Int J Clin Exp Pathol 2015;8(12):15895-15902
www.ijcep.com /ISSN:1936-2625/IJCEP0017393

Original Article

Overexpression of PAK-1 is an independent predictor of disease recurrence in colorectal carcinoma

Jaudah Al-Maghrabi[1,3], Eman Emam[1,2], Wafaey Gomaa[1,4], Doaa Al-Qaydy[1], Basim Al-Maghrabi[1], Abdelbaset Buhmeida[6], Adel Abuzenadah[6], Mohammed Al-Qahtani[6], Mahmoud Al-Ahwal[3,5]

[1]Department of Pathology, King Abdulaziz University, Jeddah, Saudi Arabia; [2]Department of Pathology, Alexandria University, Egypt; [3]Scientific Chair for Colorectal Cancer, King Abdulaziz University, Jeddah, Saudi Arabia; [4]Department of Pathology, Faculty of Medicine, Minia University, El-Minia, Egypt; [5]Department of Medicine, King Abdulaziz University, Jeddah, Saudi Arabia; [6]Center of Excellence in Genomic Medicine Research, King Abdulaziz University, Jeddah, Saudi Arabia

Received October 7, 2015; Accepted November 22, 2015; Epub December 1, 2015; Published December 15, 2015

Abstract: Background: Colorectal carcinoma (CRC) is a significant cause of major morbidity and mortality. PAK-1 is a protein that regulates cytoskeletal dynamics and cell motility. The purpose of the present study is to investigate the relationship between PAK-1 immunoexpression and CRC progression and its validity as an independent prognostic factor. Patients and methods: Paraffin blocks of 103 primary CRCs and 37 nodal metastases were retrieved and tissue microarrays were constructed. Immunohistochemistry was performed using anti-PAK-1 antibody. Immunostaining was scored and results were analysed in relation to clinicopathological parameters. Results: PAK-1 was overexpressed in primary CRC (P<0.001). No difference between low and high expression in nodal metastasis (P=0.139). There was no difference between PAK-1 immunoexpression in primary and nodal metastasis (P=0.275). High PAK-1 immunoexpression was associated with disease recurrence (P=0.03). However, there was no association with most clinicopathological parameters. PAK-1 overexpression was detected as an independent predictor of disease recurrence (P=0.05). No association was found between PAK-1 immunoexpression and disease free survival (log-rank =1.287, P=0.257). Conclusion: PAK-1 overexpression may be involved in CRC progression and could be considered an independent predictor of disease recurrence. Further in vivo and in vitro molecular studies are needed to investigate the role of PAK-1 in colorectal carcinogenesis.

Keywords: PAK-1, immunohistochemistry, colorectal carcinoma, prognosis

Introduction

P21-activated kinase (PAK) is a group of serine/threonine kinases that were first discovered in 1994 in a screen for proteins that interact with the small G-proteins Rac1 and Cdc42 [1]. PAKs contribute in numerous cellular signalling pathways [2]. PAKs family is sub-grouped according to structural and architectural characteristics into two categories; group I; including PAK 1, PAK 2, and PAK 3, and group II including PAK 4, PAK 5, and PAK 6. The distribution of PAKs among normal tissues is variable with PAK 1 particularly expressed in the brain, muscle and spleen, PAK 2 in variable tissues, PAK 4 in prostate, testis, colon, and finally PAK 3 and PAK 5 in brain tissue. Group I PAKs are activated by growth factors and extracellular signals through GTPase-dependent and independent mechanisms [2-4].

PAK-1 controls growth factor signals responsible for cell proliferation through the regulation of cell-cycle progression and mitotic activity [5-8]. It plays a central role in stimulating cell motility through the organization of actin cytoskeleton, cell shape and adhesion dynamics which are mandatory for invasion and metastasis [7]. Moreover, PAK-1 has a significant part in regulating cell death and survival signalling through controlling apoptosis [9]. Overexpression of PAK-1 is reported to increase migration potential and abnormal mitotic activity in variable carcinomas such as breast, ovary, thyroid and colon [10-12]. Overexpression of PAK-1 was associated with the development of mammary cell hyperplasia in animal models through

Table 1. Clinicopathological parameters of CRC patients (*n*=103)

