High expression of KIBRA in low atypical protein kinase C-expressing gastric cancer correlates with lymphatic invasion and poor prognosis

Yohei Yoshihama,[1,2] Yusuke Izumisawa,[3] Kazunori Akimoto,[1,4,8] Yoshinori Satoh,[1] Taichi Mizushima,[5] Kei Satoh,[3] Kazuhiro Chida,[2] Ryo Takagawa,[3] Hirotoshi Akiyama,[3] Yasushi Ichikawa,[3] Chikara Kunisaki,[3] Yoshiaki Inayama,[6] Itaru Endo,[3] Yoji Nagashima[7] and Shigeo Ohno[1,8]

[1]Department of Molecular Biology, Graduate School of Medical Science, Yokohama City University, Yokohama; [2]Department of Animal Resource Sciences, Graduate School of Agricultural and Life Sciences, The University of Tokyo, Tokyo; [3]Department of Gastroenterological Surgery, Graduate School of Medical Science, Yokohama City University, Yokohama; [4]Department of Molecular Medical Science, Faculty of Pharmaceutical Sciences, Tokyo University of Science, Chiba; [5]Department of Obstetrics and Gynecology, Graduate School of Medical Science; [6]Division of Anatomical and Surgical Pathology, [7]Deparment of Molecular Pathology, Graduate School of Medical Science, Yokohama City University, Yokohama, Japan

Overexpression of atypical protein kinase Cλ/ι (aPKCλ/ι), a regulator of cell polarity, is frequently associated with the poor prognoses of several cancers, including gastric cancer. Recent studies revealed a molecular link between aPKC and KIBRA, an upstream regulator of tumor suppressor Hippo pathway that regulates cell proliferation and apoptosis. Further, KIBRA directly inhibits the kinase activity of aPKC to regulate epithelial cell polarity. These observations suggest that the KIBRA-aPKC connection plays a role in cancer progression; however, clinical significance of the correlation between these factors remains unclear. Here we examined the correlation between KIBRA/aPKCλ/ι expression, as detected by immunohistochemistry, and clinicopathological outcomes in 164 gastric cancer patients using Fisher's exact test and Kaplan–Meier log-rank test. We found an intimate correlation between the expression level of KIBRA and aPKCλ/ι ($P = 0.012$). Furthermore, high expression of KIBRA is correlated with lymphatic ($P = 0.046$) and venous invasion ($P = 0.039$). The expression level of KIBRA by itself did not correlate with the prognosis; however, high expression of KIBRA in low aPKCλ/ι-expressing gastric cancer correlated with disease-specific ($P = 0.037$) and relapse-free survival ($P = 0.041$) by Kaplan–Meier with log-rank test and higher lymphatic invasion cases by Fisher's exact test ($P = 0.042$). Furthermore, overexpression of the aPKC-binding region of KIBRA disrupted tight junctions in epithelial cells. These results suggest that high expression of KIBRA in low aPKC-expressing cells causes massive loss of aPKC activity, leading to loss of polarity and invasiveness of gastric cancer cells. (*Cancer Sci* 2013; 104: 259–265)

Gastric cancer is the fourth most common cancer and the second highest cause of cancer-related death in the world.[1–4] Five-year survival rates for gastric cancer are approximately 20% in most areas of the world, and only 60% in Japan, where an extensive mass screening program has been implemented.[5] Although several combined chemotherapeutic regimens have been used for advanced and recurrent cases, their clinical outcomes are still unsatisfactory.[6,7] Therefore, new therapeutic targets, clinical markers and the establishment of more effective therapeutic strategies for gastric cancer are still urgently required.

Loss of epithelial cell polarity is one of the hallmarks of cancer, and recent studies have shown loss of polarity itself can cause malignancy.[8–12] Among the regulators of epithelial cell polarity, a serine/threonine kinase known as atypical protein kinase C (aPKC) plays pivotal roles in the establishment of epithelial cell polarity binding with several partner proteins such as partitioning defective proteins (PARs).[13,14] Overexpression of aPKCλ/ι is observed in several malignancies, including non-small cell lung, ovarian, colon, breast and prostate cancers and glioma.[15–20] We previously reported that high expression of aPKCλ/ι has been suggested as a prognostic factor for recurrence of gastric cancer.[21] In contrast, downregulation of aPKCλ/ι has also been observed in several cancers.[16,22] Overactivation and/or inactivation of aPKCλ/ι can cause loss of polarity in several biological contexts,[23–25] suggesting that precise control of the amount or activity of aPKCλ/ι is crucial in the progression of a variety of cancers. The activity of aPKC is regulated by its binding partner proteins; however, little is known about the expression level of these in gastric cancer tissue.

