P21 activated kinase 4 binds translation elongation factor eEF1A1 to promote gastric cancer cell migration and invasion

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Abstract. P21 activated kinase 4 (PAK4), as an effector of Cdc42, playing important roles in regulating the processes of cytoskeleton organization. PAK4 has been considered to be an oncogenic protein, which has strong relationship with gastric cancer metastasis. However, the mechanism of PAK4 in regulating gastric cancer metastasis is still not fully understood. In this study, using yeast two-hybrid system, we identified that the eukaryotic elongation factor 1 α1 (eEF1A1) is a new binding partner of PAK4. The interaction between PAK4 and eEF1A1 was confirmed by GST pull-down and co-immunoprecipitation. PAK4 co-localized with eEF1A1 in the cytoplasm of gastric cancer cells. Overexpression of PAK4 enhanced the expression level of eEF1A1 and vice versa. PAK4 and eEF1A1 could cooperate to promote gastric cancer cell migration and invasion. Furthermore, the expression of PAK4 and eEF1A1 in clinical gastric cancer samples were examined by western blotting and immunohistochemistry. Statistical analysis indicated that there was positive correlation between the expression of PAK4 and eEF1A1. This study demonstrated for the first time that PAK4 interacted with eEF1A1 to promote migration and invasion of gastric cancer cells, thereby providing new insights into the function of PAK4 and eEF1A1 in the progression of gastric cancer.

Introduction

Gastric cancer is the fifth most common cancer and the third leading cause of cancer-related death worldwide (1). Despite advances in surgery and chemotherapy, the 5-year survival rate is not more than 30% (2). The overall poor outcome of gastric cancer patients is largely because the patients are diagnosed at an advanced stages with distance metastasis. In recent years, with the understanding of molecular profiling of gastric cancer, some targeted therapies have been developed (3). However, it is still necessary to find new molecular targets in gastric cancer for the development of anticancer drugs to improve the overall survival of gastric cancer patients.

P21-activated kinases (PAKs) are series of serine/threonine kinases which were first identified as the downstream effectors of the small Rho GTPase Cdc42 and Rac1 (4). In mammalians, there are six members of PAK family proteins which are classified into two groups, group I PAKs (PAKs1-3) and group II PAKs (PAKs4-6), based on their amino acid sequences and functions (5). PAK4 is the most prevalently investigated and a representative member of group II PAKs. It was identified as Cdc42 effector that regulated filopodia protrusion (6). In addition to its functions in regulating cytoskeleton, PAK4 has important roles in promoting anchorage-independent growth, inhibiting cell adhesion, controlling cell cycle and protecting cells from apoptosis (7-10). High expression of PAK4 has been detected in several types of cancer cell lines including breast, ovarian, prostate, melanoma and lung (7). The study also demonstrated that PAK4 level was increased in gastric adenocarcinomas, which was correlated with the depth of cancer invasion and distant metastasis (11). A great deal of effort has been made to identify the downstream effectors of PAK4 in mediating gastric cancer metastasis. Our previous studies have shown that PAK4 interacted with DGCR6L or SCG10 and promoted gastric cancer migration and invasion (12,13). However, the function of PAK4 in cell motility is not yet fully understood. Therefore, exploring new binding partners of PAK4 will further illustrate the mechanism of PAK4 in mediating gastric cancer metastasis.

The eukaryotic elongation factor1A (eEF1A) is a component of polypeptide translation machinery (14). eEF1A binds with GTP and delivers aa-tRNA to the A-site of the ribosome during the protein translation elongation process (15). There are two isoforms of eEF1A: eEF1A1 and eEF1A2, which share 96% homology at amino acid level (16). eEF1A1 has been mapped on chromosome 6p14 and expressed widely, while eEF1A2 has been mapped on chromosome 20q13.3 and expressed mainly in the neurol and muscle tissues. While eEF1A1 and eEF1A2 play the same roles in protein translation, their functions in other aspects are different (17). eEF1A1 could bind to F-actin or microtubule

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to influence cytoskeletal rearrangement (18,19), eEF1A1 also acts as a component of nuclear export machinery of proteins in mammalian cells (20). It participates in proteasome-mediated protein degradation through interacting with ubiquitinated proteins (21) and some unexpected roles of eEF1A1 is in facilitating viral replication (22). eEF1A2 is an oncoprotein (23). Its high expression has been found in many cancer tissues of the breast, ovary, and lung (24-26). eEF1A2 was upregulated in gastric cancer and was an independent indicator to predict poor prognosis in patients with gastric cancer (27), but the expression of eEF1A1 in normal and cancerous gastric tissues has not been investigated.

In the current study, using yeast two-hybrid system, eEF1A1 was identified as a new binding partner of PAK4. eEF1A1 and PAK4 could cooperate to promote the migration and invasion of gastric cancer cells. The expression of eEF1A1 and PAK4 was relatively high in gastric cancer tissues compared to the adjacent normal tissues. These findings provided new insights into the function link of PAK4 and eEF1A1 in the progression of gastric cancer.