Parameter		Number (%)
Age	<60 years	60 (58.3%)
	≥60 years	43 (41.7%)
Sex	Male	57 (55.3%)
	Female	46 (44.7%)
Grade	Well-differentiated	18 (17.5%)
	Moderately-differentiated	71 (68.9%)
	Poorly-differentiated	14 (13.6%)
Tumour location	Right colon	34 (33%)
	Left colon	61 (59.2%)
	Rectum	8 (7.8%)
Tumour size	<5 cm	42 (40.8%)
	≥5 cm	61 (59.2%)
Primary tumour	T1	1 (1%)
	T2	15 (14.6%)
	T3	78 (75.7%)
	T4	9 (8.7%)
Nodal metastasis	Negative	43 (41.7%)
	Positive	58 (56.3%)
	Cannot be assessed	2 (1.9%)
Lymphovascular invasion	Positive	85 (82.5%)
	Negative	18 (17.5%)
Margin status	Free	99 (96.1%)
	Involved	4 (3.9%)
Distant metastasis	Negative	73 (72.3%)
	Positiv e	28 (27.7%)
	Not available	2 (1.9%)
Recurrence	No recurrence	77 (74.8%)
	Recurrence	26 (25.2%)
Survival	Alive	61 (59.2%)
	Dead	25 (24.3%)
	Not available	17 (16.5%)

T1: Tumour invades submucosa; T2: Tumour invades muscularis propria; T3: Tumour invades through the muscularis propria into the subserosa or into non-peritonealised pericolic or perirectal tissues; T4: Tumour directly invades other organs or structures, and/or perforates visceral peritoneum.

its correlation to increased cyclin D1 promoter activity, which in turn could be related to increased progression of breast cancer [13]. PAK-1 was included into HER-2 pathway controlling invasiveness of breast cancer cells in cooperation with other oncogenes. They showed that loss of PAK-1 leads to reduced expression of beta-catenin and its target genes with subsequent prolonged survival. These results suggest a new therapeutic strategy for HER-2 positive breast cancer patients [14]. Increased PAK-1 expression in the invasive fronts of aggressive papillary thyroid cancers in comparing to the tumour centre is supporting the hypothesis that PAKs functionally regulate thyroid cancer cell motility, and is implicated as regulator of thyroid cancer invasion [10].

The purpose of the present study is to investigate the relationship between PAK-1 immunoexpression and CRC progression and its validity as an independent prognostic factor.

Materials and methods

Patients

Hundred and three patients diagnosed as CRC constituted the material of the present study. Patients' information was gathered from the records of the Pathology Department in King Abdulaziz University, Jeddah, Saudi Arabia. Paraffin blocks of patients were sliced and stained routinely with haematoxylin and eosin to evaluate histopathological characteristics of the tumours as well as for histological grading and staging (following the AJCC staging system) [15]. For evaluation of tumour invasion, tumour stage was used as an indicator. Clinicopathological parameters of all patients as age, sex, tumour site and size, histological type, grade and stage as well as lymph node and safety margin status, and presence of distant metastasis along with follow up results were collected from the patient's medical records after obtaining the formal ethical approval. Cliniopathological data is shown in **Table 1**. The study was approved by the Research Committee of the Biomedical Ethics Unit, Faculty of Medicine, King Abdulaziz University, Jeddah, Saudi Arabia.

Tissue microarray construction

A tissue microarray was constructed from 103 primary CRC and 37 lymph nodes showing associated tumour metastasis. TMAs were constructed using an automated tissue arrayer (MASTER 3D HISTECH). Donor Paraffin blocks used in this study was retrieved from surgical

Table 2. Categories of PAK-1 immunoexpression in primary tumours and nodal metastases

	Primary tumour (*n*=103)	Nodal Metastasis (*n*=37)	*P* value
Low expression	29 (28.2%)	14 (37.8%)	0.275☂
High expression	74 (71.8%)	23 (62.2%)	
P value	<0.001*	0.139*	

*One sample non-parametric chi-square test; ☂Mann-Whitney test; Low expression: 0+1; High Expression: 2+3.