We have recently found that one of the aPKC-binding partners, KIBRA,[26,27] regulates the activity of aPKC through competitive inhibition during development of epithelial cell polarity.[28] KIBRA has also been suggested as a potential tumor suppressor gene acting as an upstream regulator of the Hippo pathway in *Drosophila*.[29–31] Epigenetic inactivation of KIBRA is associated with B-cell acute lymphocytic leukemia cell lines.[32] However, the correlation between the expression level of KIBRA and cancer progression in cancer patients remains to be evaluated.

Here, we examined the expression levels of KIBRA in gastric cancer specimens, in which aPKCλ/ι-expression has already been examined.[21] We found an intimate correlation between the expression levels of KIBRA and aPKCλ/ι. Furthermore, high expression of KIBRA correlated with lymphovascular invasion. Although KIBRA expression alone did not correlate with prognosis, high expression of KIBRA in low aPKCλ/ι-expressing gastric cancer correlated with poor prognosis and marked lymphatic invasion cases. Furthermore, the overexpression of aPKC-binding region of KIBRA disrupted tight junction formation in epithelial cells. These results suggest that high expression of KIBRA in low aPKC-expressing cells causes massive loss of aPKC activity, leading to loss of polarity and enhanced invasive behavior in gastric cancer tissues.

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[8]To whom correspondence should be addressed.
E-mails: akimoto@rs.tus.ac.jp; ohnos@med.yokohama-cu.ac.jp

Materials and Methods

Patients. This study included 164 patients who underwent resection of gastric cancer between January 1999 and December 2004 at the Department of Gastroenterological Surgery, Yokohama City University Hospital, Yokohama, Japan.[21] Patients who received preoperative chemotherapy were excluded from this study. All the patients were followed up after surgery at 3–6 month intervals, and were evaluated for disease recurrence by examining tumor markers, by endoscopy or by diagnostic imaging such as computed tomography, ultrasonography, and MRI. There were no patients who underwent endoscopic resection in this study. Follow-up data were obtained from the patients' medical records. The study protocol was approved by the Institutional Ethical Committee, Yokohama City University, and written informed consent was obtained from all the patients enrolled in this study.

Pathological diagnosis. For pathological diagnosis, 4-μm-thick formalin-fixed paraffin sections of the cases examined were stained with hematoxylin and eosin. Pathological diagnosis was performed by two experienced pathologists (Y. I. and Y.N.), according to World Health Organization guidelines.[33] Pathological tumor-node-metastasis (pTNM) stages were determined according to the International Union against Cancer (UICC).[34] The clinico-pathological features of patients enrolled in this study are summarized in Table 1. The patient group included 119 men and 45 women (aged 26–93 years, with a median age of 66 years and interquartile age range of 57–73 years). The maximum size of tumors ranged 15–195 mm, with a median of 48 mm. Ninety (55%) patients were classified as stage I,

26 (16%) as stage II, 26 (16%) as stage III, and 22 (13%) as stage IV disease.

Immunohistochemistry. Immunohistochemical staining for aPKCλ/ι and KIBRA were performed as described previously.[16] Four-micrometer-thick paraffin sections were deparaffinized, rehydrated, and then autoclaved in 10 mM citrate buffer (pH6.0) for 20 min at 121°C for antigen retrieval. After cooling, the slides were immersed in 0.3% H_2O_2 for 30 min at room temperature to quench endogenous peroxidase activity. After incubation with 10% normal goat serum in PBS, the sections were incubated with anti-mouse KIBRA antibody[27] (diluted to 1:500 with the Signal Stain Antibody Diluent [Cell Signaling Technology, Danvers, MA, USA]) at 4°C overnight. The labeled antigens were visualized with the HistFine kit (Nichirei, Tokyo, Japan) and 3, 3′-diaminobenzidine reaction. Sections were counterstained with hematoxylin and examined under a light microscope.