Materials and methods

Plasmids and cell culture. The plasmid pCDNA3 eEF1A1 was kindly provided by Stuart M. Pitson and subcloned into pCDNA3.1myc/His A (Invitrogen, Carlsbad, CA, USA). The plasmids of PAK4 were constructed in our laboratory. HEK293, BGC823 and SGC7901 cells were used in our laboratory and cultured in Dulbecco's modified Eagle's medium (DMEM) (Gibco, Los Angeles, CA, USA) supplemented with 10% fetal bovine serum (FBS) and 100 U/ml penicillin and 100 U/ml streptomycin, at 37˚C in 5% CO_2 incubator. For transient transfection, the plasmids were transfected with Lipofectamine 2000 (Invitrogen), the cells were harvested for immunoblotting 24 h after transfection.

Yeast two-hybrid screen. Yeast two-hybrid screen was performed using Matchmaker system 3 (Clontech, Mountain View, CA, USA) following to the manufacturer's instructions. The bait was constructed with the C-terminal of PAK4 (326-572 amino acids) subcloned into pGBKT7 vector. A human fetal brain cDNA library and the bait PAK4 were transfected sequentially into the yeast strain AH109. The transformants were selected under strictly nutrient deficient media using growth media lacking adenine, histidine, leucine, and tryptophan. Specificity of the interaction was confirmed by transforming yeasts with pGADT7-T and pGBKT7-53 as a positive control and pGADT7-T and pGBKT7-lam as a negative control.

GST pull-down. GST and GST-tagged PAK4 were expressed and purified from *E. coli* BL21 with GSH sepharose beads. pCDNA3.1myc/His A eEF1A1 (1 μg) were transcripted and translated in 50 μl of TNT-coupled transcripted and translated system (Promega, Madison, WI, USA). An aliquote of 20 μl reaction mixture was used for each GST pull-down assay. *In vitro* transcripted and translated myc-tagged eEF1A1 were incubated with equal amounts (10 μg) of GST or GST-tagged PAK4 at 4˚C for 2 h, and then washed three times with binding buffer (20 mM Tris-HCl, pH 7.5, 50 mM NaCl, 1% NP40 and 10% glycerol). Proteins were eluted by 2X SDS loading buffer, and separated by SDS-PAGE. The binding proteins were detected by western blotting with anti-Myc antibody.

Co-immunoprecipitation and western blotting. HEK293 cells were collected and lysed in 500 μl IP lysis buffer (25 mM Tris-HCl, pH 7.6, 150 mM NaCl, 1% NP-40, 1 mM EDTA), supplemented with proteinase and phosphatase inhibitors (2 mM dithiothreitol, 1 mM phenylmethylsulfonyl fluoride, 10 μg/ml leupeptin, 10 μg/ml aprotinin, 20 mM glycerophosphate, 1 mM Na_3VO_4). The whole cell extracts were incubated with anti-PAK4 antibody or normal IgG at 4˚C overnight. Then 50 μl 50% (v:v) of the Protein A Sepharose™ CL-4B (GE Healthcare Life Sciences, Uppsala, Sweden) beads were added for capturing the immunocomplex at 4˚C for 3 h. The beads were washed three times with IP lysis buffer and detected by western blotting.

Cells or tissue samples were washed with PBS twice and lysed with RIPA lysis buffer supplemented with cocktail protease inhibitors. The proteins were separated in 10% SDS-PAGE separation gel and transferred to PVDF membrane. The antibodies used for detecting proteins were anti-eEF1A1 (Abcam, Cambridge, UK), anti-PAK4, anti-phospho-PAK4 (ser474) (Cell Signaling Technology, Beverly, MA, USA), anti-Myc (Santa Cruz Biotechnology, Dallas, TX, USA), anti-flag and anti-GAPDH (Shanghai Kangcheng Biotechnology Co., Ltd., Shanghai, China) antibodies.

Immunofluorescence. Immunofluorescence analysis was previously described (12). Images were taken under fluorescence microscope (Perkin-Elmer, Waltham, MA, USA).

Clinical samples. Thirty-nine pairs of fresh gastric cancer tissue samples and adjacent non-cancerous tissues were collected from patients who underwent sections of gastric tumor in the First Affiliated Hospital of China Medical University. These tissues were frozen immediately in liquid nitrogen and stored at -80˚C until extraction of proteins for western blot assay. The investigation conforms to the principles outlined in the Declaration of Helsinki. Multicentre ethics approval for data collection and tissue use was granted by the Human Research ethics committee of the hospital.

Immunohistochemistry. Formalin-fixed, paraffin-embedded tissues were cut into 5-μm sections. The slides were dewaxed and rehydrated in graded alcohol solutions. Antigen retrieval was performed and endogenous peroxidases was blocked by 3% hydrogen peroxidase in methanol for 30 min at room temperature. After 5% normal bovine serum albumin (BSA) blocking, the sections were incubated with 1:100 diluted rabbit polyclonal PAK4 antibody and 1:200 diluted rabbit monoclonal eEF1A1 antibodies at 4˚C overnight. The sections were serially incubated with biotinylated anti-rabbit IgG and the super-sensitive streptavidin-biotin complex (SABC). Immune complexes were visualized by using 3, 3'-diaminobenzidine (DAB), and cell nucleus were counterstained with hematoxylin. For negative control experiments, we substituted phosphate buffer solution (PBS) for the primary antibody.

