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Review

p21-activated kinases and gastrointestinal cancer

Hong He, Graham S. Baldwin *

Department of Surgery, University of Melbourne, Austin Health, Melbourne, Victoria 3084, Australia

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ABSTRACT

p21-activated kinases (PAKs) were initially identified as effector proteins downstream from GTPases of the Rho family. To date, six members of the PAK family have been discovered in mammalian cells. PAKs play important roles in growth factor signalling, cytoskeletal remodelling, gene transcription, cell proliferation and oncogenic transformation. A large body of research has demonstrated that PAKs are up-regulated in several human cancers, and that their overexpression is linked to tumour progression and resistance to therapy. Structural and biochemical studies have revealed the mechanisms involved in PAK signalling, and opened the way to the development of PAK-targeted therapies for cancer treatment. Here we summarise recent findings from biological and clinical research on the role of PAKs in gastrointestinal cancer, and discuss the current status of PAK-targeted anticancer therapies.

1. Introduction

Since Manser and colleagues discovered the first member of the family of p21-activated kinases (PAKs), PAK1, in 1994 [1], a tremendous amount of work has revealed the roles of PAKs in diverse cellular processes, including cytoskeletal reorganisation, gene transcription, cell proliferation and survival, and oncogenic transformation (for reviews see [2–4]). Members of the p21-activated kinase family are key effectors of the Rho family of GTPases, which act as regulatory switches that control cell proliferation and motility [3–5]. PAKs are either up-regulated or hyper-activated in a variety of human cancers and abundant evidence points to roles for PAKs in tumourigenesis (for reviews see [2,3]). Here recent findings regarding the biological functions of PAK signalling in gastrointestinal cancers are summarised. The focus is on the molecular pathways activated by PAKs in the context of four gastrointestinal cancers: hepatocellular carcinoma (HCC), pancreatic cancer, gastric cancer and colorectal carcinoma (CRC).

2. PAK structure

The PAK family of 6 serine/threonine kinases are classified into two groups based on sequence, structural homology and response to activated GTPases (Fig. 1). Both group I (PAKs 1–3) and group II (PAKs 4–6) PAKs are characterised by an N-terminal regulatory domain and a conserved C-terminal kinase domain [3]. Group I PAKs are approximately 70% identical in sequence overall, but share greater than 90% identity in their kinase domains. There is however only approximately 50% identity between the kinase domains of group I and II PAKs [4]. Binding of the activated forms of the GTPases Cdc42 or Rac to the regulatory domain activates PAKs 1, 2 and 3 [6,7]. Signals from growth factor receptor tyrosine kinases and G protein-coupled receptors lead to the activation of PAKs via both GTPase-dependent and independent mechanisms. In particular, oncogenic Ras often activates PAK in cancers [8].

Structural studies have revealed that group I PAKs have a kinase domain, a p21-binding domain (PBD) and an auto-inhibitory domain (AID) which overlaps with the PBD [9]. Binding of an activated GTPase to a group I PAK disrupts PAK dimerisation leading to a series of conformational changes that unfold the AID, which in turn dissociates from the catalytic domain of the other molecule in the dimer [9–12]. All group I PAKs contain a threonine residue in their kinase domain, and dissociation of the AID permits this threonine residue to be autophosphorylated, which is necessary for full kinase activity [13]. Group I PAKs also have an N-terminal domain which binds to PIX, an important downstream effector [14]. Unlike group I PAKs, PAKs 4–6 do not have PIX-binding domains. Like the AID in group I PAKs, the group II PAKs 4–6 contain within their N-terminal regions an auto-inhibitory pseudosubstrate domain (PSD), which inhibits the kinase activity of group II PAKs in the absence of any GTPase (Fig. 1)

Abbreviations: AID, auto-inhibitory domain; CDK5RAP3, CDK5 kinase regulatory subunit-associated protein 3; CRC, colorectal carcinoma; EGFR, epithelial growth factor receptor; GAP, GTPase-activating protein; HBV, hepatitis B virus; HBx, HBV X protein; HCC, hepatocellular carcinoma; PAK, p21-activated kinase; PBD, p21-binding domain; PSD, pseudosubstrate domain

* Corresponding author at: Dept. of Surgery, University of Melbourne, Austin Health, Studley Rd., Heidelberg, Victoria 3084, Australia. Tel.: +61 3 9496 5592; fax: +61 3 9458 1650.

