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TOPIC HIGHLIGHT

2016 Gastric Cancer: Global view

Group Ⅱ p21-activated kinases as therapeutic targets in gastrointestinal cancer

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Abstract

P21-activated kinases (PAKs) are central players in various oncogenic signaling pathways. The six PAK family members are classified into group Ⅰ (PAK1-3) and group Ⅱ (PAK4-6). Focus is currently shifting from group Ⅰ PAKs to group Ⅱ PAKs. Group Ⅱ PAKs play important roles in many fundamental cellular processes, some of which have particular significance in the development and progression of cancer. Because of their important functions, group Ⅱ PAKs have become popular potential drug target candidates. However, few group Ⅱ PAKs inhibitors have been reported, and most do not exhibit satisfactory kinase selectivity and "drug-like" properties. Isoform- and kinase-selective PAK inhibitors remain to be developed. This review describes the biological activities of group Ⅱ PAKs, the importance of group Ⅱ PAKs in the development and progression of gastrointestinal cancer, and small-molecule inhibitors of group Ⅱ PAKs for the treatment of cancer.

Key words: Group Ⅱ p21-activated kinases; Signaling pathway; Gastrointestinal cancer; PAK4 inhibitor; Drug target

Core tip: Group Ⅱ p21-activated kinases (PAKs) (PAK4, 5 and 6) are pluripotent kinases that regulate many fundamental cellular processes, including cytoskeletal organization, cell cycle regulation, cell survival and cell adhesion, and have therefore been implicated in carcinogenesis and cancer progression. Members of the group Ⅱ PAKs, particularly PAK4, have emerged as attractive targets for the development of anticancer drugs. In this review, we summarize the latest findings on the biological activities of group Ⅱ PAKs, the important roles of group Ⅱ PAKs in gastrointestinal cancer and the current status of the development of

small-molecule inhibitors of PAK4 for the treatment of cancer.

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INTRODUCTION

The characteristics of a cancer cell include loss of homeostasis, altered cytoskeletal dynamics, sustained cell proliferation, resistance to cell death, and stimulation of metastasis and invasion. Substantial evidence indicates roles for the p21-activated kinase (PAK) family of serine/threonine kinases in each of these processes. PAK1, the most well-characterized member of this family, was initially identified as a binding partner of the Rho family small GTPases Cdc42 and Rac1[1]. PAKs are widely conserved and are found in yeast as well as Drosophila[2]. In mammals, six PAK family members have been identified[3]. These proteins are categorized into two subgroups on the basis of their sequence, structural homology and response to activated GTPases. Group Ⅰ includes PAK1, PAK2, and PAK3, and group Ⅱ comprises PAK4, PAK5, and PAK6 (Figure 1). Group Ⅱ PAK members have emerged as central nodes in oncogenic signaling pathways. Here, we summarize recent findings regarding the biological functions of group Ⅱ PAK signaling in gastrointestinal cancers, including hepatocellular carcinoma (HCC), pancreatic cancer, gastric cancer and colorectal carcinoma (CRC).

STRUCTURE OF GROUP Ⅱ PAKS

All PAKs are characterized by an N-terminal p21-GTPase-binding domain (PBD) and a highly conserved C-terminal kinase domain. Compared with group Ⅰ PAKs, group Ⅱ PAKs have very little sequence N-terminal to the PBD. In group Ⅰ PAKs, this proline-rich region contains binding motifs for Nck, an adapter protein that is associated with the regulation of actin dynamics[4]. Group Ⅰ PAKs also contain an autoinhibitory domain (AID) that overlaps with the PBD. Group Ⅰ PAKs can form homodimers, adopting a trans-inhibited conformation in which the AID of one molecule binds and inhibits the catalytic domain of the other[5]. Binding of an activated GTPase (Cdc42 or Rac) to the PBD disrupts dimerization and activates group Ⅰ PAKs. Group Ⅱ PAKs (except PAK5) do not possess an identifiable AID and have higher basal kinase activities than PAK1-3. Binding of GTP-Rac or GTP-Cdc42 to group Ⅱ PAKs does not promote their kinase activities[6,7], but may regulate their localization and/or their interaction with other proteins. Similar to the AID in group Ⅰ PAKs, group Ⅱ PAKs have an auto-inhibitory pseudosubstrate domain within their N-terminal regions, that inhibits their kinase activity in the absence of any GTPase (Figure 1)[8,9]. Moreover, an N-terminal domain that binds to PIX, an important downstream effector, is found in group Ⅰ PAKs but not group Ⅱ PAKs[10]. Group Ⅰ and group Ⅱ PAKs have distinct substrate specificities[11] and each group is regulated in a strikingly different manner[12].

CELLULAR ACTIVITIES OF GROUP Ⅱ PAKS

A number of studies have implicated group Ⅱ PAKs in cellular processes associated with tumorigenesis and progression.

PAK4 and anchorage-independent growth

Anchorage-independent growth is an *in vitro* hallmark of oncogenic transformation. The activated PAK4 mutants PAK4 (S445N) and PAK4 (S474E) promote anchorage-independent growth in NIH3T3 cells[13,14]. By contrast, the kinase-dead PAK4 mutant blocks Ras-mediated transformation of NIH3T3 cells and anchorage-independent growth of a human colon cancer cell line, HCT116, driven by a K-Ras activating mutation[14]. Moreover, dominant-negative PAK4 mutants partially inhibit fibroblast focus formation induced by oncogenic Dbl, a RhoGEF that mediates cell transformation[13].

Group Ⅱ PAKs and cell cycle control

PAK4 also plays an important role in cell cycle control. PAK4 is involved in the regulation of G_1 phase and G_2/M transition during the cell cycle. In immortalized fibroblasts, deletion of PAK4 markedly extends the life time of p21, a CDK (cyclin-dependent kinase) inhibitor[15], suggesting that PAK4 is important for p21 degradation. Moreover, PAK4 silencing causes G_1 phase arrest in pancreatic cancer cells by reducing the expression of cyclins A1, D1 and E1 and enhancing the expression of p27 and p21[16]. We recently demonstrated that PAK4 attenuates $p57^{Kip2}$ protein stability through the ubiquitin-proteasome pathway, leading to increased proliferation of breast cancer cells[17]. PAK4 is also required for metaphase spindle positioning and anchoring[18]. By contrast, in primary fibroblasts, PAK4 promotes cell cycle arrest and enhance the levels of the cell cycle inhibitors $p16^{INK4}$ and $p19^{ARF}$[19]. Thus, the roles of PAK4 in cell cycle control may differ between primary cells and established cell lines. PAK5 and PAK6 also function in cell cycle regulation. PAK5 knockdown inhibits cell proliferation by delaying the cell cycle at G_0/G_1 phase in human gastric cancer, hepatocellular carcinoma and glioma cells[20-22]. PAK6 silencing inhibits the cell growth of prostate cancer and causes cell cycle arrest at G_2/M phase[23].

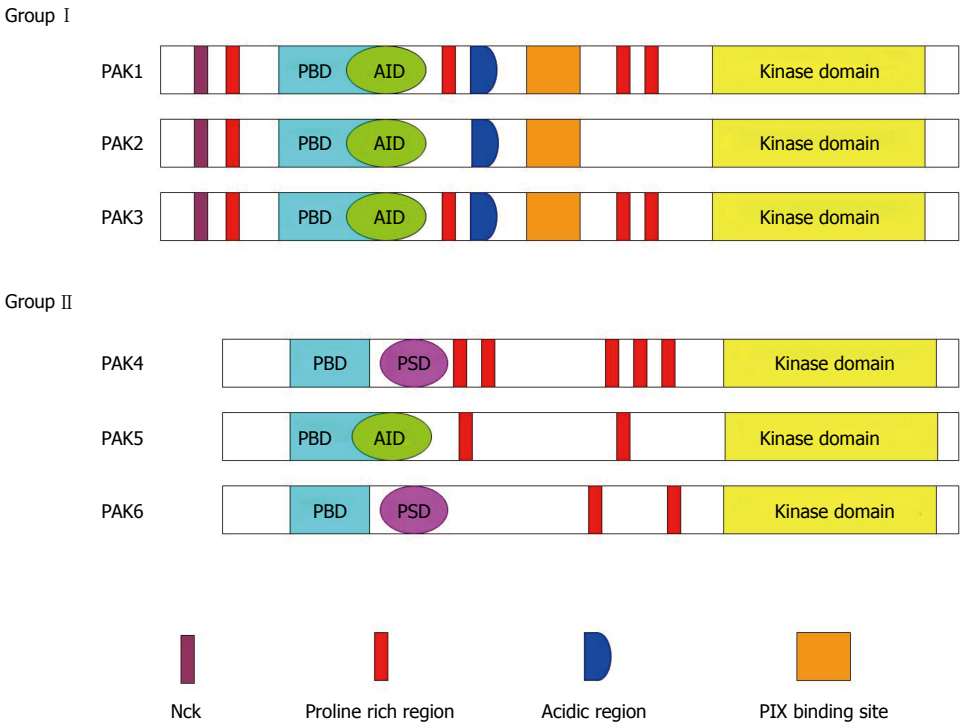

Figure 1 Structural features of the group Ⅰ and group Ⅱ p21-activated kinases family members. PAK: p21-activated kinase; PBD: p21-binding domain; AID: Autoinhibitory domain.