Transwell assay. To assess cell migration *in vitro*, the 24-well transwell chambers (Corning Costar, USA) with 8 μm pores

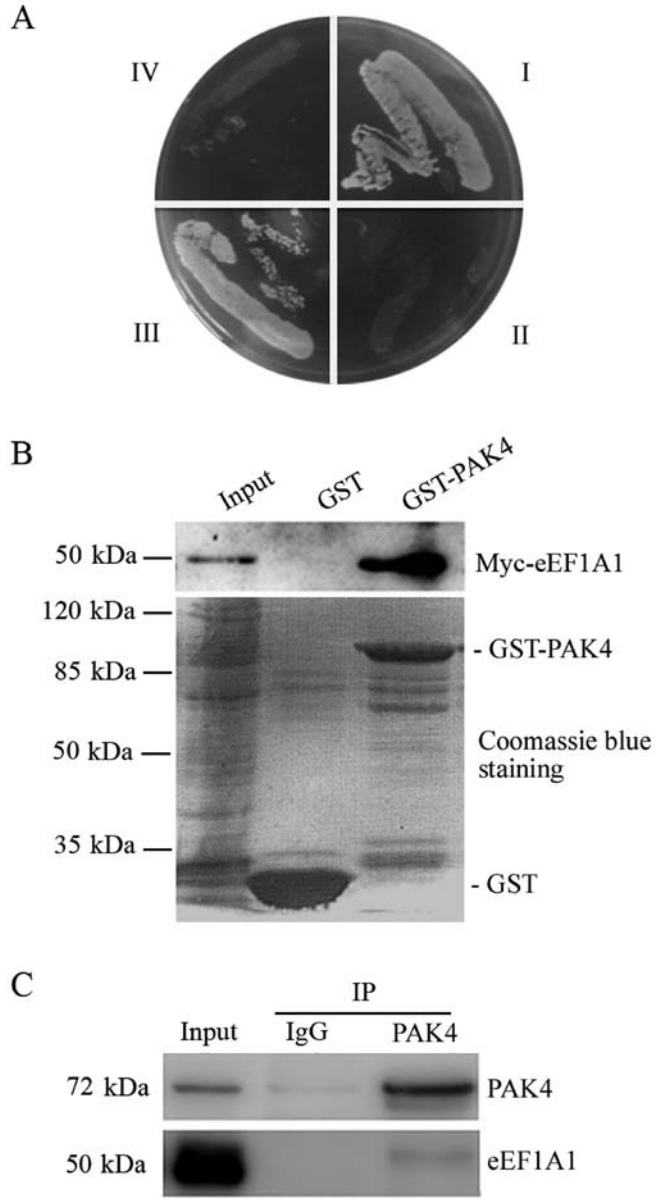

Figure 1. PAK4 interacts with eEF1A1 *in vitro* and *in vivo*. The plasmids of pGBKT7-PAK4C and pGADT7-eEF1A1 were co-transformed into yeast strain AH109 and selected on SD/-Trp/-Leu/-His/-Ade plate (III). Plasmid pGADT7-eEF1A1 and empty vector pGBKT7 were co-transformed into the yeast strain AH109 and selected on the plate SD/-Trp/-Leu/-His/-Ade (IV). pGBKT7-53 and pGADT7-T were co-transformed into yeast AH109 strain as a positive control (I). pGBKT7-lam and pGADT7-T were co-transformed into yeast AH109 strain as a negative control (II) (A). eEF1A1 directly bound GST-PAK4 *in vitro*. For GST pull-down assay, GST or GST-PAK4 fusion proteins were incubated with myc-tagged eEF1A1 transcripted and translated *in vitro*. Bound proteins were detected by western blotting with anti-Myc antibodies. Coomassie blue staining indicated the loading amount of GST or GST fusion proteins. The experiments were performed independently in triplicate (B). PAK4 interacted with eEF1A1 *in vivo*. Lysates from HEK293 cells were immunoprecipitated with the indicated antibodies or IgG. The precipitates were detected by western blotting. Three independent experiments were performed (C).

were used. Cells ($1x10^5$) diluted in 100 μl serum-free DMEM were added into each upper chambers, the lower chambers were filled with 600 μl DMEM supplemented with 10% FBS. The migrated cells were fixed in 75% ethanol after 16 h and stained by 0.4% typan blue. Five random fields per well were selected and counted under high power magnification. Three independent experiments were performed and the data are presented as mean ± SEM. The invasion assay was performed in the chamber pre-coated with matrigel to form a genuine reconstituted basement membrane, and then the same procedure as above was used.

Statistical analysis. All statistical analysis was performed using the SPSS (version 16.0) statistical software. Differences between two groups were assessed by Student's t-test. The Spearman Correlation analysis was used to measure the correlation between the expression of PAK4 and eEF1A1. The data are presented as mean ± SEM from three independent experiments. $P<0.05$ was considered to indicate a statistically significant difference.