E-mail address: grahamsb@unimelb.edu.au (G.S. Baldwin).

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[15,16]. Structural comparison of the kinase domains of PAKs 4–6 with PAK1 revealed plasticity of the catalytic domain of active group II PAKs, and suggested that there are a number of possible movements allowed within the kinase domain during catalysis [17]. Indeed, group I and group II PAKs have distinct substrate specificities [18]. The catalytic domain of PAK1 has a typical kinase fold containing N- and C-terminal lobes connected by a hinge region which forms a pocket for ATP binding and substrate catalysis [9,19]. Although the initial search for PAK inhibitors focussed on ATP competitors, because of the high degree of similarity between the ATP-binding pockets of kinases such compounds often have poor selectivity, and therefore cause unwanted side effects. PAK inhibitors that target other regions of the molecule have also been developed, and will be discussed later in this review.

3. Biological activities of PAKs

The PAK kinase family plays important roles in many biological activities, including stimulation of cell proliferation, motility and survival [2–4]. Deregulation of these cellular processes initiates and promotes carcinogenesis. PAKs stimulate cell proliferation through enhancing the activation of the MAP kinase pathway, and thereby promoting cell cycle progression. PAK1 phosphorylates two mediators of the MAP kinase pathway, MEK1 and Raf1 [20–22], and facilitates the activation of these kinases by their upstream activators Raf1 and Ras, respectively. The kinase activity of PAK1 peaks at entry into mitosis and remains sustained during mitotic progression. PAKs also promote cell cycle progression by regulation of cyclin D1 expression [23,24].

PAKs regulate cell motility by changing cytoskeletal dynamics. PAKs function as downstream effectors of Rac/Cdc42 in the regulation of the actin cytoskeleton and hence stimulate cell motility and invasion. Growth factors and other cell stimuli cause the redistribution of PAK1 from the cytoplasm into cortical actin structures and focal adhesions [25,26]. PAK1 then interacts with and phosphorylates cytoskeletal proteins, including myosin light chain kinase [27,28], LIM-kinase [29], and the p41-Arc subunit of the Arp2/3 complex [30], and thereby regulates reorganisation of the cytoskeleton.

PAKs also stimulate cell survival by inhibition of apoptosis (i.e. programmed cell death). Both PAK1 and PAK5 phosphorylate Bad, a pro-apoptotic protein, reducing its binding to and inhibition of the two anti-apoptotic proteins Bcl-2 and Bcl-xL, and thereby leading to enhancement of cell survival [31–34]. PAK1 also phosphorylates BimL, another pro-apoptotic protein, and prevents it from binding to and inhibiting Bcl-2 [35]. In rhabdomyosarcoma PAK1 additionally phosphorylates the transcription factor forkhead homolog, and suppresses its ability to activate pro-apoptotic target genes [36]. Amongst group II PAKs, PAK4 is a key effector for Cdc42 and mediates downstream signals that control cell motility, proliferation and survival [37].

4. PAKs in cancer

The PAK kinase family has a variety of effects that promote carcinogenesis including stimulation of cell proliferation, motility, survival, angiogenesis [38], the epithelial–mesenchymal transition [39] and anchorage-independent growth [40,41]. PAKs enhance tumour development by down-regulation of several pro-apoptotic pathways, as discussed in the previous paragraph. The role of PAKs in the regulation of cytoskeletal dynamics contributes significantly to their effects on cancer invasion and metastasis. Knockdown of PAK1 leads to decreased phosphorylation of myosin light chain in breast epithelial cells whilst transfection with a plasmid encoding a mutated inactive PAK1 blocks the invasiveness of breast cancer cells [42]. Expression of PAK1 increases hepatocyte growth factor-induced migration of prostate cancer cells [43]. PAKs have also been implicated in cell adhesion. PAK1 phosphorylates and stimulates the zinc finger protein, snail, which in turn represses E-cadherin promoter activity, causing cells to detach and migrate [44].