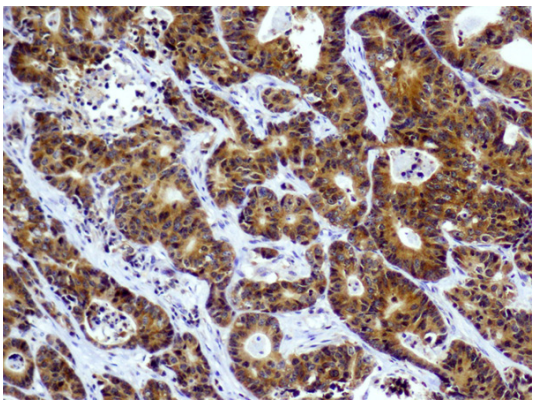

Figure 1. PAK-1 cytoplasmic immunostaining in a well differentiated primary CRC. The expression pattern of PAK-1 protein is strong (+3) and evident in both basal and apical portions of malignant cells (100×).

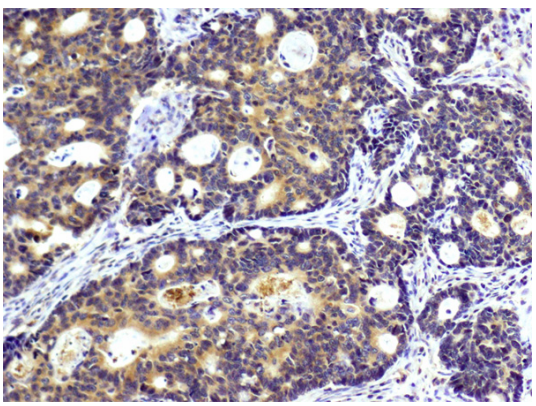

Figure 2. Moderately differentiated colorectal carcinoma showing borderline/moderate (+2) diffuse cytoplasmic expression pattern for PAK-1 (100×).

removed specimens. From selected paraffin blocks, a cylinder of tissue 1 mm in diameter was cut with a TMA instrument and inserted into a new recipient paraffin block. Serial 4-μm sections were then cut from the TMA paraffin blocks and placed on positively charged slides for immunohistochemical staining.

Immunohistochemistry

Immunohistochemical staining was done using a primary anti-p21-activated kinase-1 rabbit polyclonal antibody at a dilution of 1:100 (DakoCytomation Norden A/S, Glostrup, Denmark). Immunostaining was completed by using an automatic immunostainer (Ventana Bench Mark XT, Ventana Inc., Tucson, AZ). Positive controls were used consisting of CRC specimens previously demonstrated to stain with this antibody. Tris-buffered saline in place of the primary antibody was utilized as a negative control.

Interpretation of immunohistochemical staining

PAK-1 positivity was considered when a yellow to brown staining was found in tumour cell cytoplasm and/or nuclei. Immunostaining was evaluated semiquantitatively according to staining intensity on a scale from 0 to 3, with grade 0: negative, 1: weak, 2: moderate and 3: intense. When dichotomized for statistical analysis; negative (-) and weak (+) staining were identified as low expression, while moderate (++) and intense (+++) staining were included in high expression category [13]. Two pathologists (EE and DQ) scored immunostaining independent of clinicopathological data.

Statistical analysis

Difference between two groups of patients was tested by using Mann Whitney test. To test association procedure in three groups of patients on one independent variable the Kruskal Wallis test was used. Non-parametric chi-square was used to test variance along one variable. Multivariate logistic regression analysis was used to predict lymph node metastasis, disease recurrence, lymphovascular invasion, and distant metastasis in relation immunoexpression of PAK-1. Estimated odds ratio (exponential [16]), 95% confidence interval (CI) for exp (B), and significance denoted for each analysis. Tumour stages were dichotomised in two categories; category 1 (Stage 1+2)=superficial invasion and category 2 (Stage 3+4)=deep invasion. The Kaplan-Meier strategy was uti-

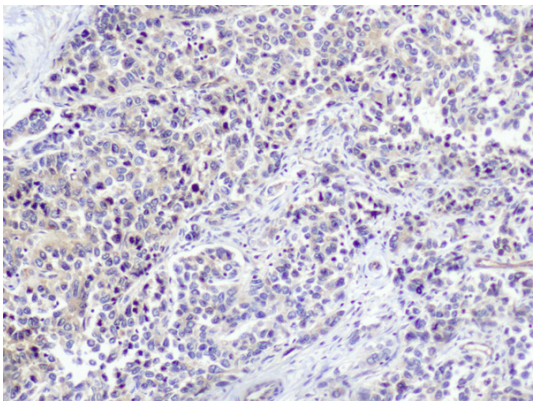

Figure 3. Poorly differentiated colorectal carcinoma showing weak (+1) diffuse cytoplasmic expression pattern for PAK-1 (100×).