Results

eEF1A1 is a new binding partner of PAK4. In order to understand the function of PAK4 in cancer cell metastasis, a yeast two-hybrid screen of a human fetal brain cDNA library with C-terminal of PAK4 (PAK4C) as bait was performed. After screening, a cDNA sequence that encoded for eEF1A1 was identified. To confirm the interaction between PAK4C and eEF1A1, full length of eEF1A1 was subcloned into pGADT7 vectors. Then eEF1A1 and PAK4C were co-transfected into yeast strain AH109. As shown in Fig. 1A, yeast strains AH109 grown on nutrient deficient plate indicated the interaction between PAK4 and eEF1A1. To further detect the interaction between eEF1A1 and PAK4, an *in vitro* GST pull-down assay was performed. GST-tagged PAK4 or GST proteins were incubated with myc-tagged eEF1A1 transcripted and translated *in vitro*. The results showed that eEF1A1 could interact with GST-tagged PAK4 (Fig. 1B). The interaction between PAK4 and eEF1A1 was then confirmed by co-immunoprecipitation. As shown in Fig. 1C, PAK4 but not an isotype-matched control IgG immunoprecipitated with endogenous eEF1A1. The above results indicated that eEF1A1 and PAK4 could interact with each other both *in vitro* and *in vivo*.

PAK4 and eEF1A1 co-localizes in the cytoplasm. To further clarify the localization and interaction of PAK4 with eEF1A1 in the cells, immunofluorescence analysis was performed. Gastric cancer SGC7901 cells were transfected with myc-tagged eEF1A1. Endogenous PAK4 was visualized under immunofluorescence microscope by anti-PAK4 antibody and Alexa 546 conjugated secondary antibody (red). Cells expressing myc-eEF1A1 were detected with anti-Myc antibody and Alexa 488 conjugated secondary antibody (green). The endogeneous PAK4 was located in cytoplasm and nucleus. eEF1A1 was mainly located in the cytosol. The co-localization of PAK4 with eEF1A1 (yellow) was largely located in the cytoplasmic region around the nucleus of the cells (Fig. 2). The data further indicated that PAK4 interacted with eEF1A1 *in vivo*.

PAK4 increases the expression of eEF1A1 independent of its kinase activity. In order to investigate whether there is relationship between eEF1A1 and PAK4 protein expression, different amounts of PAK4 were transfected into gastric cancer cells in a dose-dependent manner. The expression of

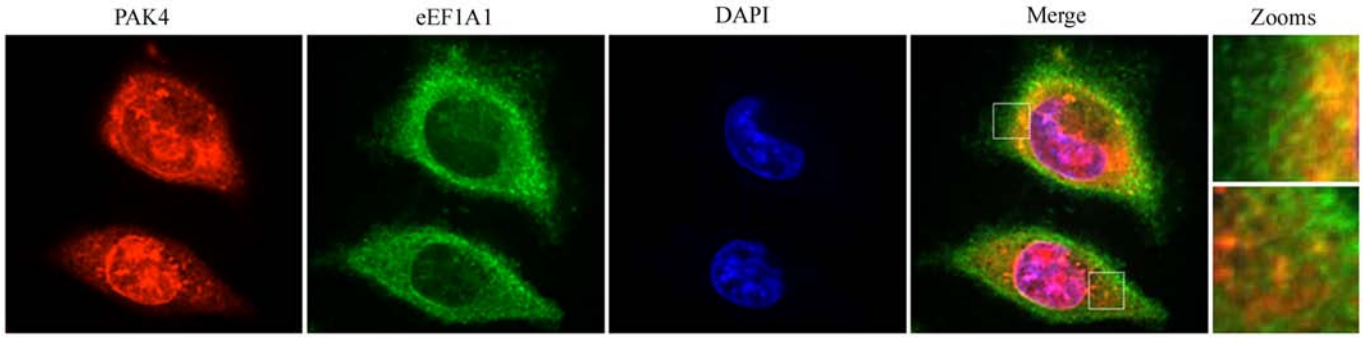

Figure 2. PAK4 co-localized with eEF1A1. SGC7901 cells were transfected with myc-tagged eEF1A1. Cells were fixed and subjected to immunofluorescence staining with antibody against PAK4 (red), myc-eEF1A1 (green) and nuclear staining with DAPI (blue), respectively. Yellow indicates co-localization. The white dot frames highlighted in merge image are zoomed to show details. Original magnification, x600.