Amongst the six PAK isoforms, the role of PAK1 in human cancer has been most thoroughly investigated. The PAK1 gene is amplified in bladder, ovarian, and breast cancers [45,46]. PAK1 expression is increased in 55% of human breast cancers and overexpression correlates with breast cancer invasiveness [2]. PAK1 promotes proliferation and survival of breast cancer cells by activation of nuclear factor kappa B (NFκB) and cyclin D1, and transgenic mice with a constitutively active PAK1 develop malignant mammary gland tumours [47]. PAK1 expression also increases with progression through the adenoma to carcinoma sequence in CRC [48]. PAK1 is critically important for the malignant growth of both neurofibromatosis types 1 and 2, which are dominantly inherited autosomal diseases caused by loss-of-function mutations of the tumour suppressor genes NF1 and NF2, respectively. Mutation carriers are predisposed to the development of multiple tumours in the central and peripheral nervous system. Neurofibromin, the product of the NF1 gene, acts as a GTPase-activating protein (GAP) for Ras by accelerating the intrinsic GTPase activity of Ras, leading to inactivation of Ras and eventually to inhibition of PAK1 activity. Merlin, the product of the NF2 gene, inhibits PAK1 activation by direct interaction with the Rac-binding domain of PAK1 [49,50]. Loss of either the NF1 or NF2 gene product leads to abnormal activation of PAK1.

The other PAK isoforms may also be up-regulated and/or hyperactivated in many human cancers, including breast, ovarian, colorectal and pancreatic cancers [2]. The PAK4 gene is amplified in colorectal and pancreatic cancers [51,52]. PAK5 expression is also increased in a panel of CRC cell lines [53], whilst increased expression of PAK6 has been detected in both prostate cancer cells and breast tumours [54,55].

5. PAKs in gastrointestinal cancers

Gastrointestinal cancer refers to cancers that affect the digestive system, and thus includes cancers of the oesophagus, gallbladder, liver, pancreas, stomach and bowel. Amplification of the genes encoding PAKs and overexpression of PAK proteins have been found in gastrointestinal cancers as listed in Table 1 [56]. The importance of PAKs in cancers of the liver, pancreas, stomach, colon and rectum will be reviewed here.

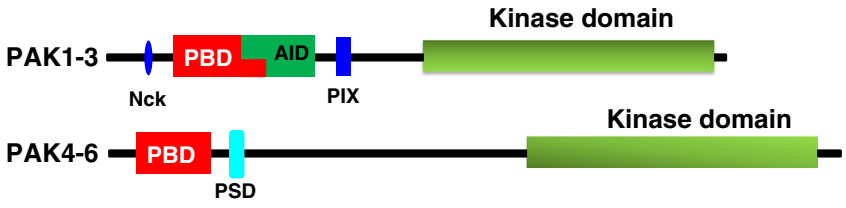

Fig. 1. Structures of PAKs. The kinase domains of group I PAKs (PAKs 1–3) and group II PAKs (PAKs 4–6) are approximately 50% identical. PAKs from both groups also contain a p21 binding domain (PBD). The group I PAKs contain an auto-inhibitory domain (AID), and binding motifs for PIX and Nck. For group I PAKs binding of an activated GTPase such as Rac or Cdc42 to the PBD disrupts PAK dimerisation leading to a series of conformational changes that unfold the AID, which then dissociates from the kinase domain of the other molecule in the dimer. Dissociation of the AID permits a conserved threonine residue in the kinase domain to be autophosphorylated, which is necessary for full kinase activity [13]. The phosphorylation sites in the activation loop differ between individual PAKs and are therefore not shown. The group II PAKs contain an autoinhibitory pseudosubstrate domain (PSD). Binding of activated Rac or Cdc42 to the PBD of group II PAKs causes conformational changes in the PSD which lead to increased kinase activity [15,16].

5.1. Liver cancer

Hepatocellular carcinoma (HCC) is the most common malignant primary cancer in the liver. The majority of cases of HCC arise in the setting of cirrhosis, which may be caused by various factors including chronic hepatitis B or C infection, or alcohol-induced liver damage. Hepatocarcinogenesis is a multistep process, associated with changes in multiple molecular signalling pathways including Wnt/β-catenin and Ras, and up to 50% of HCC carry mutations in the genes encoding components of the Wnt/β-catenin pathway [57]. Overexpression of Ras proteins has been found in cirrhotic livers and in HCC [58], although Ras mutations are not common in HCC. BRaf and phosphatidylinositol 3-kinase (PI3K) mutations have been also found in HCC patients, and BRaf mutations are significantly correlated with higher proliferation rates of HCC [59].

