The newly reengineered proteins bind to active PAK1 with micromolar affinities, which is a good starting point; however, further optimizations are needed to make this approach therapeutically useful.

## Allosteric Inhibitors That Target the PAK1 Activation Transition States

Allosteric inhibitors that can interact with non-ATP–binding sites, such as the interface of the regulatory and catalytic domain or other regulatory regions, are also being pursued. Such investigations led to the identification 2,2′-dihydroxy-1,1′-dinaphthyldisulfide (IPA-3), which binds covalently to the PAK1 regulatory domain and prevents binding to the upstream activator, Cdc42 (49, 65). Although IPA-3 showed high potency and slightly more selectivity for group I in controlled experimental conditions, it was not effective against preactivated group I kinases. Unfortunately, the disulfide bond of this compound is likely to be reduced under physiologic conditions; therefore, IPA-3 could be an effective laboratory tool to explore group I PAK functions but not useful for therapy (66).

To date, only a handful of endogenous or chemically synthesized PAK1 inhibitors have been identified. The most potent and selective PAK1 inhibitor developed so far is the organometallic FL172 compound, which exhibits a favorable selectivity profile as well as a potent ability to inhibit PAK1 activity (66). On the basis of 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 to other members of the PAK family, it is important to identify highly selective PAK1 inhibitors that can distinguish between group I and II PAKs and selectively kill cancer cells without harming normal cells. We should also 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 the 1990s and its well-established connection with cancer in 2000, investigators have made incredible progress in defining the signaling functions of PAK1 in oncogenic pathways, as well as the mechanism of activation (Fig. 1). Several recent studies have identified the role of PAK1 in human cancer, as well as PAK1 modulators, such as the allosteric inhibitors, miRNA, organometallic compounds,

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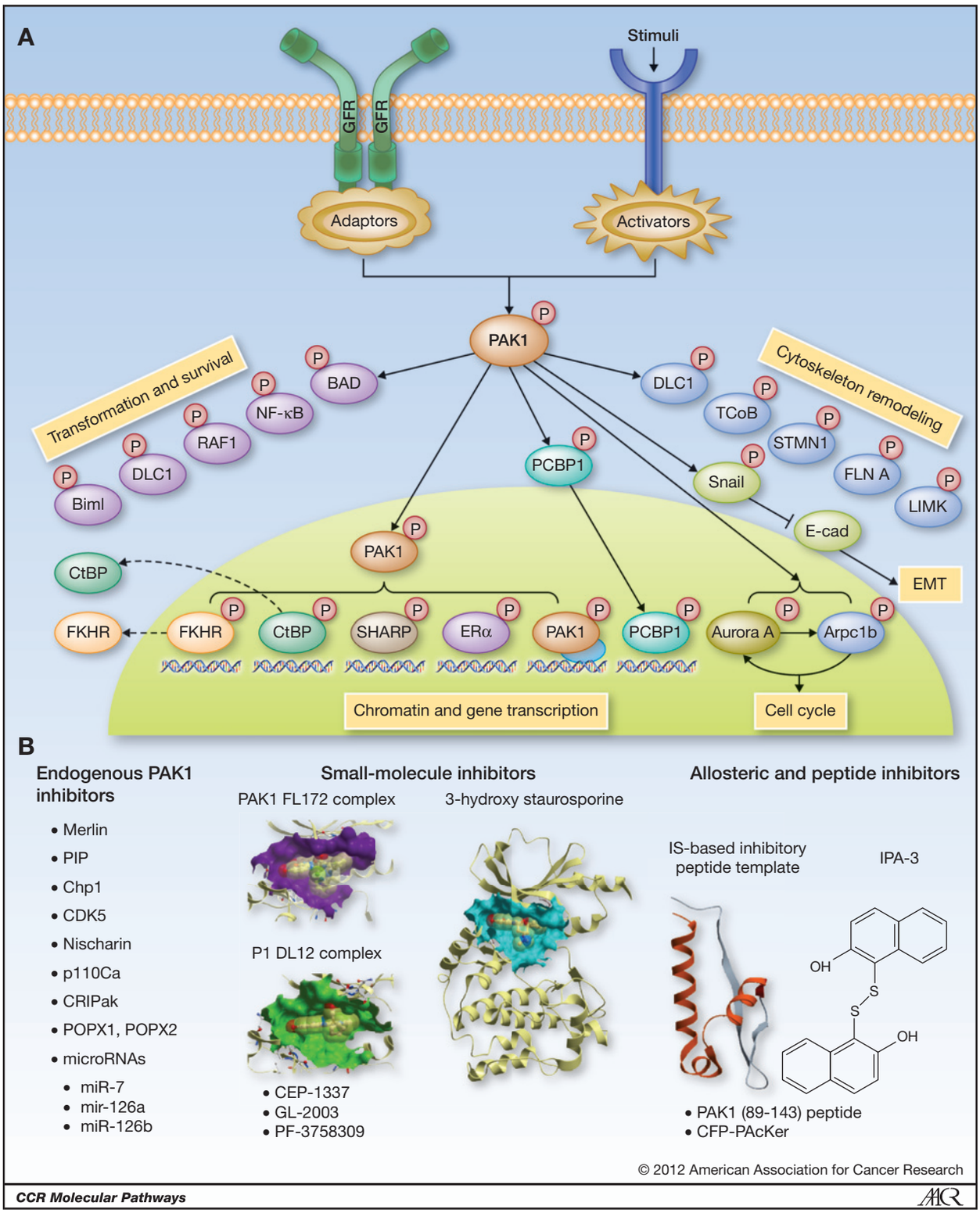


Figure 1. PAK1 molecular pathway. A, molecular pathways influenced by PAK1. PAK1 is activated in response to extracellular stimuli, and in turn the activated PAK1 initiates cascades of pathways. PAK1 influences cytoskeleton remodeling and cell survival and transformation pathways by phosphorylating the substrates shown. In the nucleus, PAK1 regulates the cell cycle and chromatin and gene transcription by phosphorylating the substrates depicted. B, current spectrum of PAK1 inhibitors. IS, inhibitory switch.

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pyrrolopyrazole inhibitor, and synthetic proteins, reflecting the intensive focus in the area of PAK1-targeted anticancer therapy. However, understanding the role of PAK1 in individual cancer types and its crucial nuclear functions will provide further opportunities to develop new therapeutic interventions. In this context, a particularly useful approach would be to translate the lessons learned from studies of RTK targeting into the development of PAK-directed anticancer therapies.

## Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

## Authors' Contributions

**Conception and design:** R. Kumar
**Writing, review, and/or revision of the manuscript:** J. Eswaran, D.-Q. Li, R. Kumar
**Administrative, technical, or material support:** A. Shah

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