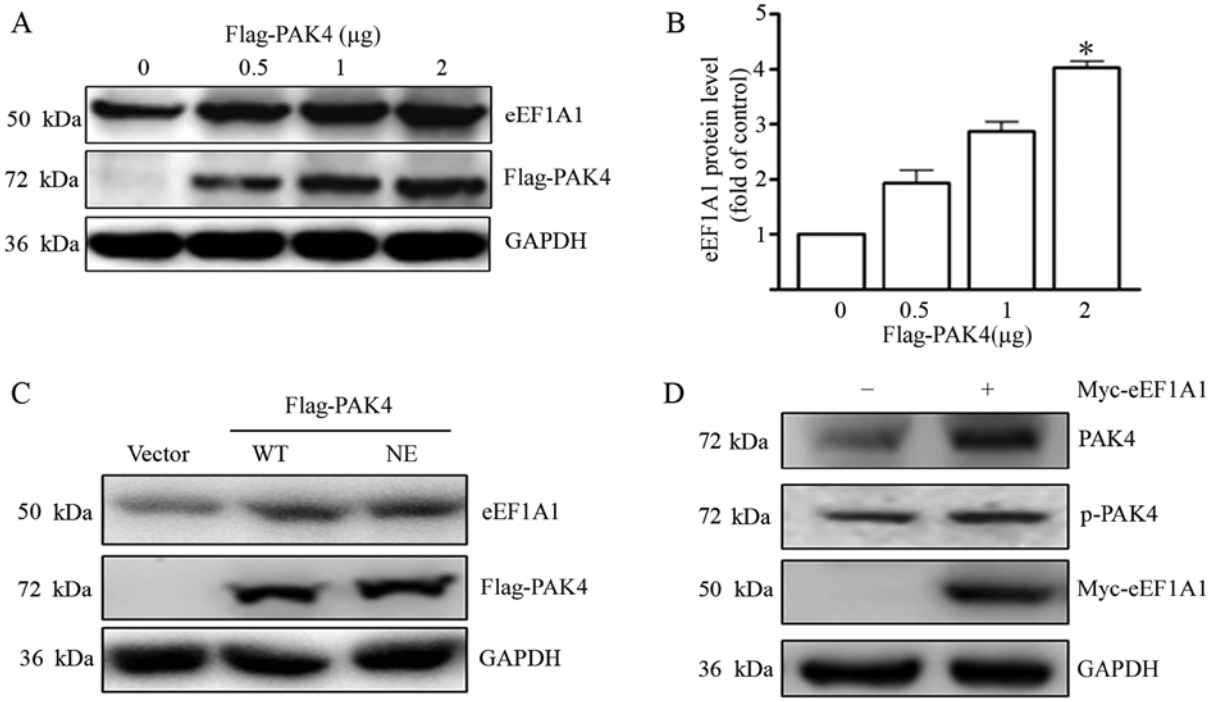

Figure 3. The regulation between PAK4 and eEF1A1. Flag-tagged PAK4 were transfected into BGC823 cells in a dose-dependent manner, and the expression of eEF1A1 was examined by western blotting (A). Quantification of eEF1A1 expression. Results were standardized to GAPDH and normalized to the values obtained from empty vector group. *P<0.05 versus the empty vector group. Data are mean ± SEM, n=3 (B). Expression of endogenous eEF1A1 was detected by western blotting (C). The expression of PAK4 and p-PAK4 were examined by western blotting (D).

endogenous eEF1A1 was examined by western blotting. As shown in Fig. 3A and B, overexpression of PAK4 significantly increased eEF1A1 expression in BGC823 cells. As a Ser/Thr kinase, PAK4 could phosphorylate downstream effectors and influence their activities. In order to detect whether PAK4 could phosphorylate eEF1A1, *in vitro* kinase assay was conducted. The autoradiography results showed that there was no obvious phosphorylation effect between PAK4 and eEF1A1 (data not shown). Then, we investigated the influence of PAK4 kinase activity on the expression of eEF1A1, the same amount of wild-type PAK4 (PAK4 WT) and the constitutively activated PAK4 (PAK4 NE) were transfected into BGC823 cells. Western blot analysis showed that the PAK4 NE could also elevate the expression of eEF1A1, but no more than PAK4 WT (Fig. 3C), which indicated that the upregulation effect of eEF1A1 by PAK4 was not dependent on the PAK4 kinase activity. Interestingly, when eEF1A1 were transfected into BGC823 cells, the endogenous PAK4 and p-PAK4 levels were increased accordingly (Fig. 3D). Therefore, it seemed that a positive regulation feedback loop was formed between PAK4 and eEF1A1.

eEF1A1 and PAK4 promotes the migration and invasion of gastric cancer cells. eEF1A1 was reported to be an actin binding protein and influenced the morphology of the cell, which implicated the function of eEF1A1 in the regulation of cell motility (17,28). In order to detect whether eEF1A1 and PAK4 could cooperate to induce the migration and invasion of gastric cancer cells, BGC823 cells were transiently transfected with PAK4 and/or eEF1A1, and transwell assays were performed. As shown in Fig. 4, overexpression of PAK4 or eEF1A1 improved the migration and invasion ability of