Evaluation of immunostaining. Two investigators (Y.I. and Y. N.) independently assessed the stained sections. The signal intensity for KIBRA was graded as 0–3 using a semi-quantitative scale: 0 representing no staining, 1 representing less intense signal than normal tissue staining, 2 representing moderate signal comparable to normal tissue staining, and 3 representing far more intense signal than in normal tissue staining. In statistical analysis, scores 0 and 1 were defined as negative, whereas 2 and 3 were positive. Sections stained for aPKCλ/ι and E-cadherin were assessed as previously described.[21]

Statistical analysis. Statistical analyses were performed using SPSS version 11.0 (SPSS Inc., Chicago, IL, USA) for Windows. Fisher's exact test was used to examine possible associations between the expression of KIBRA and clinicopathological parameters. Kaplan–Meier curves were plotted to assess the association of KIBRA expression with disease-specific and relapse-free survival rates. Survival curves were compared using the log-rank test. The data for "Depth of invasion" was categorized as pT1,2 / pT3,4 as well as "Stage" as I, II /III, IV as in the previous study[21] for comparison.

Cell culture and immunocytochemistry. Madin-Darby canine kidney II cells were obtained from Dr Shoichiro Tsukita (Kyoto University) and cultured in DMEM (Nissui, Tokyo, Japan) supplemented with 10% FBS (JRH Biosciences, Lenexa, KS, USA), 100 U/mL penicillin and streptomycin (Gibco, Grand Island, NY, USA) at 37°C/5% CO_2. Cells were transfected with pEB-CAGMCS-tagRFP (tRFP) or pEB-CAGMCS-tRFP-KIBRA-aBR (919–978).[28] Calcium switch assays were performed as described previously.[28] Cells in transwells (Costar Coning, Lowell, MA, USA) were fixed with 2% paraformaldehyde/PBS for 15 min, permeabilized with 0.5% Triton X-100/PBS for 10 min at room temperature, and stained with anti-*zonula occludens*-1 (ZO-1) antibody (Zymed Laboratories, San Francisco, CA, USA), and anti-tagRFP antibody (Evrogen, Moscow, Russia), as described previously.[25] F-actin was visualized with phalloidin conjugated with Alexa647 (Invitrogen, Carlsbad, CA, USA). RFP-positive cells that were not completely surrounded by ZO-1 staining were counted as cells with disrupted ZO-1 staining. Total RFP positive cells were also counted.

Results

Expression of KIBRA protein in gastric cancer tissue. We first performed immunohistochemical analysis for KIBRA using gastric cancer tissues previously analyzed for aPKCλ/ι expression.[21] In non-neoplastic gastric tissue, KIBRA was detected at the apical and cell–cell junctional regions (Fig. 1a). In epithelium with intestinal metaplasia, KIBRA was detected not only at apical but also in the lateral regions (Fig. 1b). Almost no signal was detected in the signet ring cells (Fig. 1c). In 164

Table 1. Clinicopathological features of patients enrolled in the study (*n* = 164)