PAK1, activated by Ras via PI3K-dependent or independent pathways, is overexpressed in HCC patients with more advanced tumours and more metastatic phenotypes [60]. The PAK1 gene is also amplified in HCC, and PAK1 stimulates HCC cell migration by activation of c-Jun NH_2-terminal kinase (JNK) and phosphorylation of paxillin. In human HCC samples, increased levels of PAK1 correlated with poor prognosis, hepatitis B virus (HBV) infection, and portal vein tumour thrombosis [61]. The human HBV X protein (HBx) is involved in the viral life cycle and exerts a direct hepatocarcinogenic effect in the development of HCC [62]. HBx induces mitochondrial translocation of Raf1 kinase by oxidative stress. This mitochondrial translocation of Raf1 is dependent on the phosphorylation of Raf1 at Ser338/339 and Tyr340/341 by PAK1 and Src kinase, respectively [63]. In HCC cells HBx up-regulates PAK1, confers resistance to anoikis (a specialised form of apoptosis that occurs in cells because of inadequate or inappropriate interaction with the cellular matrix), and promotes growth of xenografted tumours in mice. HBx-induced activation of PAK1 contributes to progression of HCC in patients with chronic HBV infection.

Currently partial resection or complete resection with liver transplantation offers the only possibility of long-term survival for HCC patients. In the case of partial resection recurrences occur in more than two-thirds of these patients. Wang and coworkers have discovered that early recurrent HCC tumours have significantly increased expression of Rac GTPase-activating protein 1 (RACGAP1) [64]. Although RACGAP1 overexpression contributes to aggressive recurrence of HCC [64], inactivation of Rac would be expected to lead to inactivation of PAK1. Further work will therefore be required to clarify the possible involvement of PAK1 in HCC recurrence. In this context it is interesting to note that down-regulation of cyclin D1, associated with decreased levels of p38 MAP kinase, AKT and PAK1, inhibits proliferation and survival of liver cancer cells [65].

Although PAK1 is clearly involved in multiple signalling pathways that significantly contribute to the progression of liver cancer, the effects of other members of the PAK family remain to be explored. Recent observations suggest that the CDK5 kinase regulatory subunit-associated protein 3 (CDK5RAP3) is overexpressed in more than 50% of HCC specimens, that CDK5RAP3 binds to and activates PAK4, and that overexpression of CDK5RAP3 promotes HCC metastasis [66].

Table 1
Genetic alterations of PAKs in gastrointestinal cancers.

Cancer location	PAKs	Alteration		References
		Gene amplification	Protein overexpression	
Oesophagus	PAK4		✓	[56]
Liver	PAK1	✓	✓	[60]
Pancreas	PAK1		✓	[71]
	PAK4	✓	✓	[51]
Stomach	PAK1		✓	[80,81]
	PAK4		✓	[82,85]
Colon	PAK1		✓	[48]
	PAK4	✓		[52]
	PAK5		✓	[53]

5.2. Pancreatic cancer

Pancreatic cancer has the highest mortality rate amongst all carcinomas, with an overall 5-year survival rate of less than 5%. The high mortality has been attributed to the lack of reliable methods for early detection and to the molecular mechanisms underlying the aggressive pathogenesis [67]. Activating mutations of KRas are amongst the most common genetic alterations in pancreatic cancer with an incidence of approximately 100% [68,69], and oncogenic Ras activates both PAK1 and PAK4. PAK4 gene amplification is found in pancreatic cancer, and is associated with significantly higher kinase activity of the PAK4 protein [51]. PAK4 promotes pancreatic cancer cell motility and invasion [70]. MUC13, a transmembrane mucin, has recently been found to be overexpressed in pancreatic cancer, and increased expression of MUC13 in pancreatic cancer cell lines stimulated xenograft growth, and was correlated with increased expression and activation of PAK1 [71]. Conversely Smad4, which is a tumour suppressor gene frequently mutated in human pancreatic cancer, induces cell death by suppression of PAK1 [72]. Although the effect of PAK1 knockout on pancreatic tumourigenesis in mice has not been reported, the importance of PAK1 in pancreatic physiology is highlighted by the observation that islets from $PAK1^{-/-}$ mice are deficient in the second sustained phase of insulin secretion [73].