Table 3. Relation of PAK-1 immunoexpression to clinicopathological parameters

Parameter	*P* value
Age	0.625✪
Sex	0.196✪
Grade	0.826●
Tumour location	0.686●
Tumour size	0.714✪
Depth of invasion (pT)	0.371●
Nodal metastasis	0.878●
Distant metastasis	0.611✪
Lymphovascular invasion	0.235✪
Margin status	0.462✪
Recurrence	0.03✪

●Kruskal-Wallis Test; ✪Mann-Whitney test.

lised to ascertain the survival probabilities where the Log Rank test was used to look at the contrast between survivals. The end-point for patients was death from tumour (disease-free). Disease-free survival (DFS) was calculated as the time from diagnosis to the appearance of recurrent disease (or date last seen disease-free). Statistical procedures were performed using SPSS Release 16.0. Statistical significance was determined at *P* value of ≤0.05 and was 2-sided.

Results

PAK-1 immunoexpression profile

Homogenous yellow-brown PAK-1 immunostaining was shown exclusively in the cytoplasm of malignant cells and in the occasional inflammatory cells. Interestingly, no nuclear staining was noted. Seventy four CRCs (71.8%) showed high PAK-1 expression while 29 CRCs (28.2%) showed low PAK-1 expression; PAK-1 was overexpressed in primary tumours ($P<0.001$). In nodal metastasis, there was no distinction between low and high expression ($P=0.139$). There was no statistically significant difference between PAK-1 immunoexpression in primary and nodal metastasis ($P=0.275$) (**Table 2**). **Figures 1**-**3** demonstrate the immunostaining expression patterns of PAK-1 protein in different primary tumours.

Relationship between PAK-1 expression and clinicopathological features of CRCs

There was no significant association between PAK-1 immuno expression and age, sex, degree of differentiation, depth of tumour invasion, stage, nodal metastasis, lymphovascular invasion, status of surgical resection margins and distant metastasis. However, overexpression of PAK-1 in CRCs is associated with disease recurrence ($P=0.03$) (**Table 3**).

Survival analysis

In Kaplan-Meier survival analysis, at 60 month (5 years) follow up time for example, **Figure 4** showed that PAK-1 expression patterns revealed an association with disease recurrence in that 50% of patients who developed tumor recurrence had high PAK-1 expression pattern of original tumor, as contrasted to only 20% patients who experienced disease recurrence had low PAK-1 expression pattern. In other words, there was a clear trend of difference in DFS between patients with tumors with low PAK-1 expression patterns (longer DFS) and those tumors with high PAK-1 expression patterns (shorter DFS), even though it was not statistically significant ($P<0.2$, log rank).

Relationship between PAK-1 expression and prognosis

Regression analysis for cytoplasmic immunoexpression revealed that PAK-1 overexpression was an independent predictor of disease recurrence ($P=0.05$) (**Table 4**). PAK-1 immunoexpression was not related to survival (log-rank =1.287, $P=0.257$) (**Figure 4**).