Group Ⅱ PAKs and cell survival

Increased levels of cell survival under different apoptotic stimuli are often associated with oncogenesis. PAK4 plays a key role in cell survival and protection from apoptosis. PAK4 promotes cell survival and prevents apoptosis *via* both kinase-dependent and -independent mechanisms. In response to serum starvation, PAK4 phosphorylates the pro-apoptotic protein BAD at Ser112 and promotes cell survival[24]. Furthermore, in response to cytokines that activate death domain-containing receptors, such as tumor necrosis factor and Fas receptors, PAK4 abrogates the activation of initiator caspase 8 by inhibiting caspase 8 recruitment to the death domain receptors, thereby preventing apoptosis[25]. In addition, knockdown of PAK4 leads to a reduction of the activation of several pro-survival pathways, including the NFκB, ERK and JNK pathways[26]. Like PAK4, PAK5 and PAK6 are also associated with the protection of cells from apoptosis. PAK5 induces resistance to apoptosis induced by camptothecin and C2-ceramide by phosphorylating BAD at Ser112[27]. PAK5 is constitutively localized to the mitochondria, *via* its phosphorylation activity, PAK5 can prevent BAD translocation to the mitochondria, thereby inhibiting the apoptotic cascade[27]. Overexpression of PAK5 also inhibits camptothecin-induced apoptosis by inhibiting the activity of caspase-8 in colorectal cancer cells[28]. PAK5 overexpression markedly inhibits cisplatin-induced apoptosis by increasing the expression of pre-caspase 3 in hepatocellular carcinoma cells[29]. Moreover, inhibition of PAK6 results in a decrease in Ser112 phosphorylation of BAD, leading to enhanced binding of BAD to Bcl-2 and Bcl-X(L) and the release of cytochrome c, which culminates in caspase activation and apoptosis[30].

Group Ⅱ PAKs and cell migration and invasion

Migration and invasion are essential aspects of the oncogenic process, and they are required for metastasis. Based on its well characterized functions in actin cytoskeletal organization, cell adhesion, and integrin phosphorylation[31], PAK4 plays a central role in cancer cell migration and invasion. Overexpression of a constitutively active PAK4 mutant promotes pancreatic ductal cell migration and invasion. By contrast, PAK4 silencing reduces cell invasion in a pancreatic tumor cell line[32]. PAK4 overexpression also promotes the migration, invasion and proliferation of choriocarcinoma cells[33]. PAK4 knockdown inhibites invasion and migration by downregulating MMP-2, αvβ3-integrin and phospho-epidermal growth factor receptor (phospho-EGFR) in glioma xenograft cells[34]. PAK4 enhances endometrial cancer cell migration and invasion in an ERK1/2-MMP-2-dependent manner[35]. Moreover, PAK4 can promote cell migration and invasion through the HGF/LIMK1/cofilin pathways in prostate cancer cells[36] and through the MEK-1/ERK1/2/MMP2 pathways in ovarian cancer cells[37]. We recently demonstrated that PAK4-mediated superior cervical ganglia 10 (SCG10) phosphorylation regulates microtubule dynamics to promote gastric cancer cell migration and invasion *in vitro* and metastasis in a xenograft mouse models[38] (Figure 2). These results indicates that PAK4 is closely associated with migration

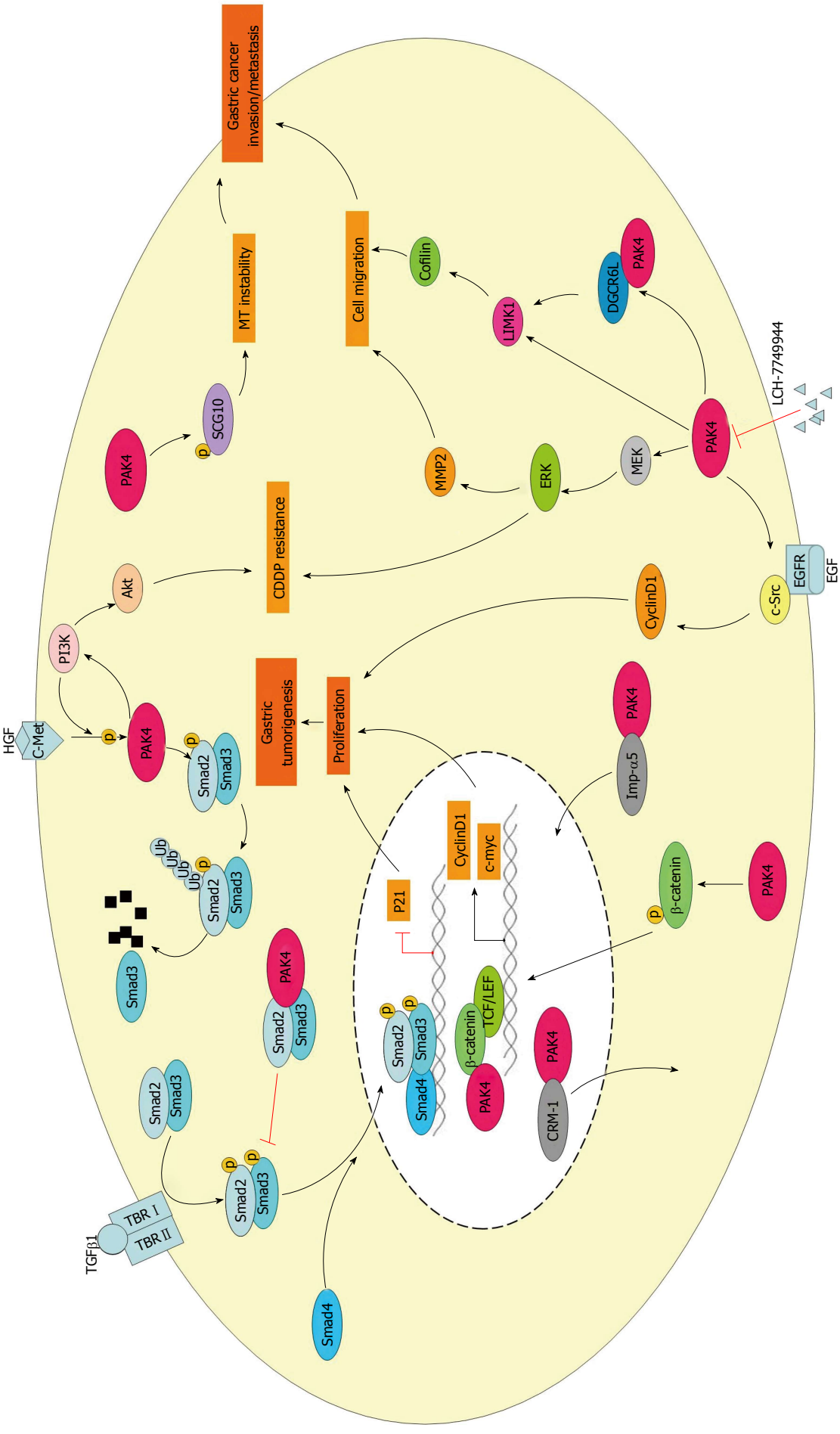

Figure 2 p21-activated kinase 4 signaling pathways in gastric cancer. Schematic depicting some of the signaling cascades mediated by PAK4. PAK4 is a nucleo-cytoplasmic shuttling protein that is exported by the CRM-1-dependent pathway and imported into the nucleus in an importin α5-dependent manner. We have demonstrated that PAK4 contributes to gastric tumorigenesis and gastric cancer invasion and metastasis by phosphorylating Smad2, SCG10 or β-catenin or by interacting with DGCR6L. PAK4-mediated substrate phosphorylation is indicated by the letter P. PAK4 activation and inhibition of proteins are depicted using arrows and blocked lines, respectively. Specific details of the pathways are described in the text. CRM-1: Chromosome region maintenance-1; Imp-α5: Importin α5; SCG10: Superior cervical ganglia 10; DGCR6L: DiGeorge critical region 6 L; PI3K: Phosphoinositide 3-kinase; Akt: Protein kinase B or PKB; MEK: Mitogen-activated protein kinase kinase; MAPKK; ERK: Extracellular-signal regulated protein kinase; MT: Microtubule; LIMK1: LIM domain kinase 1; MMP2: Matrix metallopeptidase 2; CDDP: Cisplatin or cis-diamminedichloroplatinum(Ⅱ).

and invasion in several different types of cancer cells. PAK5 and PAK6 are also involved in cancer cell migration and invasion. PAK5 promotes glioma and breast cancer cell migration and invasion through the PAK5-Egr1-MMP2 signaling pathway[39,40]. Androgen-stimulated PAK6 activation promotes prostate cancer cells motility and invasion[41]. miR-23a overexpression has been proposed to suppress the invasion and migration of prostate cancer cells by targeting the PAK6-LIMK1 signaling pathway[42].