| Pathologic factors	
Age, years (range)	65.9 (26–93)
Gender	
Male/female	119/45
Tumor diameter, mm (range)	48.0 (15–195)
Histological type	
Intestinal/diffuse	94/70
Depth of invasion	
pT1/pT2/pT3/pT4	65/59/29/11
Nodal metastasis	
Negative/positive	91/73
Distant metastasis	
Negative/positive	162/2
Lymphatic invasion	
Absent/positive	87/77
Venous invasion	
Absent/positive	102/62
pStage	
I/II/III/IV	90/26/26/22
Resectability	
R0/R1/R2	117/38/9
Adjuvant chemotherapy (R0, R1 patients)	
Negative/positive	116/48
Recurrence (R0, R1 patients)	
Negative/positive	117/47
aPKC expression	
low/high	50/114
E-cadherin expression	
Negative/positive	87/77
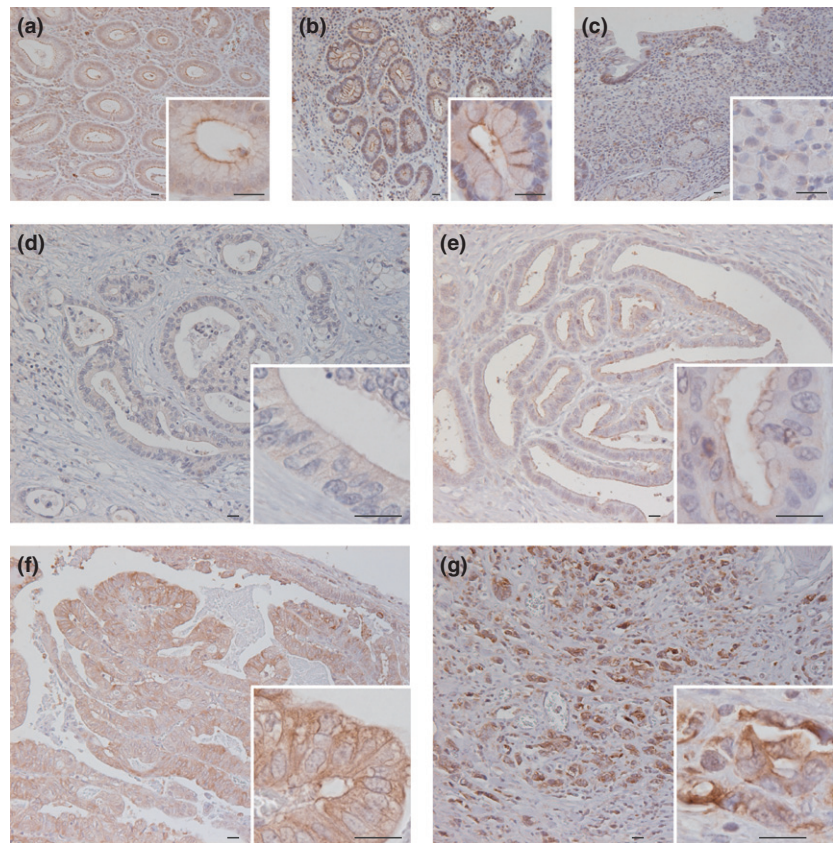
Fig. 1. Expression and localization of KIBRA in non-neoplastic and cancerous gastric tissues. Representative images of non-neoplastic and cancerous gastric tissues immunostained for KIBRA are shown: non-neoplastic epithelium (a), gastric epithelium with intestinal (b), signet ring cells (c), gastric cancer tissue diagnosed as 0 + (d), 1 + (e), 2 + (f), and 3 + (g). Bars: 20 μm.

gastric cancer cases, increased expression of KIBRA (to moderately or strongly positive levels) was detected in 109 cases (66.5%), and the signal was detected not only in apical and junctional regions but also in cytoplasmic regions (Fig. 1d–g).

Correlation between KIBRA expression and clinicopathological features of gastric cancer tissues. We next analyzed the association between KIBRA expression and clinicopathological features of gastric cancer tissues. As shown in Table 2, aPKCλ/ι expression showed a significantly positive correlation with KIBRA expression ($P = 0.012$). Increased staining of KIBRA significantly correlated with increased probability of lymphatic invasion ($P = 0.046$) and venous invasion ($P = 0.039$). Although not statistically significant, there was a tendency for increased expression of KIBRA in patients older than 70 years compared to younger ones ($P = 0.058$), and in intestinal-type cancer compared to diffuse-type ($P = 0.069$). There was no deviation between KIBRA expression and gender, tumor diameter, depth of invasion, lymph node metastasis, distant metastasis, stage, curability or E-cadherin expression. As shown in Figure 2, there was no correlation between 5-year disease specific survival (DSS) and relapse free survival (RFS) between KIBRA-positive and -negative cancers.

Correlation between KIBRA/aPKCλ/ι expression and clinicopathological features of gastric cancer patients. We next separated the samples into two groups, aPKCλ/ι-high or -low,[21] and examined the association between KIBRA and clinical characteristics in these groups. We first analyzed the association between the expression of KIBRA and DSS or RFS. Neither DSS nor RFS were significantly correlated with KIBRA expression in the aPKCλ/ι-high group (Fig. 3a,b); however, there was a significant difference in the 5-year DSS between the KIBRA-positive and KIBRA-negative samples in the aPKCλ/ι-low patient group (73.1% and 95.8%, respectively; $P = 0.037$; Fig. 3c). The 5-year RFS was also significantly dif-