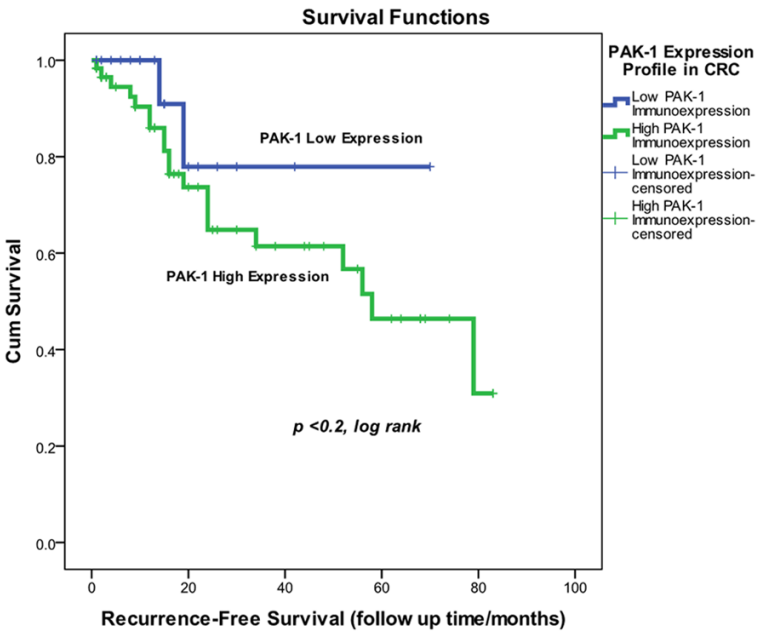

Figure 4. Kaplan-Meier curve showing the recurrence free survival outcomes of patients with low and high PAK-1 expression patterns (log-rank=1.16, *P*=0.28).

Table 4. Regression analysis for cytoplasmic PAK-1 immunoexpression

Variable	Exp (B)	95% CI for exp (B)	*P* value
Nodal Metastasis	1.180	0.448-3.106	0.738
Distant metastasis	1.223	0.448-3.338	0.694
Tumour invasion	0.614	0.182-2.079	0.434
Lymphovascular invasion	0.606	0.147-2.491	0.487
Margin status	2.567	0.299-22.035	0.390
Recurrence	0.269	0.072-1.006	0.05

Discussion

CRC arises and progresses as a result of cumulative genetic and epigenetic changes in tumour cells [17]. Detection of novel prognostic molecules involved in CRC is required to develop new targeted therapy for CRC. PAK-1 is claimed to be implicated as central player in transformation of normal cell into a malignant one through diverse mechanisms including alteration of cell motility, induction of abnormal cell proliferation and escape from apoptotic signals [3-6, 18]. PAK-1 was found to be overexpressed in a wide variety of cancers including; brain [19], breast [20], liver [21], kidney [22], bladder [23], lung [9], ovary [24], and T-cell lymphoma [25]. Previous reports adopted PAK-1 as a promising potential therapeutic agent in thyroid carcinoma, melanoma and gastric carcinoma [26-28].

In CRC, expression of PAK1 increases with progression through the adenoma to carcinoma sequence, with the most dramatic increases in invasive and metastatic CRC [29]. In the present cohort, the aim was to explore the relationship between PAK-1 overexpression and CRC progression and its validity as independent prognostic factor. PAK-1 was found to be overexpressed in CRCs. This finding is comparable to previous studies [12, 29-31]. In the current study, there was no statistically significant difference between PAK-1 expression in primary tumours and lymph node deposits. This means that PAK-1 may be involved in the progression of nodal metastasis in the same way like in primary tumour. This notion may be supported when knockdown of PAK1 controls growth and metastasis of CRC cell lines and mice models with liver metastasis [32]. In the current study, PAK-1 overexpression is significantly associated with disease recurrence. This further supports the suggested role of PAK-1 in tumour cells proliferation, survival, and migration. In vitro evidence came from PAK-1 gene transfection which increases PAK-1 expression in SW480 cell line and augmented the invasiveness and metastasis of CRC cells [33].

On the other hand, there is no significant association with other clinicopathological factors. This finding is contradictory to those of Li et al. who reported that PAK-1 activity is associated with depth of invasion, lymph node metastasis, distant metastasis, tumour grade and tumour stage [34].

In the present study, overexpression of PAK-1 is found to be an independent predictor of disease recurrence. Comparably, Carter [29] and

others [35, 36] concluded that PAK-1 expression is significantly increasing with tumour progression and metastasis supporting its functional role in CRC invasiveness and motility. In the current study, there was a clear trend of association, even though it was not statistically significant, between PAK-1 overexpression in primary CRCs and patients' survival i.e., in other words, there was a clear drift of difference in DFS between patients with tumors with low PAK-1 expression patterns (longer DFS) and those patients with tumors of high PAK-1 expression patterns (shorter DFS). This is in conformity with study by Li, L.H., et al. [34] who showed the association between PAK-1 expression and phosphorylation with patient's survival.