Group Ⅱ PAKs in cancer

Accumulating evidence suggests that PAKs regulate a variety of biological processes including cytoskeletal remodeling, anchorage-independent growth, apoptosis and transcriptional/translational regulation, thereby contributing to tumorigenesis and progression. Group Ⅱ PAKs also influence the behaviour of cancer cells. Among the three group Ⅱ PAKs, the role of PAK4 in human cancer has been best investigated. PAK4 is overexpressed, amplified, and/or point mutated in a number of cancer cell lines[14] and tumors including breast[14,43], gallbladder[44], pancreas[16,32,45,46], colon[47], lung[14], ovarian[37], prostate, melanoma, CNS, renal, leukaemia[14], gastric[48] and oral squamous-cell carcinoma[49]. PAK4 overexpression in iMMECs (immortalized mouse mammary epithelial cells) leads to changes in 3D acinar architecture that are typically associated with tumorigenesis, including reduced central acinar cell death, abrogation of lumen formation, loss of cell polarity, and increased acinar size and cell number[43]. Importantly, PAK4-overexpressing cells form tumors when implanted into the mammary fat pads of mice[43], indicating that PAK4 is sufficient to trigger oncogenic transformation in these cells. Other oncogenes, such as Her2Neu (ErbB2) and Ras, also cause iMMECs to generate tumors in mice[50]. PAK4 is also strongly upregulated in response to Her2Neu and Ras in iMMECs[43]. PAK4 plays a critical role in Her2Neu-driven breast cancer cell invasion[51]. These results suggest a role for PAK4 as a key downstream target of these oncogenes in breast cancer. In the MCF10A progression series (a unique cell culture model that recapitulates the different stages of breast cancer progression using MCF10A, AT1, DCIS.com and CA1a cells), PAK4 levels are higher in more tumorigenic cell lines[52]. The *PAK4* gene is frequently amplified in high-grade serous and endometrioid ovarian cancers, whereas PAK4 silencing causes a statistically significant decrease in cell viability[53,34]. PAK4 overexpression and activation have been associated with ovarian cancer progression and prognosis in patients[37]. PAK4 is also upregulated in gliomas, and increasing PAK4 expression is associated with glioma pathological grades[54]. Taken together, these observations suggest that PAK4 functions as an oncogenic gene in the tumorigenesis and progression of some cancer types.

PAK5 and PAK6 may also be associated with cancer. PAK5-Egr1-MMP2 signaling promotes the migration and invasion of breast cancer[40] and glioma cells[39]. Importantly, our recent evidence suggests that PAK5 phosphorylates GATA1 at Ser161 and Ser187. Phosphorylated GATA1 recruits more HDAC3/4 to the promoter of *E-cadherin*, consequently suppressing *E-cadherin* transcription and promoting the EMT of breast cancer cells[55]. PAK5 overexpression has been detected in colorectal cancers[56], gliomas[22] and during human epithelial ovarian cancer (EOC) progression and metastasis[57]. Moreover, PAK5 promotes paclitaxel-chemoresistance in EOC[57] and PAK5 silencing efficiently inhibits the development of human glioma[22].

High *PAK6* mRNA level has been detected in certain cancer cells, including prostate, colon, ovary and lung cancer cells[14]. PAK6 protein expression is elevated in some prostate cancers and is further increased in prostate tumors that have relapsed after androgen deprivation therapy; thus, PAK6 may play a role in tumor cell motility and in stress responses[58]. PAK6 may also influence the radiosensitivity and chemosensitivity of prostate cancer cells because PAK6 inhibition in combination with irradiation or docetaxel significantly decreases the survival or growth of prostate cancer cells, respectively[23]. Hypermethylation is commonly associated with gene silencing of genes that suppress tumorigenesis. Because the *PAK6* gene is sometimes hypermethylated in prostate cancer[59], the exact role of PAK6 in prostate cancer is not entirely clear. Moreover, our experimental data have indicated that PAK6 inhibits prostate cancer growth by phosphorylating AR and Mdm2 and promoting the ubiquitin-mediated degradation of AR[60]. In summary, there is no direct or solid evidence suggesting that either PAK5 or PAK6 is essential for the malignant growth of human cells.

Group Ⅱ PAKs in gastrointestinal cancers

Gastrointestinal cancers include cancers that affect the digestive system, such as oesophagus, gallbladder, liver, pancreatic, gastric and colorectal cancers. Amplification of the genes encoding PAKs and over-expression of PAK genes or proteins have been found in gastrointestinal cancers (Table 1)[61]. Here we will review the importance of group Ⅱ PAKs in cancers of the liver, pancreas, stomach, colon and rectum.

LIVER CANCER

Hepatocellular carcinoma (HCC) is a leading cause of cancer-related death in many countries, particularly in Asia, and its burden is expected to increase[62]. HCC may be induced by many factors, including hepatitis B virus (HBV) or hepatitis C virus (HCV) infection or liver damage induced by alcohol. The high invasiveness and metastasis of HCC cells are a major challenge for HCC treatment. The carcinogenesis and progression of HCC is complex and is associated with multiple deregulated signaling pathways, including MAPK, PI3K-mTOR and

Table 1 Genetic alterations of group Ⅱ p21-activated kinases in gastrointestinal cancers

Cancer location	PAKs	Alteration		Ref.
		Gene amplification	Gene or protein overexpression	
Oesophagus	PAK4		√	[86]
Gallbladder	PAK4		√	[44]
Liver	PAK4		√	[64]
	PAK5		√	[21]
	PAK6		√	[67]
Pancreas	PAK4	√	√	[16,32,45,46]
Stomach	PAK4		√	[48,79]
	PAK5		√	[20]
Colon	PAK4	√		[47]
	PAK5		√	[56]
	PAK6		√	[95]

PAKs: p21-activated kinases.

Wnt/β-Catenin[63]. PAK1 is involved in several signaling pathways that markedly contribute to the progression of HCC, including the PAK1/JNK/paxillin, HBV X protein/PAK1, PAK1/Raf-1/BAD and PAK1(p38 MAP kinase, AKT)/cyclinD1 pathways[61].

Members of group Ⅱ PAKs have also been determined to play pivotal roles in HCC invasion and metastasis. CDK5 kinase-associated protein 3 (CDK5RAP3) and PAK4 overexpression are correlated in human HCCs, and CDK5RAP3 binds to and activates PAK4 to promote HCC metastasis[64]. Essential roles of microRNAs (miRNAs) in HCC cell invasion and metastasis have been reported. Several miRNAs function through PAK4 inhibition[65]. miRNome analyses have revealed that the third most-highly expressed miRNA in the human liver is miR-199a/b-3p, which is consistently decreased in HCC. miR-199a/b-3p targets PAK4 to inhibit HCC growth by suppressing the PAK4/Raf/MEK/ERK pathway both *in vitro* and *in vivo*[65]. Recent observations suggest that miR-224 is overexpressed in HCC cells and that miR-224 promotes HCC cell migration and invasion through the miR-224/HOXD10/p-PAK4/MMP-9 signaling pathway[66]. Moreover, PAK5 is strongly upregulated in more than 83% of HCC specimens and promotes cell proliferation and tumorigenicity in human HCCs[21]. PAK5 significantly suppresses cisplatin-induced apoptosis and promotes cell proliferation by downregulating p-chk2 in HCC cells[29]. These results indicate that PAK5 may play an essential role in the initiation of human HCC. PAK6 is upregulated in HCC samples and is positively correlated with HCC cell proliferation. High PAK6 expression is associated with Edmondsone-Steiner grade and the number of tumor nodules and poor prognosis[67]. These findings suggest that PAK6 overexpression is involved in HCC pathogenesis. However, the complex mechanisms by which PAK6 contributes to HCC remain to be explored.

PANCREATIC CANCER

Pancreatic cancer is one of the most aggressive and the least curable malignancies. The overall 5-year survival rate is less than 5%, largely due to its rapid progression, early metastasis and resistance to chemotherapy and/or radiotherapy[68]. Clearly, there is an urgent need to develop reliable methods for its early detection and to improve treatment strategies based on a better understanding of the mechanisms underlying the aggressive nature of this cancer. *KRas* oncogene activating mutations are the most frequent genetic alterations in pancreatic cancer, occurring in approximately 100% of primary tumors[69]. The *KRas* oncogene product binds to and activates PAK4. The *PAK4* gene is located at chromosome 19q13, a region amplified in some pancreatic cancers[46]. The amplification of the *PAK4* gene is related to markedly higher PAK4 kinase activity[46]. PAK4 protein overexpression has also been observed in pancreatic cancer tissue specimens and cell lines[32]. PAK4 promotes pancreatic cancer cell proliferation and survival *via* AKT- and ERK-dependent activation of the NF-κB pathway[16]. PAK4 also stimulates pancreas ductal cell motility and invasion[32].

Tyrosine kinase receptors, such as epidermal growth factor receptor (EGFR) and the *KRas* oncogene are of interest in targeted therapies against pancreatic cancer[70]. Ras acts downstream of EGFR, and the mutation status of *KRas* is a potential predictor of response to EGFR inhibitors in pancreatic cancer. *KRas* mutations are exceptionally common in patients with pancreatic cancer[71]. Because *KRas* gene mutants are constitutively active independently of EGFR, a single *KRas* mutation can circumvent the anti-tumor activity of EGFR inhibitors. *KRas* mutations are involved in the initiation or early phase of pancreatic tumorigenesis[72]. Importantly, Collins *et al*[73] have recently demonstrated that oncogenic KRas is required not only for the initiation, but also for the maintenance of pancreatic cancer in mice. Therefore, KRas oncoprotein and/or its downstream effectors are potential important therapeutic targets in pancreatic cancer. As PAK4 is activated by and acts downstream of KRas[45], PAK inhibitors, particularly in combination with classic chemotherapeutic drugs, may hold promise for the future management of pancreatic cancer. Glaucarubinone and gemcitabine, two anticancer agent, synergistically inhibit pancreatic cancer cell growth both *in vitro* and *in vivo*, at least in part by inhibiting pathways involving PAK1 and PAK4[74]. The effects of other group Ⅱ PAKs on the development and progression of pancreatic cancer remain to be explored.