In the past two decades, pancreatic cancer therapy has focussed on several signalling molecules, with particular interest in membrane receptors such as the epithelial growth factor receptor (EGFR) [74]. The EGFR acts, at least in part, thorough Ras, and the outcome of clinical trials with EGFR inhibitors in pancreatic cancer is dependent on the mutation status of Ras. A single Kras mutation, which is the most common genetic alteration in pancreatic cancer, is able to circumvent the anti-tumour activity of anti-EGFR therapies as mutant forms of KRas are constitutively active independently of the EGFR. Since oncogenic Ras activates PAK1 and PAK4, both of which are overexpressed and hyper-activated in pancreatic cancer [51,70,71], PAK inhibitors, especially when combined with classic chemotherapeutic drugs, may provide a promising therapeutic strategy for pancreatic cancer.

5.3. Gastric cancer

Gastric cancer is one of the most common causes of cancer-related death. In Asian countries including China, Japan and Korea, the morbidity and mortality of gastric cancer are the highest amongst malignant carcinomas [75]. The majority of gastric cancers in the East Asian population are associated with infection by virulent strains of the gastric bacterium *Helicobacter pylori* [76,77]. The strains carry unique variants of the bacterial protein CagA, which activates PAK1 in host cells through PIX, a PAK1-activating SH3 adaptor protein (as shown in Fig. 1). Hence PIX-specific siRNA blocks the activation of PAK1 by CagA [77]. Activated PAK1 in turn has been shown to activate NF-κB, which triggers the release of proinflammatory cytokines [78,79].

Both PAK1 and PAK4 are overexpressed in gastric cancer and play important roles in its metastasis [80–82]. In human gastric cancer cell lines, PAK1 regulates the expression and activity of cyclins D1 and B1 via NF-κB, and inhibition of PAK1 suppresses proliferation and migration by decreasing the expression of cyclins D1 and B1 [83,84]. In gastric cancer patients, overexpression of PAK1 is associated with advanced and metastatic tumour stages, and increased PAK1 activity is related to reduced survival [81]. PAK1 affects metastasis of gastric cancer cells by activation of ERK and JNK [81], whilst PAK4 stimulates the migration of gastric cancer cells via activation of Lim kinase 1 [85]. Gastric cancer patients with PAK4 overexpression do not respond to capecitabine/cisplatin treatment and have poor survival [82]. Given their important roles in the viability and mobility of gastric cancer

cells, PAKs are becoming attractive therapeutic targets, especially since the outcome of current chemotherapy remains disappointing, with the median survival ranging between 9 and 11 months only [86].

5.4. Colorectal carcinoma

Colorectal carcinoma (CRC) arises and progresses as a result of cumulative genetic and epigenetic changes in tumour cells. Mutations in the genes encoding components of the Ras and Wnt/β-catenin signalling pathways occur in 50% and 90% of CRCs [87,88]. In animal models constitutive activation of Wnt/β-catenin signalling initiates growth of benign adenomas. Mutations in KRas, BRaf and related pathways stimulate adenoma growth and contribute to invasive and other malignant behaviours. Despite the high frequency of Wnt/β-catenin mutations in CRC, no therapy targeted to the Wnt pathway has yet been developed because of the lack of suitable enzyme targets in this pathway [89].

Activation of KRas by mutation increases cell proliferation and motility, and KRas mutations are clinically associated with a poor prognosis of CRC [90]. Ras activates multiple signalling pathways, including PAKs, the Raf/MEK/MAPK/ERK cascade and PI3K/AKT [91]. Mutations of KRas, BRaf and PI3K occur in 50%, 10% and 15% of CRC respectively [92]. Whereas KRas mutations do not usually occur in CRC cells that have BRaf mutations, they may coexist with PI3K mutations [92]. Because of the inhibitory effects between the PI3K and Raf/MEK/MARK/ERK pathways [93], blocking one signal alone may increase the signal from the other pathway. Therefore combined inhibition of both PI3K and Raf/MEK/MARP/ERK is necessary when considering targeted therapy.

KRas mutations are often associated with mutations that activate the Wnt/β-catenin pathway [94]. Synchronous detection of activated KRas and of nuclear β-catenin, the hallmark of active Wnt signalling, identifies a group of patients with poor prognosis and resistance to chemotherapy [95]. Most CRC contain collections of mutations, most frequently in Ras, Raf, PI3K and components of the Wnt/β-catenin signalling pathway. Although advanced knowledge of the signalling network involved in these pathways has provided useful information for the development of targeted therapy, the clinical benefits so far have been limited, indicating a need for improvement in the rational design of new therapies.