Previous reports supported some molecular mechanisms involving PAK-1 role in CRC pathogenesis. PAK-1 is implicated in proliferation, survival, migration, and VEGF secretion of CRC cells which had Ras, PI3K, and Apc mutations. Also, PAK-1 knockdown suppressed growth, survival and migration of CRC cell lines through inactivation of ERK and AKT, the downstream targets of Ras [35, 37-39]. This observation is consistent with the report by Li and co-workers that PAK-1 regulates CRC metastasis through ERK-dependent phosphorylation of FAK [34]. PAK-1 was found to have a stimulatory effect on HIF-1a expression and VEGF secretion by CRC cells in response to hypoxia provide a molecular basis for the stimulation by PAK1 of CRC survival and metastasis [40]. Others found that of PAK-1 downregulation in CRC cell lines reduced beta-catenin levels and cell proliferation via directly phosphorylated beta-catenin [12]. According to the current findings, PAK-1 is an expected as a target for therapy of CRC. Some PAK-1 inhibitors are being tried. Pitts et al., established that this novel agent demonstrates activity against preclinical CRC models cell lines with a range of molecular aberrations [41]. This PAK inhibited CRC cell lines by inhibiting proliferation and migration.

Limitations of the study include the lack of tissues from normal and dysplastic colonic mucosa and short survival period of latest patients included in the study.

Conclusion

In Conclusion, our observations along with previous data supported that PAK-1 overexpression is involved in CRC invasiveness and disease recurrence. PAK-1 overexpression was also an independent prognostic predictor of disease recurrence. Subsequently, targeting PAK-1 as therapy in CRC is promising. Further studies are needed to investigate molecular mechanistic role of PAK-1 in human tumorigenesis. Also testing PAK-1 inhibitors as potential therapeutic target for interruption of tumour cell proliferation and invasion is required to be investigated intensively.

Acknowledgements

This project was funded by the National Plan for Science, Technology and Innovation (MAARIFAH) - King Abdulaziz City for Science and Technology - the Kingdom of Saudi Arabia - award number (11-BIO1524-03). The authors also, acknowledge with thanks Science and Technology Unit, King Abdulaziz University for technical support.

Disclosure of conflict of interest

None.

Address correspondence to: Dr. Jaudah Al-Maghrabi, Department of Pathology, King Abdulaziz University, Jeddah 21589, Saudi Arabia. Tel: +9662 6401000 Ext. 17069; Fax: +966 2 6401000 Ext. 17223; E-mail: jalmaghrabi@hotmail.com

References

[1] Manser E, Leung T, Salihuddin H, Zhao ZS and Lim L. A brain serine/threonine protein kinase activated by Cdc42 and Rac1. Nature 1994; 367: 40-46.
[2] Arias-Romero LE and Chernoff J. A tale of two Paks. Biol Cell 2008; 100: 97-108.
[3] Molli PR, Li DQ, Murray BW, Rayala SK and Kumar R. PAK signaling in oncogenesis. Oncogene 2009; 28: 2545-2555.
[4] Bokoch GM. Biology of the p21-activated kinases. Annu Rev Biochem 2003; 72: 743-781.
[5] Dummler B, Ohshiro K, Kumar R and Field J. Pak protein kinases and their role in cancer. Cancer Metastasis Rev 2009; 28: 51-63.
[6] Shrestha Y, Schafer EJ, Boehm JS, Thomas SR, He F, Du J, Wang S, Barretina J, Weir BA, Zhao JJ, Polyak K, Golub TR, Beroukhim R and Hahn WC. PAK1 is a breast cancer oncogene that coordinately activates MAPK and MET signaling. Oncogene 2012; 31: 3397-3408.
[7] Ye DZ and Field J. PAK signaling in cancer. Cell Logist 2012; 2: 105-116.

[8] Koh CG, Manser E, Zhao ZS, Ng CP and Lim L. Beta1PIX, the PAK-interacting exchange factor, requires localization via a coiled-coil region to promote microvillus-like structures and membrane ruffles. J Cell Sci 2001; 114: 4239-4251.

[9] Ong CC, Jubb AM, Haverty PM, Zhou W, Tran V, Truong T, Turley H, O'Brien T, Vucic D, Harris AL, Belvin M, Friedman LS, Blackwood EM, Koeppen H and Hoeflich KP. Targeting p21-activated kinase 1 (PAK1) to induce apoptosis of tumor cells. Proc Natl Acad Sci U S A 2011; 108: 7177-7182.

[10] McCarty SK, Saji M, Zhang X, Jarjoura D, Fusco A, Vasko VV and Ringel MD. Group I p21-activated kinases regulate thyroid cancer cell migration and are overexpressed and activated in thyroid cancer invasion. Endocr Relat Cancer 2010; 17: 989-999.