Table 2. Correlation between KIBRA expression and clinicopathological features of gastric cancer patients

|	KIBRA expression		P-value
	Positive $n = 109$ (%)	Negative $n = 55$ (%)	
Age (years) <70/≥70	64/45 (59/41)	41/14 (75/25)	0.058
Gender			
Male/Female	81/28 (74/26)	38/17 (69/31)	0.578
Tumor diameter (mm) <50/≥50	59/50 (54/46)	33/22 (60/40)	0.508
Histological type			
Intestinal/Diffuse	68/41 (62/38)	26/29 (47/53)	0.069
Depth of invasion			
pT1,2/pT3,4	84/25 (77/23)	40/15 (73/27)	0.567
Lymph node metastasis			
Negative/Positive	61/48 (56/44)	30/25 (55/45)	0.869
Distant metastasis			
Negative/Positive	108/1 (99/1)	54/1 (98/2)	1.000
Lymphatic invasion			
Negative/Positive	52/57 (48/52)	36/19 (65/35)	0.046*
Venous invasion			
Negative/Positive	62/47 (57/43)	41/14 (75/25)	0.039*
Stage			
I,II/III,IV	78/31 (72/28)	38/17 (69/31)	0.856
Curability			
R0,R1/R2	103/6 (94/6)	52/3 (95/5)	1.000
aPKC expression			
low/high	26/83 (24/76)	24/31 (44/56)	0.012*
E-cadherin expression			
Negative/Positive	56/53 (51/49)	31/24 (56/44)	0.620

*$P < 0.05$ for Fisher's exact test.
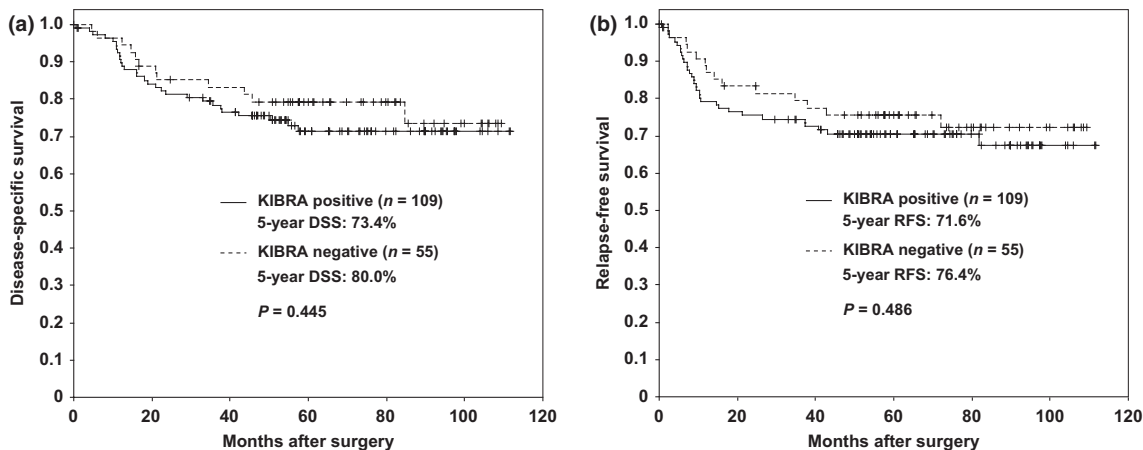
Fig. 2. No correlation between the expression of KIBRA and disease-specific survival or relapse-free survival. Kaplan–Meier plot comparing positive (line) and negative (dashed line) staining of KIBRA for disease-specific survival (DSS) (a) and relapse-free survival (RFS) (b) are shown. P-values were calculated by log-rank test.
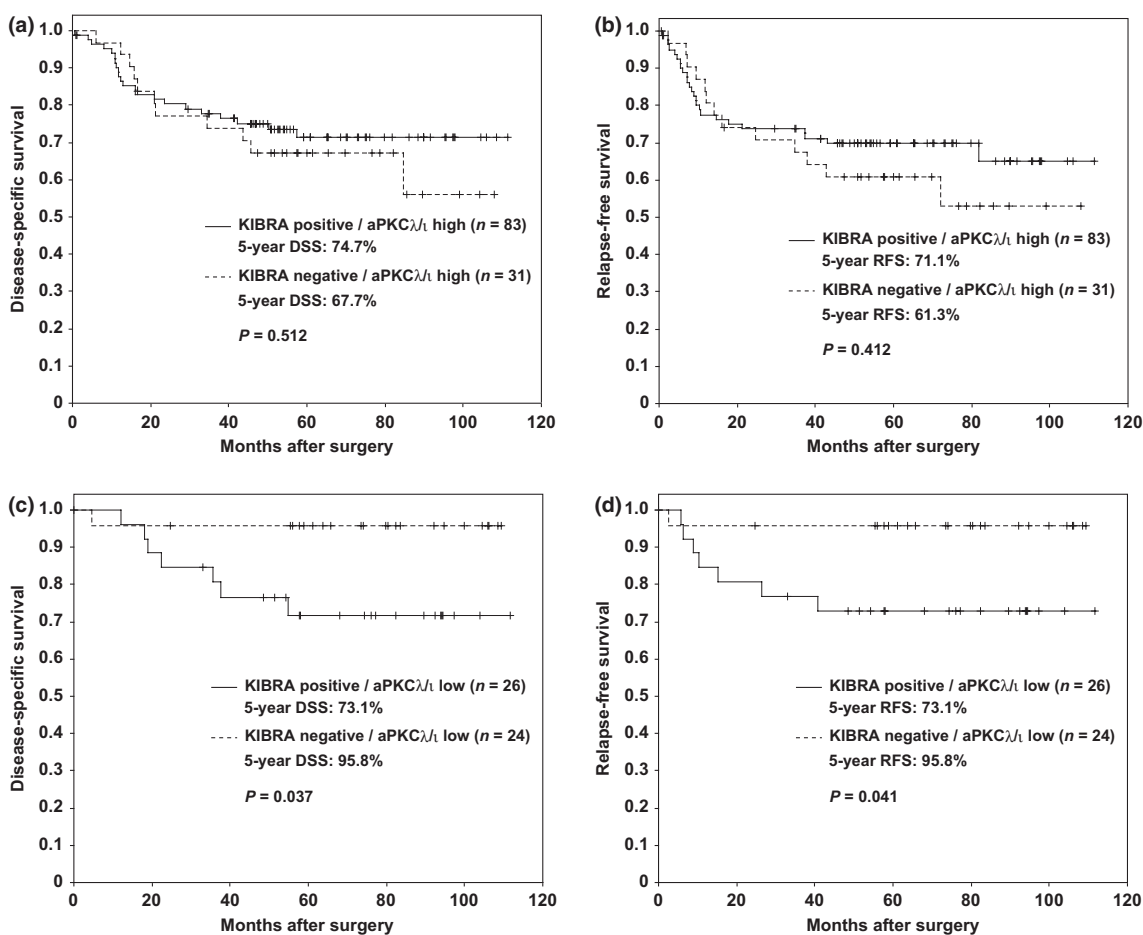