GASTRIC CANCER

Gastric cancer is the most common malignant gastrointestinal tumor and the leading cause of cancer-related mortality in Asian countries, including China, Japan and South Korea[75]. As the majority of patients suffering from gastric cancer present with advanced disease, the outcomes of conventional therapies

such as surgery, chemotherapy and radiotherapy are usually poor. Numerous molecules targeting specific pathways are involved in the tumorigenesis and progression of gastric cancer[76]. Thus, it is essential to investigate proteins controlling invasion and metastasis and to identify valuable diagnostic markers and novel therapeutic targets for gastric cancer treatment.

PAK1 downregulation inhibits the proliferation of gastric cancer cells by decreasing the expression of cyclins D1 and B1[77,78]. PAK4 also plays essential roles in gastric cancer tumorigenesis and progression. We recently documented an oncogenic role of PAK4 in the repression of TGF-β-mediated growth inhibition in gastric cancer cells[79]. PAK4 interacts with Smad2/3 *via* a kinase-independent mechanism, blocking TGF-β-induced phosphorylation of Smad2 Ser465/467 or Smad3 Ser423/425 and its consequent activation. PAK4 also phosphorylates Smad2 at Ser465 *via* a kinase-dependent mechanism, leading to the degradation of Smad2 through the ubiquitin-proteasome pathway under hepatocyte growth factor (HGF) stimulation[79]. These results indicate that PAK4 may contribute to the development of gastric cancer (Figure 2).

PAK4 is overexpressed in metastatic gastric cancer patients[48], implicating a role for PAK4 in gastric cancer metastasis. Using a yeast two-hybrid screen, we identified novel PAK4 binding proteins, including DGCR6L and SCG10. We determined that the interaction of PAK4 with DGCR6L promotes the migration of gastric cancer cells by enhancing the phosphorylation of LIMK1[80] (Figure 2). We recently reported that phosphorylation of SCG10 at Ser50 by PAK4 promotes gastric cancer cell invasion *in vitro* and metastasis *in vivo*[38] (Figure 2). Moreover, PAK4 and phospho-Ser50 SCG10 expression are positively correlated in gastric cancer samples, and high expression of SCG10 phospho-Ser50 is positively correlated with an aggressive clinical gastric cancer phenotype[38]. In addition, high level of activated PAK4 correlates with poor prognosis in gastric cancer patients[81]. These findings suggest that PAK4 may be an attractive therapeutic target and a potential prognostic marker for gastric cancer. Consistent with this suggestion, we observed that LCH-7749944, a novel PAK4 inhibitor, effectively suppresses the proliferation, migration and invasion of human gastric cancer cells[82] (Figure 2). Importantly, LCH-7749944 successfully inhibits EGFR activity *via* inhibition of PAK4[82]. Although cisplatin and cisplatin-based combination chemotherapy are effective against gastric cancer, gastric cancer patients with PAK4 overexpression do not respond to capecitabine/cisplatin chemotherapy and have poor survival rates[48]. PAK4 confers cisplatin resistance in gastric cancer cells *via* the activation of the MEK/ERK and PI3K/Akt pathways[83] (Figure 2). Therefore, PAK4 may be a potential target for overcoming cisplatin resistance in gastric cancer.

PAK5, another group Ⅱ PAK, is upregulated in different gastric cancer cell lines and gastric cancer tissues[20]. *PAK5* knockdown inhibits human gastric cancer cell proliferation by downregulating CDK2, CDC25A, and cyclin D1[20]. These data indicate that PAK5 may be a novel therapeutic target in gastric cancer. Further studies are needed to demonstrate the importance of PAK5 in the carcinogenesis and progression of gastric cancer.

COLORECTAL CARCINOMA

Colorectal carcinoma (CRC) development results from interactions between genetic and epigenetic alterations at many levels. Mutations in *APC*, a negative regulator of the Wnt/β-catenin signaling pathway, and *KRas* play a central role in CRC. *APC* gene mutation induces the activation of Wnt/β-catenin signaling and initiates the growth of benign adenomas. Mutations in *KRas* and related pathways promote adenoma growth and contribute to invasive and other malignant behaviours[84]. KRas activates multiple signaling pathways, including PAKs, the Raf/MEK/ERK cascade and PI3K/AKT[85].

As a downstream effector of KRas, PAKs are involved in CRC cell proliferation, apoptosis, adhesion and migration. PAK4 overexpression has been identified in primary mouse colon tumors[86], the *PAK4* gene is also amplified in CRC patient samples[47]. PAK4 expression is associated with colorectal cancer metastasis and infiltration[87]. In mutant *KRAS*-driven HCT116 colon cancer cells, overexpression of a kinase-inactive mutant form of PAK4 severely abrogates anchorage-independent growth[14], indicating that PAK4 is essential for HCT116 colon cancer cell proliferation in anchorage-independent culture. PAK4 RNA inhibits the proliferation of HCT116 cells independently of RAF/MEK/ERK and PI3K/AKT signaling[88]. Furthermore, a small-molecule inhibitor of PAK4, PF-3758309, potently inhibits the proliferation of a range of human cancer cell lines, including multiple colon cancer cell lines, and modulates numerous PAK4-dependent signaling nodes[89].

The constitutive activation of Wnt/β-catenin signaling through mutation of the *APC* gene is considered an important event in the development and progression of colon cancer. However, other dysregulated mechanisms may underlie the increased nuclear translocation of β-catenin and constitutive activation of Wnt/β-catenin signaling in colon cancer cells. We recently reported that PAK4 interacts with and phosphorylates β-catenin at Ser675, which promotes TCF/LEF transcriptional activity and stabilizes β-catenin by inhibiting its degradation[90] (Figure 2). Moreover, PAK4 nuclear accumulation enhances β-catenin nuclear import and increases TCF/LEF transcriptional activity[90] (Figure 2). These findings indicate a novel role of PAK4 in modulating β-catenin intracellular translocation and signaling.

Altered miRNA expression plays a crucial role in CRC. miR-145 is down-regulated in colon cancer cells[91]. miR-145 targets PAK4 and inhibits the PAK4/Raf/MEK/ERK pathway, leading to growth inhibition of SW480

and HT-29 cells[92]. Interestingly, miR-145 directly targets catenin δ-1, contributing to the impaired nuclear translocation of β-catenin by disturbing PAK4 nuclear import[93].

PAK5 and PAK6 also participate in CRC progression. PAK5 is overexpressed during CRC malignant progression and decreases CRC cell adhesion while promoting its migration[56], which may contribute to CRC metastasis. In addition, PAK5 inhibits camptothecin-induced apoptosis by suppressing the activity of caspase-8 and the phosphorylation of Bad in CRC cells[28]. Importantly, we recently reported that PAK5-mediated E47 phosphorylation promotes EMT and metastasis of colon cancer[94]. In contrast to other group Ⅱ PAKs, very little is known about the function of PAK6 in CRC, except that PAK6 increases chemoresistance and is an independent prognostic marker for stage Ⅱ and Ⅲ colon cancer patients undergoing 5-FU based chemotherapy[95].

Group Ⅱ PAK inhibitors

Because of their important roles in the development of cancer, PAKs have become attractive potential candidate drug targets. Among the group Ⅱ PAKs, PAK4 is considered a first-in-class anti-cancer drug target because of its elevated expression in cancers. The Pfizer compound PF-3758309, a pan-PAK ATP-competitive inhibitor, was originally described as a PAK4 inhibitor[89]. PF-3758309 blocks the growth of several human tumor xenografts[89]. Unfortunately, during clinical trials in patients with advanced solid tumors, undesirable pharmacokinetic characteristics were observed, and the clinical trial was terminated (clinicaltrials.gov). We recently reported a novel potent PAK4 inhibitor, LCH-7749944[82]. This inhibitor suppresses the proliferation of human gastric cancer cells through a pathway mediated by PAK4/c-Src/EGFR and cyclin D1. LCH-7749944 also impedes the migration and invasion of human gastric cancer cells by simultaneously blocking the PAK4/LIMK1/cofilin and PAK4/MEK-1/ERK1/2/MMP2 pathways. Specifically, this PAK4 inhibitor affects cell morphology by blocking the formation of filopodia and inducing cell elongation. It also inhibits EGFR activity by inhibiting PAK4[82]. These results suggest that LCH-7749944 may offer a novel therapeutic strategy for advanced metastatic gastric cancer. However, the biochemical potency of LCH-7749944 must be refined before it can be considered as a therapeutic agent. Another PAK4 inhibitor, KY-04031, was recently identified in a high-throughput screen[96]. Structural analysis indicated that both the indole and indazole moieties of KY-04031 are required for the PAK4 hinge interaction and that the triazine core mimics the ribose of the natural ATP substrate[96]. Although the cell-based anti-cancer activity of this compound is less efficient than that of PF-3758309[96], the unique molecular features of this compound can be used for the development of new PAK4 inhibitors. Recently, our group and another group cooperatively designed and synthesized a series of novel 1-phenanthryl-tetrahydroisoquinoline analogues as a new class of small-molecule PAK4 inhibitors that fit into the PAK4 cavity[97]. Lead optimization identified all derivatives with greater potency than the lead compound, particularly compound 21a[97]. Compound 21a markedly induced the cell cycle in G_1/S phase and suppressed the migration and invasion of MCF-7 breast cancer cells through the PAK4-LIMK1-cofilin pathway[97]. Possible novel binding modes between PAK4 and compound 21a were revealed in a molecular modeling study, providing a structural basis for further structure-based design of PAK4 inhibitors[97].