[11] Siu MK, Wong ES, Chan HY, Kong DS, Woo NW, Tam KF, Ngan HY, Chan QK, Chan DC, Chan KY and Cheung AN. Differential expression and phosphorylation of Pak1 and Pak2 in ovarian cancer: effects on prognosis and cell invasion. Int J Cancer 2010; 127: 21-31.

[12] Zhu G, Wang Y, Huang B, Liang J, Ding Y, Xu A and Wu W. A Rac1/PAK1 cascade controls beta-catenin activation in colon cancer cells. Oncogene 2012; 31: 1001-1012.

[13] Balasenthil S, Sahin AA, Barnes CJ, Wang RA, Pestell RG, Vadlamudi RK and Kumar R. p21-activated kinase-1 signaling mediates cyclin D1 expression in mammary epithelial and cancer cells. J Biol Chem 2004; 279: 1422-1428.

[14] Arias-Romero LE, Villamar-Cruz O, Huang M, Hoeflich KP and Chernoff J. Pak1 kinase links ErbB2 to beta-catenin in transformation of breast epithelial cells. Cancer Res 2013; 73: 3671-3682.

[15] Edge S, Byrd D, Compton C and al. e. AJCC Cancer Staging Handbook. New York: Springer; 2010.

[16] NIH consensus conference. Adjuvant therapy for patients with colon and rectal cancer. JAMA 1990; 264: 1444-1450.

[17] He H and Baldwin GS. p21-activated kinases and gastrointestinal cancer. Biochim Biophys Acta 2013; 1833: 33-39.

[18] Zhou GL, Zhuo Y, King CC, Fryer BH, Bokoch GM and Field J. Akt phosphorylation of serine 21 on Pak1 modulates Nck binding and cell migration. Mol Cell Biol 2003; 23: 8058-8069.

[19] Aoki H, Yokoyama T, Fujiwara K, Tari AM, Sawaya R, Suki D, Hess KR, Aldape KD, Kondo S, Kumar R and Kondo Y. Phosphorylated Pak1 level in the cytoplasm correlates with shorter survival time in patients with glioblastoma. Clin Cancer Res 2007; 13: 6603-6609.

[20] Bostner J, Ahnstrom Waltersson M, Fornander T, Skoog L, Nordenskjold B and Stal O. Amplification of CCND1 and PAK1 as predictors of recurrence and tamoxifen resistance in postmenopausal breast cancer. Oncogene 2007; 26: 6997-7005.

[21] Tse EY and Ching YP. The role of p21-activated kinases in hepatocellular carcinoma metastasis. J Mol Signal 2014; 9: 7.

[22] O'Sullivan GC, Tangney M, Casey G, Ambrose M, Houston A and Barry OP. Modulation of p21-activated kinase 1 alters the behavior of renal cell carcinoma. Int J Cancer 2007; 121: 1930-1940.

[23] Ito M, Nishiyama H, Kawanishi H, Matsui S, Guilford P, Reeve A and Ogawa O. P21-activated kinase 1: a new molecular marker for intravesical recurrence after transurethral resection of bladder cancer. J Urol 2007; 178: 1073-1079.

[24] Brown LA, Kalloger SE, Miller MA, Shih Ie M, McKinney SE, Santos JL, Swenerton K, Spellman PT, Gray J, Gilks CB and Huntsman DG. Amplification of 11q13 in ovarian carcinoma. Genes Chromosomes Cancer 2008; 47: 481-489.

[25] Mao X, Onadim Z, Price EA, Child F, Lillington DM, Russell-Jones R, Young BD and Whittaker S. Genomic alterations in blastic natural killer/extranodal natural killer-like T cell lymphoma with cutaneous involvement. J Invest Dermatol 2003; 121: 618-627.

[26] Liu F, Li X, Wang C, Cai X, Du Z, Xu H and 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.

[27] Ma Y, McCarty SK, Kapuriya NP, Brendel VJ, Wang C, Zhang X, Jarjoura D, Saji M, Chen CS and Ringel MD. Development of p21 activated kinase-targeted multikinase inhibitors that inhibit thyroid cancer cell migration. J Clin Endocrinol Metab 2013; 98: E1314-1322.