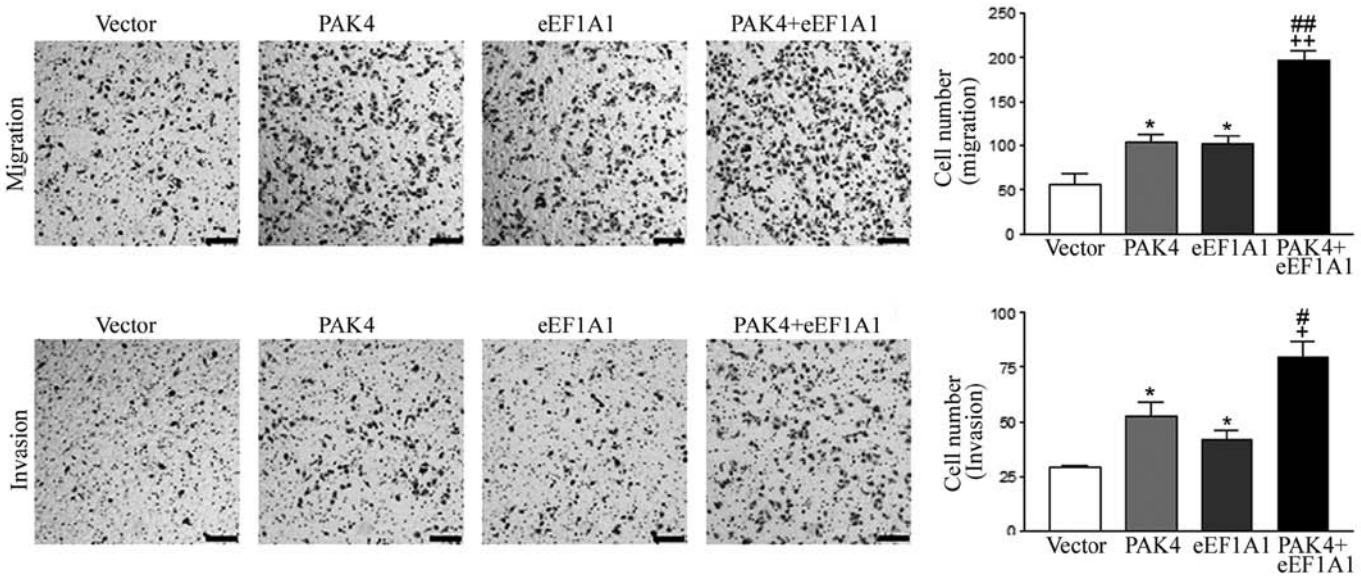

Figure 4. PAK4 and eEF1A1 promote the migration and invasion of gastric cancer cells. Gastric cancer BGC823 cells overexpression PAK4 and/or eEF1A1 were conducted with transwell migration and invasion assay. Representative images were taken under x100 original magnification. Scale bar=100 μm. *P<0.05 versus the empty vector group; $^{+}$P<0.05, $^{++}$P<0.01 versus the PAK4 group; $^{\#}$P<0.05, $^{\#\#}$P<0.01 versus the eEF1A1 group. Data are mean ± SEM, n=3.

BGC823 cells compared with empty vector groups. With co-transfection of eEF1A1 and PAK4, the migration and invasion ability of gastric cancer cells increased markedly compared with the cells transfected with either PAK4 or eEF1A1 alone. Therefore, PAK4 and eEF1A1 could cooperate to induce gastric cancer cell migration and invasion.

Expression of eEF1A1 and PAK4 are increased in human gastric cancer tissues. In order to confirm the correlation between PAK4 and eEF1A1 in human gastric cancer tissues, 39 pairs of clinical gastric cancer tissues and adjacent non-tumor tissues were collected and measured by western blotting. The representative results were shown in Fig. 5A. Compared with normal gastric tissues (N), the expression level of eEF1A1 was increased in gastric tumor tissues (T). The expression level of PAK4 in gastric tumor tissues (T) was also higher than that of adjacent normal tissues (N) (Fig. 5A and B). Furthermore, the immunohistochemistry was also applied for detection of the localization and the expression of PAK4 and eEF1A1 in gastric cancer tissues. PAK4 and eEF1A1 were mainly detected in the cytosol of gastric cancer tissues (Fig. 5C), which was consistent with the cell study (Fig. 2). Furthermore, the high expression of eEF1A1 was associated with the intense presence of PAK4 (Fig. 5C). The correlation between expression of PAK4 and eEF1A1 was analyzed. As shown in Fig. 5D, the expression of eEF1A1 was positively correlated with the expression of PAK4 in gastric cancer tissues (P<0.0001).

Discussion

Metastasis is a major cause of death among gastric cancer patients. Therefore, it is very important to understand the molecular mechanisms of this aggressive behavior during the progression of gastric cancers. In this study, we demonstrated for the first time that PAK4 could interact with eEF1A1. In human gastric cancers tissues, the expression of eEF1A1 and PAK4 were increased. The correlation analysis revealed that there was a positive correlation between eEF1A1 and PAK4. The cooperation of PAK4 and eEF1A1 could promote the migration and invasion of gastric cancer cells.

PAK4 was first discovered as a downstream effector of Cdc42. PAK4 is a multifunctional protein acting as a modulator of F-actin organization, filapodia formation, stress fiber dissolution and the process of cell motility (29). It is well known that PAK4 is overexpressed and hyperactivated in clinical samples of gastric cancers (30,31). PAK4 expression was positively correlated with gastric tumor invasion and lymph node metastasis (30). PAK4 could regulate cancer cell migration through different signaling pathways (32,33). As to gastric cancer cells, our recent work demonstrated that PAK4 could promote gastric cancer cell migration through binding with DGCR6L or phosphorylating SCG10 (12,13). Exploring PAK4's new interacting proteins would help us to understand its regulatory process on cancer cell migration and invasion.

In the current study, we chose the C-terminal of PAK4 as bait and the fetal brain cDNA library for the yeast two-hybrid experiment. The C-terminal portion of PAK4 contained kinase (catalytic) domain which could bind and phosphorylate downstream effectors. The N-terminal region of PAK4 contained autoinhibited domain which could suppress the kinase activity. The kinase domain of PAK4 had increased kinase activity when compared with the full-length protein (6). Thus, the C-terminal domain of PAK4 represented the highly activated form of the kinase. The fetal brain cDNA library was applied based on previous publications (12). The human fetal brain library contained cDNAs that encoded for the most abundant different types of proteins. It provided almost all the proteins for binding with PAK4, and improved the positive rate of the results in screening for interacted proteins.