To develop PAK4 or other PAKs inhibitors for cancer therapy, several potential problems must be considered. First, it is important to distinguish the kinase-dependent and kinase-independent functions of PAK4 or other PAKs before developing PAK inhibitors, particularly ATP-competitive inhibitors. PAK4 and other PAKs sometimes function in a kinase-independent manner. Moreover, a PAK4 mutation (E329K) confers PAK4 resistance to competitive ATP inhibitors[98]. Thus, an alternative approach might be to develop allosteric PAKs inhibitors. Second, PAK inhibitors must overcome the specificity and redundancy problem caused by the various and overlapping characteristics of the PAKs themselves, their regulators and effectors. For PAK4 to be an effective drug target, a better understanding of PAK signaling in normal and cancer cells is needed.

CONCLUSION

Group Ⅱ PAKs play important roles in cellular processes including cytoskeletal organization, cell cycle control, and cell survival. Not surprisingly, group Ⅱ PAKs have been implicated in cancer progression. PAK4 is overexpressed in a number of cancers[14,43,47,64,79], and the *PAK4* gene is amplified in pancreatic cancer, colon cancer and oral squamous-cell carcinoma[32,45-47,49]. PAK4 is both essential and sufficient to lead to cancer in a mouse breast cancer model[43]. These observations have made PAK4 a popular candidate both as a diagnostic tool for the early detection of cancer and as a drug target for cancer therapy. Although several PAK4 small-molecule inhibitors have produced desirable results during *in vitro* experiments, satisfactory effects have not been observed in clinical trials. Further studies of PAK4 biology will provide new avenues for therapeutic interventions.

PAK5 is overexpressed in some cancers, and PAK5 overexpression has been detected in cancers that are prone to invasion and migration, such as colorectal cancer[56], gliomas[22] and epithelial ovarian cancer[57]. However, PAK5 overexpression is rarely observed in normal tissues or tumor growth in situ. The mechanisms underlying PAK5 overexpression in cancers and the modulation of its activity remain

unclear. Coupled with the identification of the PAK5-Egr1-MMP2 pathway in cancer cell invasion, migration and intercellular functions[39,40], PAK5 may become a potential drug target to suppress cancer invasion and metastasis.

Overexpression of PAK6 is detected mainly in prostate cancer. PAK6 also contributes to prostate cancer development and progression after androgen deprivation therapy[58]. However, the role of PAK6 in prostate cancer is not entirely clear, let alone gastrointestinal cancer or other cancers.

Further study is needed to fully elucidate the functions of PAK4-6 in normal and cancer cells. Group Ⅱ PAK research remains exciting as we further unravel the functions of PAK4-6 in the development of gastrointestinal and other cancers and validate their effectiveness as drug targets.

REFERENCES

1 **Manser E**, Leung T, Salihuddin H, Zhao ZS, Lim L. A brain serine/threonine protein kinase activated by Cdc42 and Rac1. *Nature* 1994; **367**: 40-46 [PMID: 8107774 DOI: 10.1038/367040a0]

2 **Bokoch GM**. Biology of the p21-activated kinases. *Annu Rev Biochem* 2003; **72**: 743-781 [PMID: 12676796 DOI: 10.1146/annurev.biochem.72.121801.161742]

3 **Kumar R**, Gururaj AE, Barnes CJ. p21-activated kinases in cancer. *Nat Rev Cancer* 2006; **6**: 459-471 [PMID: 16723992 DOI: 10.1038/nrc1892]

4 **Zhao ZS**, Manser E, Lim L. Interaction between PAK and nck: a template for Nck targets and role of PAK autophosphorylation. *Mol Cell Biol* 2000; **20**: 3906-3917 [PMID: 10805734 DOI: 10.1128/MCB.20.11.3906-3917.2000]

5 **Lei M**, Lu W, Meng W, Parrini MC, Eck MJ, Mayer BJ, Harrison SC. Structure of PAK1 in an autoinhibited conformation reveals a multistage activation switch. *Cell* 2000; **102**: 387-397 [PMID: 10975528 DOI: 10.1016/S0092-8674(00)00043-X]

6 **Abo A**, Qu J, Cammarano MS, Dan C, Fritsch A, Baud V, Belisle B, Minden A. PAK4, a novel effector for Cdc42Hs, is implicated in the reorganization of the actin cytoskeleton and in the formation of filopodia. *EMBO J* 1998; **17**: 6527-6540 [PMID: 9822598 DOI: 10.1093/emboj/17.22.6527]

7 **Dan C**, Nath N, Liberto M, Minden A. PAK5, a new brain-specific kinase, promotes neurite outgrowth in N1E-115 cells. *Mol Cell Biol* 2002; **22**: 567-577 [PMID: 11756552 DOI: 10.1128/MCB.22.2.567-577.2002]

8 **Baskaran Y**, Ng YW, Selamat W, Ling FT, Manser E. Group I and II mammalian PAKs have different modes of activation by Cdc42. *EMBO Rep* 2012; **13**: 653-659 [PMID: 22653441]

9 **Ha BH**, Davis MJ, Chen C, Lou HJ, Gao J, Zhang R, Krauthammer M, Halaban R, Schlessinger J, Turk BE, Boggon TJ. Type II p21-activated kinases (PAKs) are regulated by an autoinhibitory pseudosubstrate. *Proc Natl Acad Sci USA* 2012; **109**: 16107-16112 [PMID: 22988085 DOI: 10.1073/pnas.1214447109]

10 **Hoelz A**, Janz JM, Lawrie SD, Corwin B, Lee A, Sakmar TP. Crystal structure of the SH3 domain of betaPIX in complex with a high affinity peptide from PAK2. *J Mol Biol* 2006; **358**: 509-522 [PMID: 16527308 DOI: 10.1016/j.jmb.2006.02.027]

11 **Rennefahrt UE**, Deacon SW, Parker SA, Devarajan K, Beeser A, Chernoff J, Knapp S, Turk BE, Peterson JR. Specificity profiling of Pak kinases allows identification of novel phosphorylation sites. *J Biol Chem* 2007; **282**: 15667-15678 [PMID: 17392278 DOI: 10.1074/jbc.M700253200]

12 **Ha BH**, Morse EM, Turk BE, Boggon TJ. Signaling, Regulation, and Specificity of the Type II p21-activated Kinases. *J Biol Chem* 2015; **290**: 12975-12983 [PMID: 25855792 DOI: 10.1074/jbc.R115.650416]

13 **Qu J**, Cammarano MS, Shi Q, Ha KC, de Lanerolle P, Minden A. Activated PAK4 regulates cell adhesion and anchorage-independent growth. *Mol Cell Biol* 2001; **21**: 3523-3533 [PMID: 11313478 DOI: 10.1128/MCB.21.10.3523-3533.20011]

14 **Callow MG**, Clairvoyant F, Zhu S, Schryver B, Whyte DB, Bischoff JR, Jallal B, Smeal T. Requirement for PAK4 in the anchorage-independent growth of human cancer cell lines. *J Biol Chem* 2002; **277**: 550-558 [PMID: 11668177 DOI: 10.1074/jbc.M105732200]

15 **Nekrasova T**, Minden A. PAK4 is required for regulation of the cell-cycle regulatory protein p21, and for control of cell-cycle progression. *J Cell Biochem* 2011; **112**: 1795-1806 [PMID: 21381077 DOI: 10.1002/jcb.23092]

16 **Tyagi N**, Bhardwaj A, Singh AP, McClellan S, Carter JE, Singh S. p-21 activated kinase 4 promotes proliferation and survival of pancreatic cancer cells through AKT- and ERK-dependent activation of NF-κB pathway. *Oncotarget* 2014; **5**: 8778-8789 [PMID: 25238288]

17 **Li Y**, Wang D, Zhang H, Wang C, Dai W, Cheng Z, Wang G, Li F. P21-activated kinase 4 regulates the cyclin-dependent kinase inhibitor p57(kip2) in human breast cancer. *Anat Rec* (Hoboken) 2013; **296**: 1561-1567 [PMID: 23873832 DOI: 10.1002/ar.22754]

18 **Bompard G**, Rabeharivelo G, Cau J, Abrieu A, Delsert C, Morin N. P21-activated kinase 4 (PAK4) is required for metaphase spindle positioning and anchoring. *Oncogene* 2013; **32**: 910-919 [PMID: 22450748 DOI: 10.1038/onc.2012.98]

19 **Cammarano MS**, Nekrasova T, Noel B, Minden A. Pak4 induces premature senescence via a pathway requiring p16INK4/p19ARF and mitogen-activated protein kinase signaling. *Mol Cell Biol* 2005; **25**: 9532-9542 [PMID: 16227603 DOI: 10.1128/MCB.25.21.9532-9542.2005]

20 **Gu J**, Li K, Li M, Wu X, Zhang L, Ding Q, Wu W, Yang J, Mu J, Wen H, Ding Q, Lu J, Hao Y, Chen L, Zhang W, Li S, Liu Y. A role for p21-activated kinase 7 in the development of gastric cancer. *FEBS J* 2013; **280**: 46-55 [PMID: 23106939 DOI: 10.1111/febs.12048]