In CRC, expression of PAK1 increases with progression through the adenoma to carcinoma sequence, with the most dramatic increases in invasive and metastatic CRC [48]. PAK1 phosphorylates Raf and facilitates the Ras/Raf/MAPK signalling pathway which, as mentioned above, plays a crucial role in CRC development [88,96]. Conversely PAK1 knockdown downregulates JNK and cyclin D1 [97]. A kinase-inactive PAK4 blocks oncogenic Ras-induced transformation and inhibits the anchorage-independent growth of HCT116 colon cancer cells [40]. The PAK4 gene is also amplified in CRC patient samples [52]. PAK5 is overexpressed during CRC progression and regulates CRC cell adhesion and migration [53], and apoptosis [31]. The expression of PAK2 in hepatic stellate cells has been suggested to contribute to liver metastasis of CRC cells [98].

Recently we have reported that knockdown of PAK1 abrogates growth and metastasis of CRC cell lines in xenograft and liver metastasis models in mice [99]. PAK1 is also required for the proliferation, survival, migration, and VEGF secretion of CRC cells harbouring mutations in Ras, PI3K and Apc [100]. Furthermore PAK1 knockdown inhibited growth, survival and migration of CRC cell lines by inactivation of ERK and AKT, the downstream targets of Ras [100]. This observation is consistent with the report by Li and coworkers that PAK1 regulates CRC metastasis through ERK-dependent phosphorylation of FAK [101]. Interestingly, a recent report has shown that PAK1 is also required for KRas-driven skin cancer, and that inhibition of PAK1 causes tumour regression and loss of activity of ERK and AKT [102]. The conclusions of this study are definitive because the cells used were derived from $PAK^{-/-}$ mice and hence, although being more expensive to generate, will not have the off-target effects associated with siRNA-treated cells. Taken together, these results indicate that instead of combination therapies targeting both ERK with a MEK inhibitor and AKT with a PI3K inhibitor, targeting PAK1 alone could be an alternative approach in CRC treatment. We have further reported that PAK1 associates with β-catenin in CRC cells, and that PAK1 knockdown inhibited β-catenin activation by reducing the expression of β-catenin and c-myc (one of the downstream targets of β-catenin signalling) and by suppressing β-catenin/TCF4 transcriptional activity [99]. Consistent with our findings, Zhu and coworkers have shown that PAK1 phosphorylated β-catenin at Ser675 leading to a more stable and transcriptionally active β-catenin in CRC cells [103]. Together these results indicate that PAK1 is required for β-catenin activation in CRC and plays a key role in mediating the cross-talk between Ras and Wnt/β-catenin signalling. However a recent report that PAK1 negatively regulates Wnt signalling in the nematode *Caenorhabditis elegans* [104] indicates that PAK1 may act differently in different species.

To summarise, oncogenic Ras activates PAK1, and enhances Wnt/β-catenin signalling in CRC initiation and progression via activation of ERK- and AKT-pathways. PAK1 is required for the activation of ERK, AKT and β-catenin signalling in CRC cells, and is critical for CRC growth and metastasis *in vivo* (Fig. 2). These findings indicate that PAK1 acts as a convergence point in multiple signalling pathways important for CRC development.

6. Perspectives in PAK-targeted therapy

The conclusion that PAKs in general, and PAK1 in particular, act as central nodes in multiple signalling pathways that control cell proliferation, mobility, survival and transformation, makes them attractive targets in the treatment of diseases including cancers. Functional inhibition of PAK1 has been achieved experimentally using several forms of dominant-negative mutants, RNA interference and a number of chemical inhibitors with various degrees of specificity (Table 2). Several small molecule inhibitors such as CEP-1347, and the SRC and