Fig. 3. Association between high expression of KIBRA in aPKCλ/-low gastric cancer and poor prognosis. Kaplan–Meier plot comparing positive (line) and negative (dashed line) staining of KIBRA for disease-specific survival (DSS) and relapse-free survival (RFS) in a aPKCλ/ι-high group (a & b) or in a aPKCλ/ι-low group (c & d) are shown. P-values were calculated by log-rank test.

ferent between the two groups (73.1% and 95.8%, respectively; P = 0.041; Fig. 3d). As shown in Table 3, KIBRA expression did not correlate with any clinical characteristics in the aPKCλ/ι-high group; however, the increased staining of KIBRA showed significant correlation with increased probability

of lymphatic invasion (P = 0.042) and not significant but strong correlation with increased probability of venous invasion (P = 0.067) in the aPKCλ/ι-low group. There was also a correlation of increased KIBRA expression patients older than 70 years old compared to younger ones (P = 0.028), and in

Table 3. Association between atypical protein kinase Cλ/ι (aPKCλ/ι)/KIBRA expression and clinicopathological features of gastric cancer patients

| | aPKCλ/ι high | | | aPKCλ/ι low | | |
| | KIBRA expression | | | KIBRA expression | | |
	Positive n = 83 (%)	Negative n = 31 (%)	P-value	Positive n = 26 (%)	Negative n = 24 (%)	P-value
Age <70/ ≥ 70 (years)	49/34 (59/41)	20/11 (65/35)	0.670	15/11 (58/42)	21/3 (87/13)	0.028*
Gender						
Male/Female	65/18 (78/22)	25/6 (81/19)	1.000	16/10 (62/38)	13/11 (54/46)	0.405
Tumor diameter <50/ ≥ 50 (mm)	44/39 (53/47)	16/15 (52/48)	1.000	15/11 (58/42)	17/7 (71/29)	0.388
Histological type						
Intestinal/Diffuse	56/27 (67/33)	22/9 (71/29)	0.823	12/14 (46/54)	4/20 (17/83)	0.035*
E-cadherin expression						
Negative/Positive	44/39 (53/47)	15/16 (48/52)	0.679	12/14 (46/54)	16/8 (67/33)	0.166
Depth of invasion						
pT1,2/pT3,4	63/20 (76/24)	20/11 (65/35)	0.243	21/5 (81/19)	20/4 (83/17)	1.000
Lymph node metastasis						
Negative/Positive	49/34 (59/41)	16/15 (52/48)	0.527	12/14 (46/54)	14/10 (58/42)	0.413
Distant metastasis						
Negative/Positive	83/0 (100/0)	30/1 (97/3)	0.272	25/1 (96/4)	24/0 (100/0)	1.000
Lymphatic invasion						
Negative/Positive	39/44 (47/53)	17/14 (55/45)	0.530	13/13 (50/50)	19/5 (79/21)	0.042*
Venous invasion						
Negative/Positive	47/36 (57/43)	21/10 (68/32)	0.391	15/11 (58/42)	20/4 (83/17)	0.067
Stage						
I,II/III,IV	60/23 (72/28)	19/12 (61/39)	0.264	18/8 (69/31)	19/5 (79/21)	0.526
Curability						
R0,R1/R2	78/5 (94/6)	29/2 (94/6)	1.000	25/1 (96/4)	23/1 (96/4)	1.000

*P < 0.05 for Fisher's exact test.

intestinal-type cancer compared to diffuse-type (P = 0.035) in the aPKCλ/ι-low group. There was no deviation between KIBRA expression and gender, tumor diameter, depth of invasion, lymph node metastasis, distant metastasis, stage, curability and E-cadherin expression in the aPKCλ/ι-low group (Table 3).

Effect of KIBRA-aPKC interaction on cell-cell contact formation. To further understand the biological significance of KIBRA expression in the aPKCλ/ι-low group, we next examined the effect of KIBRA-aPKC interactions in cell–cell contact formation. To this purpose, we used calcium switch (CS) assays with Madin-Darby canine kidney (MDCK) cells, a dog kidney epithelial cell line commonly used for polarity research. MDCK cells were depolarized in low calcium medium to disrupt cell–cell contact (Fig. 4a, 0 h). Then cells were cultured in normal calcium medium to reform cell–cell contact. In MDCK cells expressing tRFP, ZO-1, a tight junction protein, was recruited to the primordial cell–cell junction 0.5 h after a CS, and formed linear staining at cell–cell junctional regions 2 –6 h after a CS (Fig. 4a). We tried to examine the effect of overexpression of full-length KIBRA; however, we somehow failed to overexpress full-length KIBRA in MDCK cells, possibly due to cytotoxic effect such as Hippo pathway activation (data not shown).[29–31] Thus we used overexpression of tRFP-tagged aPKC-binding region of KIBRA (tRFP-aBR).[28] In contrast to tRFP overexpression, overexpression of tRFP-aBR delayed the recruitment of ZO-1 to cell–cell junction, and disrupted junctional staining of ZO-1 up to 6 h after CS (Fig. 4a). Almost 40% of tRFP-aBR expressing MDCK cells showed disrupted ZO-1 staining, whereas only 4% of tRFP expressing MDCK cells did 6 h after a CS (Fig. 4b). Given that aBR can competitively inhibit the kinase activity of aPKC,[28] the results suggest that overexpression of KIBRA can disrupt cell–cell junctions through inhibition of aPKC activity in epithelial cells.

Discussion

Previous studies have suggested the importance of the deregulation of aPKCλ/ι in the progression of various cancer types. In the present study, we extended this notion and provided evidence suggesting the importance of aPKCλ/ι activity for loss of polarity and invasiveness in gastric cancer tissues.