By yeast two-hybrid screening method, we found that eEF1A1 was a new interacting protein of PAK4. The eukary-

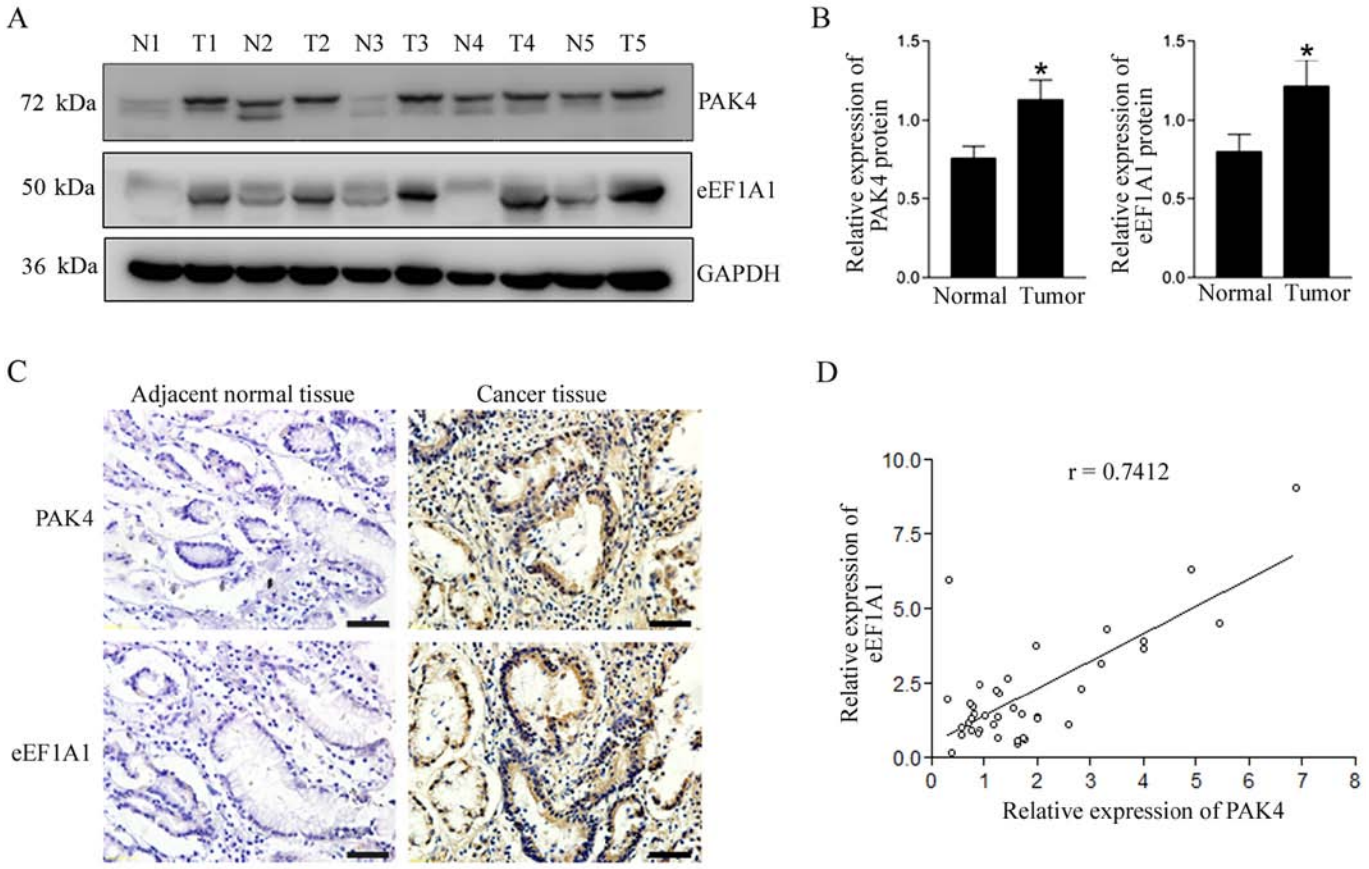

Figure 5. The expression of PAK4 and eEF1A1 in gastric cancer tissues. The expression levels of eEF1A1 and PAK4 in gastric tumor tissues (T) and matched adjacent non-cancerous tissues (N) were analyzed by western blotting and the representative results are shown (A). Quantification of PAK4 and eEF1A1 expression. Results were standardized to GAPDH. *P<0.05 versus the normal group (B). Representative images of immunohistochemical analysis of the expression of PAK4 and eEF1A1 in the adjacent normal gastric tissues and gastric cancer tissues, scale bar, 50 μm (C). Spearman analysis was used to analyze the correlation between relative expression of PAK4 and eEF1A1 (D).