[28] Ong CC, Jubb AM, Jakubiak D, Zhou W, Rudolph J, Haverty PM, Kowanetz M, Yan Y, Tremayne J, Lisle R, Harris AL, Friedman LS, Belvin M, Middleton MR, Blackwood EM, Koeppen H and Hoeflich KP. P21-activated kinase 1 (PAK1) as a therapeutic target in BRAF wild-type melanoma. J Natl Cancer Inst 2013; 105: 606-607.

[29] Carter JH, Douglass LE, Deddens JA, Colligan BM, Bhatt TR, Pemberton JO, Konicek S, Hom J, Marshall M and Graff JR. Pak-1 expression increases with progression of colorectal carcinomas to metastasis. Clin Cancer Res 2004; 10: 3448-3456.

[30] Liu Y, Xiao H, Tian Y, Nekrasova T, Hao X, Lee HJ, Suh N, Yang CS and 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.

[31] 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 and Velculescu VE. Colorectal cancer: mutations in a signalling pathway. Nature 2005; 436: 792.

[32] He H, Huynh N, Liu KH, Malcontenti-Wilson C, Zhu J, Christophi C, Shulkes A and Baldwin GS. P-21 activated kinase 1 knockdown inhibits beta-catenin signalling and blocks colorectal cancer growth. Cancer Lett 2011; 317: 65-71.

[33] Wu JB, Han YJ, Nan QZ, Zhang ZS, Zhang HQ and Song YG. Effect of p21-activated kinase-1 on the invasiveness of colorectal carcinoma cells in vitro. Nan Fang Yi Ke Da Xue Xue Bao 2009; 29: 1341-1343.

[34] Li LH, Zheng MH, Luo Q, Ye Q, Feng B, Lu AG, Wang ML, Chen XH, Su LP and Liu BY. P21-activated protein kinase 1 induces colorectal cancer metastasis involving ERK activation and phosphorylation of FAK at Ser-910. Int J Oncol 2010; 37: 951-962.

[35] Huynh N, Liu KH, Baldwin GS and He H. P21-activated kinase 1 stimulates colon cancer cell growth and migration/invasion via ERK- and AKT-dependent pathways. Biochim Biophys Acta 2010; 1803: 1106-1113.

[36] Li LH, Luo Q, Zheng MH, Pan C, Wu GY, Lu YZ, Feng B, Chen XH and Liu BY. P21-activated protein kinase 1 is overexpressed in gastric cancer and induces cancer metastasis. Oncol Rep 2012; 27: 1435-1442.

[37] Chow HY, Jubb AM, Koch JN, Jaffer ZM, Stepanova D, Campbell DA, Duron SG, O'Farrell M, Cai KQ, Klein-Szanto AJ, Gutkind JS, Hoeflich KP and Chernoff J. p21-Activated kinase 1 is required for efficient tumor formation and progression in a Ras-mediated skin cancer model. Cancer Res 2012; 72: 5966-5975.

[38] Fransen K, Klintenas M, Osterstrom A, Dimberg J, Monstein HJ and Soderkvist P. Mutation analysis of the BRAF, ARAF and RAF-1 genes in human colorectal adenocarcinomas. Carcinogenesis 2004; 25: 527-533.

[39] Yuen ST, Davies H, Chan TL, Ho JW, Bignell GR, Cox C, Stephens P, Edkins S, Tsui WW, Chan AS, Futreal PA, Stratton MR, Wooster R and Leung SY. Similarity of the phenotypic patterns associated with BRAF and KRAS mutations in colorectal neoplasia. Cancer Res 2002; 62: 6451-6455.

[40] Liu KH, Huynh N, Patel O, Shulkes A, Baldwin G and He H. P21-activated kinase 1 promotes colorectal cancer survival by up-regulation of hypoxia-inducible factor-1alpha. Cancer Lett 2013; 340: 22-29.

[41] Pitts TM, Kulikowski GN, Tan AC, Murray BW, Arcaroli JJ, Tentler JJ, Spreafico A, Selby HM, Kachaeva MI, McPhillips KL, Britt BC, Bradshaw-Pierce EL, Messersmith WA, Varella-Garcia M and Eckhardt SG. Association of the epithelial-to-mesenchymal transition phenotype with responsiveness to the p21-activated kinase inhibitor, PF-3758309, in colon cancer models. Front Pharmacol 2013; 4: 35.