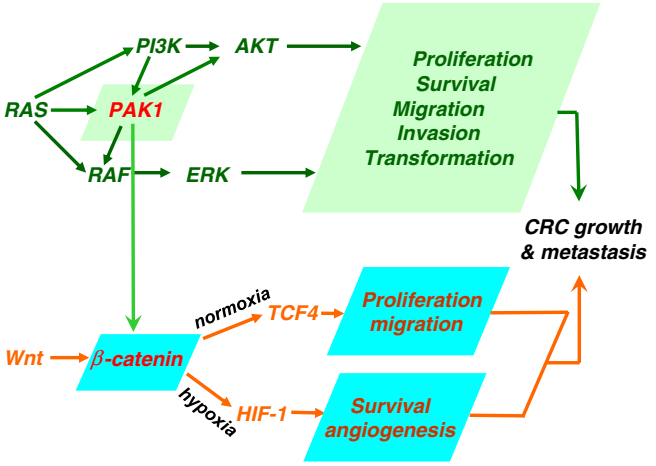

Fig. 2. PAK1 mediates cross-talk between Ras and Wnt/β-catenin signalling in CRC initiation and progression. Oncogenic Ras activates PAK1, which in turn stimulates the proliferation, survival, and migration/invasion of CRC cells via activation of ERK- and AKT-dependent pathways, which are critical for CRC progression. The Wnt-β-catenin pathway also stimulates CRC cell proliferation and migration under normoxia, via formation of a complex between β-catenin and the transcription factor TCF4 in the nucleus. Under hypoxia, β-catenin interacts with hypoxia-inducible factor 1 (HIF-1) instead, to stimulate survival and angiogenesis. PAK1 provides a critical connection between Ras and Wnt-β-catenin pathways by associating with β-catenin and promoting β-catenin/TCF4 activity.

Table 2
PAK inhibitors.

Inhibitor	Kd (nM)		Reference
	PAK1	PAK4	
Competitive with ATP			
CEP-1347	>1000	NA	[105]
OSU-03012	1000	NA	[115]
FL172	110	NA	[116]
PF-3758309	14	2.7	[114]
LCH-7749944	NA	15,000	[109]
Not competitive with ATP			
WR-PA18	NA	NA	[110]
IPA3	2500	>10,000	[112]
Mechanism undefined			
FRAX597	7.7	>1000	[102]

NA, not available. The recently developed group I-selective inhibitor FRAX597 not only inhibits PAK1 activity, but also reduces the amount of PAK1 and PAK2 protein in treated cells and animals [102].

ETK tyrosine kinase inhibitors AG 879 and FK228, respectively, have been shown to inhibit PAK activity [105–107]. The organometallic compound FL172 inhibits PAK1 kinase activity with an IC_{50} of 110 nM by blocking the ATP-binding site [108], and LCH-7749944 suppresses PAK4 kinase activity with an IC_{50} of 15 μM [109]. The recently developed group I-selective inhibitor FRAX597 not only inhibits PAK1 activity, but also reduces the amount of PAK1 and PAK2 protein in treated cells and animals [102].

Some functions of PAK1 are mediated via kinase-independent, protein–protein interactions. Targeting short fragments of PAK1 other than the kinase domain inhibits PAK1 function by blocking its interaction with other proteins. For example, a small cell-permeable peptide called WR-PAK18, which includes the PIX-binding site of PAK1, selectively blocks the PAK–PIX interaction and suppresses Ras transformation in vitro [110,111]. PAK1 activation has also been suppressed by the allosteric inhibitor IPA-3 [112], which interacts with a domain distinct from the ATP-binding site. Although IPA-3 showed high potency and selectivity for group I PAKs, it was not effective against pre-activated group I kinases.

Although suppression of PAK1 expression by RNAi provides better discrimination between the individual PAK isoforms, this approach cannot be used effectively for clinical application because of the instability of RNAi in vivo. Recently potent pyrroloaminopyrazole-based PAK inhibitors have been developed by Pfizer [113]. The affinity of PF-3758309 is greatest for PAK4 (Kd = 2.7 nM), intermediate for PAKs 1, 5 and 6 (Kd = 14–18 nM), and lowest for PAKs 2 and 3 (Kd = 100–190 nM). The observation that PF-3758309 at a daily oral dose of 20 mg/kg suppressed the growth of several human cancers including CRC xenografts in mice [114] suggested that PAK4 might be an attractive therapeutic target in cancer patients. However, a clinical trial in patients with advanced solid tumours was prematurely terminated in 2011 because of "the undesirable PK characteristics of PF-03758309 and the lack of an observed dose–response relationship" (http://clinicaltrials.gov/show/NCT00932126).

In future efforts to develop PAK inhibitors for cancer therapy, several potential problems will need to be taken into consideration. Firstly the inhibitor must overcome the problem of specificity and redundancy caused by the diverse and overlapping nature of the PAKs themselves, and of their regulators and effectors. Secondly inhibitor toxicity caused by off-target effects must be low. Thirdly the development of acquired resistance to PAK inhibitor therapy must be limited. With increasing knowledge of PAK signalling in normal and cancer cells, the development of PAK inhibitors should further elucidate the role of PAKs in tumourigenesis and help to establish the PAK family as targets for clinical therapy.

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