otic translation elongation factor eEF1A1 was a component of polypeptide translation and elongation apparatus that delivered aa-tRNA to the ribosomes (14). Besides its role in facilitating the translation of newly synthesized mRNAs, eEF1A1 also played roles in cytoskeleton organization, viral replication, nuclear export of proteins and apoptosis (17). The interaction of PAK4 and eEF1A1 was confirmed by GST pull-down and co-immunoprecipitation assays *in vitro* and *in vivo*. Moreover, PAK4 and eEF1A1 were co-localized in the cytoplasm of gastric cancer cells in immunofluorescence analysis. The binding and site-directed mutation assays will be performed to further illustrate the binding motif of eEF1A1 and PAK4. eEF1A2, as one of the two isoforms of eEF1A, was an oncoprotein and overexpressed in various cancer types (17). eEF1A2 stimulated cancer cell migration and invasion through AKT-dependent manner (34). Moreover, eEF1A2 participated in phospholipid signaling pathway and regulated actin remodeling (35). eEF1A2 also was involved in cell proliferation, cell transformation and apoptosis inhibition (36). Due to the high sequence similarity of eEF1A1 and eEF1A2, we did not rule out the possibility that eEF1A2 was an interacting partner of PAK4. So besides eEF1A1, the mechanisms of interaction between PAK4 and eEF1A2 and need to be further investigated in future studies.

As a serine and threonine kinase, PAK4 exerted its influence on cellular events mainly through phosphorylating downstream substrates (37). PAK4 promoted liver cancer cell metastasis through phosphorylating p53 (38). PAK4 acted by phosphorylating Bad to inhibit apoptosis (39). On the other hand, it was reported that PAK4 could work as a scaffold protein that mediated protein-protein interaction through kinase-independent pathway (10,40). Our previous work demonstrated that PAK4 could bind Smad2/3 to promote gastric cancer growth through kinase denpendent or independent mechanism (41), and PAK4 directly bind to DGCR6L and β-actin to promote gastric metastasis, which did not rely on its kinase activity (12).

In our experiment, an *in vitro* kinase assay was performed to detect whether PAK4 could phosphorylate eEF1A1. However, the results of autoradiography showed that there was no obvious phosphorylation effect between PAK4 and eEF1A1. It could be that the eEF1A1 was not a direct substrate of PAK4, that PAK4 played its function through binding with eEF1A1. We further detected whether PAK4 could regulate the expression of eEF1A1, and found that PAK4 enhanced the expression of eEF1A1 protein independent of its kinase activity. This raises the possibility that PAK4 might bind to eEF1A1 and thereby prevent eEF1A1's degradation through inhibiting recruitment of eEF1A1 to the proteasomes or PAK4 might inhibit eEF1A1 unbiquitination and therefore inhibited its degradation. Of note, with eEF1A1 overexpression, PAK4

and p-PAK4 expression levels were also elevated. The above results suggested the formation of a positive feedback loop between PAK4 and eEF1A1.

Previous studies have demonstrated that eEF1A was an actin binding protein, and was essential for the cytoskeleton organization and cell morphology changes (28,42,43). These results suggested that eEF1A1 might be involved in the regulation of cell migration. To detect whether eEF1A1 participated in the process of migration and invasion in gastric cancer, transwell assay was performed. Our results demonstrated that the ability of migration and invasion of BGC823 cells was increased with overexpression of eEF1A1. Specifically, with PAK4 and eEF1A1 co-expression in gastric cancer cells, the migration and invasion ability of gastric cancer cells were markedly increased compared with the cells transfected with PAK4 or eEF1A1 alone. These results implied that the cooperation of PAK4 and eEF1A1 would further enhance the migration and invasion of gastric cancer cells. The mechanism on eEF1A1 increasing gastric cancer cell migration and invasion has not been reported yet. From the results of our experiments, we speculate that eEF1A1 might increase cell migration and invasion through directly binding with PAK4. However, the mechanism of their interaction in mediating cell migration and invasion remains to be further detected. The downstream effectors of PAK4, such as LIMK1 and cofilin, should be examined to confirm whether eEF1A1 participated in the PAK4 mediating cancer cell migration and invasion.

eEF1A1 is involved in oncogenic transformation. It has been reported that an eEF1A1 truncation encoded by PTI-1 genes has oncogenic roles in transformation of NIH3T3 cells (44). Several differentially expressed gene screening have identified eEF1A1 involved in many types of cancers, such as cervical squamous cell carcinomas, lung squamous-cell carcinoma and breast cancer (45-47). In addition, eEF1A1 expression was increased in metastatic rat mammary adenocarcinoma (48). However, the expression of eEF1A1 in gastric cancer tissue and its relationship with the progression of gastric cancer has not been reported.

In the current study, human gastric cancer tissues were collected and the expression of eEF1A1 and PAK4 were examined. Results showed that eEF1A1 and PAK4 were localized in cytoplasm and the expression increased in gastric cancers rather than in the corresponding normal gastric tissues. Furthermore, the correlation analysis revealed that there was a positive correlation between the expression of eEF1A1 and PAK4. However, further analysis of the correlation between the expression of eEF1A1 and PAK4 with the clinicopathologic characteristics of gastric cancer patients needed to be done when the size and number of clinical samples are expanded.

In summary, we demonstrated that eEF1A1 was a new binding partner of PAK4 in gastric cancer cells. This study provided a possible functional link between PAK4 and eEF1A1 in mediating gastric cancer cell migration and invasion. Targeting eEF1A1 and PAK4 might be a promising method in anticancer treatment of gastric cancer patients.

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