21 **Fang ZP**, Jiang BG, Gu XF, Zhao B, Ge RL, Zhang FB. P21-activated kinase 5 plays essential roles in the proliferation and tumorigenicity of human hepatocellular carcinoma. *Acta Pharmacol Sin* 2014; **35**: 82-88 [PMID: 23685956 DOI: 10.1038/aps.2013.31]

22 **Gu X**, Wang C, Wang X, Ma G, Li Y, Cui L, Chen Y, Zhao B, Li K. Efficient inhibition of human glioma development by RNA interference-mediated silencing of PAK5. *Int J Biol Sci* 2015; **11**: 230-237 [PMID: 25632266 DOI: 10.7150/ijbs.9193]

23 **Wen X**, Li X, Liao B, Liu Y, Wu J, Yuan X, Ouyang B, Sun Q, Gao X. Knockdown of p21-activated kinase 6 inhibits prostate cancer growth and enhances chemosensitivity to docetaxel. *Urology* 2009; **73**: 1407-1411 [PMID: 19362342 DOI: 10.1016/j.urology.2008.09.041]

24 **Gnesutta N**, Qu J, Minden A. The serine/threonine kinase PAK4 prevents caspase activation and protects cells from apoptosis. *J Biol Chem* 2001; **276**: 14414-14419 [PMID: 11278822 DOI: 10.1074/jbc.M011046200]

25 **Gnesutta N**, Minden A. Death receptor-induced activation of initiator caspase 8 is antagonized by serine/threonine kinase PAK4. *Mol Cell Biol* 2003; **23**: 7838-7848 [PMID: 14560027 DOI: 10.1128/MCB.23.21.7838-7848.2003]

26 **Li X**, Minden A. PAK4 functions in tumor necrosis factor (TNF) alpha-induced survival pathways by facilitating TRADD binding to the TNF receptor. *J Biol Chem* 2005; **280**: 41192-41200 [PMID: 16227624 DOI: 10.1074/jbc.M506884200]

27 **Cotteret S**, Jaffer ZM, Beeser A, Chernoff J. p21-Activated kinase 5 (Pak5) localizes to mitochondria and inhibits apoptosis by phosphorylating BAD. *Mol Cell Biol* 2003; **23**: 5526-5539 [PMID: 12897128 DOI: 10.1128/MCB.23.16.5526-5539.2003]

28 **Wang X**, Gong W, Qing H, Geng Y, Wang X, Zhang Y, Peng L, Zhang H, Jiang B. p21-activated kinase 5 inhibits camptothecin-induced apoptosis in colorectal carcinoma cells. *Tumour Biol* 2010;

31: 575-582 [PMID: 20567954 DOI: 10.1007/s13277-010-0071-3]
29 **Zhang DG**, Zhang J, Mao LL, Wu JX, Cao WJ, Zheng JN, Pei DS. p21-Activated kinase 5 affects cisplatin-induced apoptosis and proliferation in hepatocellular carcinoma cells. *Tumour Biol* 2015; **36**: 3685-3691 [PMID: 25560489]
30 **Zhang M**, Siedow M, Saia G, Chakravarti A. Inhibition of p21-activated kinase 6 (PAK6) increases radiosensitivity of prostate cancer cells. *Prostate* 2010; **70**: 807-816 [PMID: 20054820 DOI: 10.1002/pros.21114]
31 **Dart AE**, Wells CM. P21-activated kinase 4--not just one of the PAK. *Eur J Cell Biol* 2013; **92**: 129-138 [PMID: 23642861 DOI: 10.1016/j.ejcb.2013.03.002]
32 **Kimmelman AC**, Hezel AF, Aguirre AJ, Zheng H, Paik JH, Ying H, Chu GC, Zhang JX, Sahin E, Yeo G, Ponugoti A, Nabioullin R, Deroo S, Yang S, Wang X, McGrath JP, Protopopova M, Ivanova E, Zhang J, Feng B, Tsao MS, Redston M, Protopopov A, Xiao Y, Futreal PA, Hahn WC, Klimstra DS, Chin L, DePinho RA. Genomic alterations link Rho family of GTPases to the highly invasive phenotype of pancreas cancer. *Proc Natl Acad Sci USA* 2008; **105**: 19372-19377 [PMID: 19050074 DOI: 10.1073/pnas.0809966105]
33 **Zhang HJ**, Siu MK, Yeung MC, Jiang LL, Mak VC, Ngan HY, Wong OG, Zhang HQ, Cheung AN. Overexpressed PAK4 promotes proliferation, migration and invasion of choriocarcinoma. *Carcinogenesis* 2011; **32**: 765-771 [PMID: 21325635 DOI: 10.1093/carcin/bgr033]
34 **Kesanakurti D**, Chetty C, Rajasekhar Maddirela D, Gujrati M, Rao JS. Functional cooperativity by direct interaction between PAK4 and MMP-2 in the regulation of anoikis resistance, migration and invasion in glioma. *Cell Death Dis* 2012; **3**: e445 [PMID: 23254288 DOI: 10.1038/cddis.2012.182]
35 **Lu W**, Xia YH, Qu JJ, He YY, Li BL, Lu C, Luo X, Wan XP. p21-activated kinase 4 regulation of endometrial cancer cell migration and invasion involves the ERK1/2 pathway mediated MMP-2 secretion. *Neoplasma* 2013; **60**: 493-503 [PMID: 23790167 DOI: 10.4149/neo_2013_064]
36 **Ahmed T**, Shea K, Masters JR, Jones GE, Wells CM. A PAK4-LIMK1 pathway drives prostate cancer cell migration downstream of HGF. *Cell Signal* 2008; **20**: 1320-1328 [PMID: 18424072 DOI: 10.1016/j.cellsig.2008.02.021]
37 **Siu MK**, Chan HY, Kong DS, Wong ES, Wong OG, Ngan HY, Tam KF, Zhang H, Li Z, Chan QK, Tsao SW, Strömblad S, Cheung AN. p21-activated kinase 4 regulates ovarian cancer cell proliferation, migration, and invasion and contributes to poor prognosis in patients. *Proc Natl Acad Sci USA* 2010; **107**: 18622-18627 [PMID: 20926745 DOI: 10.1073/pnas.0907481107]
38 **Guo Q**, Su N, Zhang J, Li X, Miao Z, Wang G, Cheng M, Xu H, Cao L, Li F. PAK4 kinase-mediated SCG10 phosphorylation involved in gastric cancer metastasis. *Oncogene* 2014; **33**: 3277-3287 [PMID: 23893240 DOI: 10.1038/onc.2013.296]
39 **Han ZX**, Wang XX, Zhang SN, Wu JX, Qian HY, Wen YY, Tian H, Pei DS, Zheng JN. Downregulation of PAK5 inhibits glioma cell migration and invasion potentially through the PAK5-Egr1-MMP2 signaling pathway. *Brain Tumor Pathol* 2014; **31**: 234-241 [PMID: 24062079 DOI: 10.1007/s10014-013-0161-1]
40 **Wang XX**, Cheng Q, Zhang SN, Qian HY, Wu JX, Tian H, Pei DS, Zheng JN. PAK5-Egr1-MMP2 signaling controls the migration and invasion in breast cancer cell. *Tumour Biol* 2013; **34**: 2721-2729 [PMID: 23696025 DOI: 10.1007/s13277-013-0824-x]
41 **Liu X**, Busby J, John C, Wei J, Yuan X, Lu ML. Direct interaction between AR and PAK6 in androgen-stimulated PAK6 activation. *PLoS One* 2013; **8**: e77367 [PMID: 24130878 DOI: 10.1371/journal.pone.0077367]
42 **Cai S**, Chen R, Li X, Cai Y, Ye Z, Li S, Li J, Huang H, Peng S, Wang J, Tao Y, Huang H, Wen X, Mo J, Deng Z, Wang J, Zhang Y, Gao X, Wen X. Downregulation of microRNA-23a suppresses prostate cancer metastasis by targeting the PAK6-LIMK1 signaling pathway. *Oncotarget* 2015; **6**: 3904-3917 [PMID: 25714010]
43 **Liu Y**, Chen N, Cui X, Zheng X, Deng L, Price S, Karantza V, Minden A. The protein kinase Pak4 disrupts mammary acinar architecture and promotes mammary tumorigenesis. *Oncogene* 2010; **29**: 5883-5894 [PMID: 20697354 DOI: 10.1038/onc.2010.329]
44 **Kim JH**, Kim HN, Lee KT, Lee JK, Choi SH, Paik SW, Rhee JC, Lowe AW. Gene expression profiles in gallbladder cancer: the close genetic similarity seen for early and advanced gallbladder cancers may explain the poor prognosis. *Tumour Biol* 2008; **29**: 41-49 [PMID: 18497548 DOI: 10.1159/000132570]
45 **Chen S**, Auletta T, Dovirak O, Hutter C, Kuntz K, El-ftesi S, Kendall J, Han H, Von Hoff DD, Ashfaq R, Maitra A, Iacobuzio-Donahue CA, Hruban RH, Lucito R. Copy number alterations in pancreatic cancer identify recurrent PAK4 amplification. *Cancer Biol Ther* 2008; **7**: 1793-1802 [PMID: 18836286]
46 **Mahlamäki EH**, Kauraniemi P, Monni O, Wolf M, Hautaniemi S, Kallioniemi A. High-resolution genomic and expression profiling reveals 105 putative amplification target genes in pancreatic cancer. *Neoplasia* 2004; **6**: 432-439 [PMID: 15548351]
47 **Parsons DW**, Wang TL, Samuels Y, Bardelli A, Cummins JM, DeLong L, Silliman N, Ptak J, Szabo S, Willson JK, Markowitz S, Kinzler KW, Vogelstein B, Lengauer C, Velculescu VE. Colorectal cancer: mutations in a signalling pathway. *Nature* 2005; **436**: 792 [PMID: 16094359 DOI: 10.1038/436792a]
48 **Ahn HK**, Jang J, Lee J, Se Hoon P, Park JO, Park YS, Lim HY, Kim KM, Kang WK. P21-activated kinase 4 overexpression in metastatic gastric cancer patients. *Transl Oncol* 2011; **4**: 345-349 [PMID: 22190998]
49 **Begum A**, Imoto I, Kozaki K, Tsuda H, Suzuki E, Amagasa T, Inazawa J. Identification of PAK4 as a putative target gene for amplification within 19q13.12-q13.2 in oral squamous-cell carcinoma. *Cancer Sci* 2009; **100**: 1908-1916 [PMID: 19594544 DOI: 10.1111/j.1349-7006.2009.01252.x]
50 **Karantza-Wadsworth V**, Patel S, Kravchuk O, Chen G, Mathew R, Jin S, White E. Autophagy mitigates metabolic stress and genome damage in mammary tumorigenesis. *Genes Dev* 2007; **21**: 1621-1635 [PMID: 17606641 DOI: 10.1101/gad.1565707]
51 **Rafn B**, Nielsen CF, Andersen SH, Szyniarowski P, Corcelle-Termeau E, Valo E, Fehrenbacher N, Olsen CJ, Daugaard M, Egebjerg C, Bøttzauw T, Kohonen P, Nylandsted J, Hautaniemi S, Moreira J, Jäättelä M, Kallunki T. ErbB2-driven breast cancer cell invasion depends on a complex signaling network activating myeloid zinc finger-1-dependent cathepsin B expression. *Mol Cell* 2012; **45**: 764-776 [PMID: 22464443 DOI: 10.1016/j.molcel.2012.01.029]
52 **So JY**, Lee HJ, Kramata P, Minden A, Suh N. Differential Expression of Key Signaling Proteins in MCF10 Cell Lines, a Human Breast Cancer Progression Model. *Mol Cell Pharmacol* 2012; **4**: 31-40 [PMID: 24558516]
53 **Davis SJ**, Sheppard KE, Pearson RB, Campbell IG, Gorringe KL, Simpson KJ. Functional analysis of genes in regions commonly amplified in high-grade serous and endometrioid ovarian cancer. *Clin Cancer Res* 2013; **19**: 1411-1421 [PMID: 23362323 DOI: 10.1158/1078-0432.CCR-12-3433]
54 **Gorringe KL**, George J, Anglesio MS, Ramakrishna M, Etemadmoghadam D, Cowin P, Sridhar A, Williams LH, Boyle SE, Yanaihara N, Okamoto A, Urashima M, Smyth GK, Campbell IG, Bowtell DD. Copy number analysis identifies novel interactions between genomic loci in ovarian cancer. *PLoS One* 2010; **5**: pii: e11408 [PMID: 20844748 DOI: 10.1371/journal.pone.0011408]
55 **Li Y**, Ke Q, Shao Y, Zhu G, Li Y, Geng N, Jin F, Li F. GATA1 induces epithelial-mesenchymal transition in breast cancer cells through PAK5 oncogenic signaling. *Oncotarget* 2015; **6**: 4345-4356 [PMID: 25726523]
56 **Gong W**, An Z, Wang Y, Pan X, Fang W, Jiang B, Zhang H. P21-activated kinase 5 is overexpressed during colorectal cancer progression and regulates colorectal carcinoma cell adhesion and migration. *Int J Cancer* 2009; **125**: 548-555 [PMID: 19415746 DOI: 10.1002/ijc.24428]
57 **Li D**, Yao X, Zhang P. The overexpression of P21-activated kinase 5 (PAK5) promotes paclitaxel-chemoresistance of epithelial ovarian cancer. *Mol Cell Biochem* 2013; **383**: 191-199 [PMID:

