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Review

p21-activated kinases and gastrointestinal cancer

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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

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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.

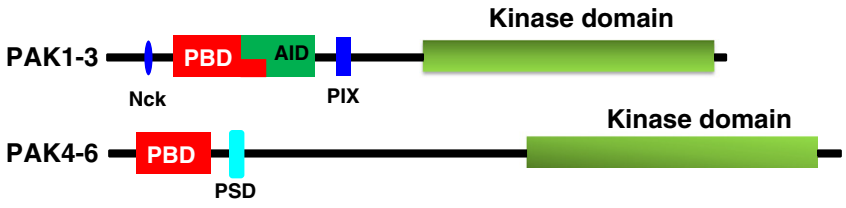

Fig. 1. Structures of PAKs. The kinase domains of group I PAKs (PAKs 1–3) and group II PAKs (PAKs 4–6) are approximately 50% identical. PAKs from both groups also contain a p21 binding domain (PBD). The group I PAKs contain an auto-inhibitory domain (AID), and binding motifs for PIX and Nck. For group 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].