23877225 DOI: 10.1007/s11010-013-1767-7]
58 **Kaur R**, Yuan X, Lu ML, Balk SP. Increased PAK6 expression in prostate cancer and identification of PAK6 associated proteins. *Prostate* 2008; **68**: 1510-1516 [PMID: 18642328 DOI: 10.1002/pros.20787]
59 **Wang Y**, Yu Q, Cho AH, Rondeau G, Welsh J, Adamson E, Mercola D, McClelland M. Survey of differentially methylated promoters in prostate cancer cell lines. *Neoplasia* 2005; **7**: 748-760 [PMID: 16207477]
60 **Liu T**, Li Y, Gu H, Zhu G, Li J, Cao L, Li F. p21-Activated kinase 6 (PAK6) inhibits prostate cancer growth via phosphorylation of androgen receptor and tumorigenic E3 ligase murine double minute-2 (Mdm2). *J Biol Chem* 2013; **288**: 3359-3369 [PMID: 23132866 DOI: 10.1074/jbc.M112.384289]
61 **He H**, Baldwin GS. p21-activated kinases and gastrointestinal cancer. *Biochim Biophys Acta* 2013; **1833**: 33-39 [PMID: 23092728 DOI: 10.1016/j.bbamcr.2012.10.015]
62 **Giordano S**, Columbano A. Met as a therapeutic target in HCC: facts and hopes. *J Hepatol* 2014; **60**: 442-452 [PMID: 24045150 DOI: 10.1016/j.jhep.2013.09.009]
63 **Whittaker S**, Marais R, Zhu AX. The role of signaling pathways in the development and treatment of hepatocellular carcinoma. *Oncogene* 2010; **29**: 4989-5005 [PMID: 20639898 DOI: 10.1038/onc.2010.236]
64 **Mak GW**, Chan MM, Leong VY, Lee JM, Yau TO, Ng IO, Ching YP. Overexpression of a novel activator of PAK4, the CDK5 kinase-associated protein CDK5RAP3, promotes hepatocellular carcinoma metastasis. *Cancer Res* 2011; **71**: 2949-2958 [PMID: 21385901 DOI: 10.1158/0008-5472.CAN-10-4046]
65 **Hou J**, Lin L, Zhou W, Wang Z, Ding G, Dong Q, Qin L, Wu X, Zheng Y, Yang Y, Tian W, Zhang Q, Wang C, Zhang Q, Zhuang SM, Zheng L, Liang A, Tao W, Cao X. Identification of miRNomes in human liver and hepatocellular carcinoma reveals miR-199a/b-3p as therapeutic target for hepatocellular carcinoma. *Cancer Cell* 2011; **19**: 232-243 [PMID: 21316602 DOI: 10.1016/j.ccr.2011.01.001]
66 **Li Q**, Ding C, Chen C, Zhang Z, Xiao H, Xie F, Lei L, Chen Y, Mao B, Jiang M, Li J, Wang D, Wang G. miR-224 promotion of cell migration and invasion by targeting Homeobox D 10 gene in human hepatocellular carcinoma. *J Gastroenterol Hepatol* 2014; **29**: 835-842 [PMID: 24219032 DOI: 10.1111/jgh.12429]
67 **Chen H**, Miao J, Li H, Wang C, Li J, Zhu Y, Wang J, Wu X, Qiao H. Expression and prognostic significance of p21-activated kinase 6 in hepatocellular carcinoma. *J Surg Res* 2014; **189**: 81-88 [PMID: 24576777 DOI: 10.1016/j.jss.2014.01.049]
68 **Hidalgo M**. Pancreatic cancer. *N Engl J Med* 2010; **362**: 1605-1617 [PMID: 20427809 DOI: 10.1056/NEJMra0901557]
69 **Bardeesy N**, DePinho RA. Pancreatic cancer biology and genetics. *Nat Rev Cancer* 2002; **2**: 897-909 [PMID: 12459728 DOI: 10.1038/nrc949]
70 **Costello E**, Greenhalf W, Neoptolemos JP. New biomarkers and targets in pancreatic cancer and their application to treatment. *Nat Rev Gastroenterol Hepatol* 2012; **9**: 435-444 [PMID: 22733351 DOI: 10.1038/nrgastro.2012.119]
71 **Jones S**, Zhang X, Parsons DW, Lin JC, Leary RJ, Angenendt P, Mankoo P, Carter H, Kamiyama H, Jimeno A, Hong SM, Fu B, Lin MT, Calhoun ES, Kamiyama M, Walter K, Nikolskaya T, Nikolsky Y, Hartigan J, Smith DR, Hidalgo M, Leach SD, Klein AP, Jaffee EM, Goggins M, Maitra A, Iacobuzio-Donahue C, Eshleman JR, Kern SE, Hruban RH, Karchin R, Papadopoulos N, Parmigiani G, Vogelstein B, Velculescu VE, Kinzler KW. Core signaling pathways in human pancreatic cancers revealed by global genomic analyses. *Science* 2008; **321**: 1801-1806 [PMID: 18772397 DOI: 10.1126/science.1164368]
72 **Klimstra DS**, Longnecker DS. K-ras mutations in pancreatic ductal proliferative lesions. *Am J Pathol* 1994; **145**: 1547-1550 [PMID: 7992857]
73 **Collins MA**, Bednar F, Zhang Y, Brisset JC, Galbán S, Galbán CJ, Rakshit S, Flannagan KS, Adsay NV, Pasca di Magliano M. Oncogenic Kras is required for both the initiation and maintenance of pancreatic cancer in mice. *J Clin Invest* 2012; **122**: 639-653 [PMID: 22232209 DOI: 10.1172/JCI59227]
74 **Yeo D**, Huynh N, Beutler JA, Christophi C, Shulkes A, Baldwin GS, Nikfarjam M, He H. Glaucarubinone and gemcitabine synergistically reduce pancreatic cancer growth via down-regulation of P21-activated kinases. *Cancer Lett* 2014; **346**: 264-272 [PMID: 24491405 DOI: 10.1016/j.canlet.2014.01.001]
75 **Jemal A**, Bray F, Center MM, Ferlay J, Ward E, Forman D. Global cancer statistics. *CA Cancer J Clin* 2011; **61**: 69-90 [PMID: 21296855 DOI: 10.3322/caac.20107]
76 **Schinzari G**, Cassano A, Orlandi A, Basso M, Barone C. Targeted therapy in advanced gastric carcinoma: the future is beginning. *Curr Med Chem* 2014; **21**: 1026-1038 [PMID: 24304282 DOI: 10.2174/09298673216613129124054]
77 **Liu F**, Li X, Wang C, Cai X, Du Z, Xu H, Li F. Downregulation of p21-activated kinase-1 inhibits the growth of gastric cancer cells involving cyclin B1. *Int J Cancer* 2009; **125**: 2511-2519 [PMID: 19610058 DOI: 10.1002/ijc.24588]
78 **Cai XZ**, Wang J, Li XD, Wang GL, Liu FN, Cheng MS, Li F. Curcumin suppresses proliferation and invasion in human gastric cancer cells by downregulation of PAK1 activity and cyclin D1 expression. *Cancer Biol Ther* 2009; **8**: 1360-1368 [PMID: 19448398]
79 **Wang C**, Li Y, Zhang H, Liu F, Cheng Z, Wang D, Wang G, Xu H, Zhao Y, Cao L, Li F. Oncogenic PAK4 regulates Smad2/3 axis involving gastric tumorigenesis. *Oncogene* 2014; **33**: 3473-3484 [PMID: 23934187 DOI: 10.1038/onc.2013.300]
80 **Li X**, Ke Q, Li Y, Liu F, Zhu G, Li F. DGCR6L, a novel PAK4 interaction protein, regulates PAK4-mediated migration of human gastric cancer cell via LIMK1. *Int J Biochem Cell Biol* 2010; **42**: 70-79 [PMID: 19778628 DOI: 10.1016/j.biocel.2009.09.008]
81 **Li D**, Zhang Y, Li Z, Wang X, Qu X, Liu Y. Activated Pak4 expression correlates with poor prognosis in human gastric cancer patients. *Tumour Biol* 2015; **36**: 9431-9436 [PMID: 26124003]
82 **Zhang J**, Wang J, Guo Q, Wang Y, Zhou Y, Peng H, Cheng M, Zhao D, Li F. LCH-7749944, a novel and potent p21-activated kinase 4 inhibitor, suppresses proliferation and invasion in human gastric cancer cells. *Cancer Lett* 2012; **317**: 24-32 [PMID: 22085492 DOI: 10.1016/j.canlet.2011.11.007]
83 **Fu X**, Feng J, Zeng D, Ding Y, Yu C, Yang B. PAK4 confers cisplatin resistance in gastric cancer cells via PI3K/Akt- and MEK/Erk-dependent pathways. *Biosci Rep* 2014; **34**: 59-67 [PMID: 24471762 DOI: 10.1042/BSR20130102]
84 **Zoratto F**, Rossi L, Verrico M, Papa A, Basso E, Zullo A, Tomao L, Romiti A, Lo Russo G, Tomao S. Focus on genetic and epigenetic events of colorectal cancer pathogenesis: implications for molecular diagnosis. *Tumour Biol* 2014; **35**: 6195-6206 [PMID: 25051912 DOI: 10.1007/s13277-014-1845-9]
85 **Repasky GA**, Chenette EJ, Der CJ. Renewing the conspiracy theory debate: does Raf function alone to mediate Ras oncogenesis? *Trends Cell Biol* 2004; **14**: 639-647 [PMID: 15519853 DOI: 10.1016/j.tcb.2004.09.014]
86 **Liu Y**, Xiao H, Tian Y, Nekrasova T, Hao X, Lee HJ, Suh N, Yang CS, Minden A. The pak4 protein kinase plays a key role in cell survival and tumorigenesis in athymic mice. *Mol Cancer Res* 2008; **6**: 1215-1224 [PMID: 18644984 DOI: 10.1158/1541-7786]
87 **Song B**, Wang W, Zheng Y, Yang J, Xu Z. P21-activated kinase 1 and 4 were associated with colorectal cancer metastasis and infiltration. *J Surg Res* 2015; **196**: 130-135 [PMID: 25791829 DOI: 10.1016/j.jss.2015.02.035]
88 **Tabusa H**, Brooks T, Massey AJ. Knockdown of PAK4 or PAK1 inhibits the proliferation of mutant KRAS colon cancer cells independently of RAF/MEK/ERK and PI3K/AKT signaling. *Mol Cancer Res* 2013; **11**: 109-121 [PMID: 23233484 DOI: 10.1158/1541-7786.MCR-12-0466]
89 **Murray BW**, Guo C, Piraino J, Westwick JK, Zhang C, Lamerdin J, Dagostino E, Knighton D, Loi CM, Zager M, Kraynov E, Popoff I, Christensen JG, Martinez R, Kephart SE, Marakovits J, Karlicek S, Bergqvist S, Smeal T. Small-molecule p21-activated kinase inhibitor PF-3758309 is a potent inhibitor of oncogenic signaling

and tumor growth. *Proc Natl Acad Sci USA* 2010; **107**: 9446-9451 [PMID: 20439741 DOI: 10.1073/pnas.0911863107]

90 **Li Y**, Shao Y, Tong Y, Shen T, Zhang J, Li Y, Gu H, Li F. Nucleo-cytoplasmic shuttling of PAK4 modulates β-catenin intracellular translocation and signaling. *Biochim Biophys Acta* 2012; **1823**: 465-475 [PMID: 22173096 DOI: 10.1016/j.bbamcr.2011.11.013]

91 **Michael MZ**, O' Connor SM, van Holst Pellekaan NG, Young GP, James RJ. Reduced accumulation of specific microRNAs in colorectal neoplasia. *Mol Cancer Res* 2003; **1**: 882-891 [PMID: 14573789]

92 **Wang Z**, Zhang X, Yang Z, Du H, Wu Z, Gong J, Yan J, Zheng Q. MiR-145 regulates PAK4 via the MAPK pathway and exhibits an antitumor effect in human colon cells. *Biochem Biophys Res Commun* 2012; **427**: 444-449 [PMID: 22766504 DOI: 10.1016/j.bbrc.2012.06.123]

93 **Yamada N**, Noguchi S, Mori T, Naoe T, Maruo K, Akao Y. Tumor-suppressive microRNA-145 targets catenin δ-1 to regulate Wnt/β-catenin signaling in human colon cancer cells. *Cancer Lett* 2013; **335**: 332-342 [PMID: 23499891 DOI: 10.1016/j.canlet.2013.02.060]

94 **Zhu G**, Li X, Guo B, Ke Q, Dong M, Li F. PAK5-mediated E47 phosphorylation promotes epithelial-mesenchymal transition and metastasis of colon cancer. *Oncogene* 2015; Epub ahead of print [PMID: 26212009 DOI: 10.1038/onc.2015.259]

95 **Chen J**, Lu H, Yan D, Cui F, Wang X, Yu F, Xue Y, Feng X, Wang J, Wang X, Jiang T, Zhang M, Zhao S, Yu Y, Tang H, Peng Z. PAK6 increase chemoresistance and is a prognostic marker for stage II and III colon cancer patients undergoing 5-FU based chemotherapy. *Oncotarget* 2015; **6**: 355-367 [PMID: 25426562]

96 **Ryu BJ**, Kim S, Min B, Kim KY, Lee JS, Park WJ, Lee H, Kim SH, Park S. Discovery and the structural basis of a novel p21-activated kinase 4 inhibitor. *Cancer Lett* 2014; **349**: 45-50 [PMID: 24704155 DOI: 10.1016/j.canlet.2014.03.024]

97 **Song S**, Li X, Guo J, Hao C, Feng Y, Guo B, Liu T, Zhang Q, Zhang Z, Li R, Wang J, Lin B, Li F, Zhao D, Cheng M. Design, synthesis and biological evaluation of 1-phenanthryl-tetrahydroisoquinoline derivatives as novel p21-activated kinase 4 (PAK4) inhibitors. *Org Biomol Chem* 2015; **13**: 3803-3818 [PMID: 25705811 DOI: 10.1039/c5ob00037h]

98 **Whale AD**, Dart A, Holt M, Jones GE, Wells CM. PAK4 kinase activity and somatic mutation promote carcinoma cell motility and influence inhibitor sensitivity. *Oncogene* 2013; **32**: 2114-2120 [PMID: 22689056 DOI: 10.1038/onc.2012.233]

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