We showed that KIBRA localizes at the apical domain of epithelial cells in normal gastric tissue in a similar manner to aPKCλ/ι.[21] Importantly, we found a correlation between the expression level of aPKCλ/ι and KIBRA (Table 2), suggesting that KIBRA might affect aPKCλ/ι in gastric carcinogenesis. We also showed that high expression of KIBRA correlated with lymphovascular invasion (Table 2). Although the expression of KIBRA did not affect the DSS or RFS in total (Fig. 2), high expression of KIBRA correlated with poor prognosis both in DSS and in RFS in low aPKCλ/ι-expressing samples (Fig. 3). Further, high KIBRA expression showed positive correlation with lymphovascular invasion only in the low aPKCλ/ι-expressing group (Table 3). Thus, we focused on the correlation between KIBRA expression with lymphovascular invasion. Considering that KIBRA inhibits the kinase activity of aPKCλ/ι in epithelial cells,[28] the kinase activity of aPKCλ/ι might be severely lowered in KIBRA-high/aPKCλ/ι-low gastric cancer tissue. Loss of aPKC activity can disrupt cell–cell adhesion by increased endocytosis of E-cadherin[35] and loss of tight junctions,[25,36] which can increase the possibility of vascular invasion. Consistently, we showed that overexpression of the aPKC-binding region of KIBRA in MDCK cells causes defects in tight junction formation during calcium switching (Fig. 4) similarly to aPKCλ/ι RNAi[36] but not to its overexpression,[25] supporting the hypothesis that imbalances in KIBRA and aPKCλ/ι expression can cause loss of polarity, presumably because the decrease in aPKC kinase activity. The correlation between high expression of aPKCλ/ι and tumor

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(a)

Time after a CS (h)

| | 0 | 0.5 | 2 | 6 |

(rows labeled tRFP and tRFP-aBR)

(b)

Cells with disrupted ZO-1 staining/RFP positive cells (%)

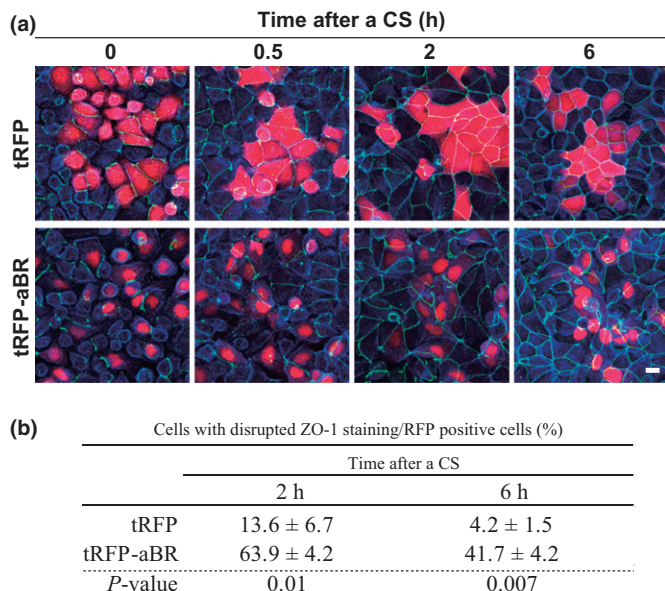|	Time after a CS	
	2 h	6 h
tRFP	13.6 ± 6.7	4.2 ± 1.5
tRFP-aBR	63.9 ± 4.2	41.7 ± 4.2
P-value	0.01	0.007

Fig. 4. Overexpression of the atypical protein kinase C (aPKC) binding region of KIBRA affects tight junction formation. (a) Madin-Darby canine kidney (MDCK) cells were transfected to express indicated proteins and then subjected to calcium switch assays. Cells were fixed at indicated times after calcium switch and stained for ZO-1 (green), tRFP (red), and F-actin (blue). Scale bars, 10 μm. (b) Cells that showed disrupted ZO-1 staining in tRFP- or tRFP-aBR-expressing MDCK cells were counted, and percentages for total RFP positive cells were calculated. Values represent the mean ± standard deviation (SD) of three independent experiments. *P*-values were calculated by Student's *t*-test.

progression is suggested in many malignancies including breast cancer.[15–20] On the other hand, in bladder cancer tissue, loss of aPKC expression is correlated with tumor recurrence.[22] Taken together with this study, these facts suggest that both increased and decreased activity of aPKCλ/ι is important for tumorigenesis and the activity of aPKCλ/ι should be properly regulated to maintain normal tissue homeostasis.

In aPKC-λ/ι low cases, KIBRA-negative gastric cancer has a more diffuse type (83%) than KIBRA positive (54%), but shows more favorable prognosis (Table 3). KIBRA-negative/aPKCλ/ι-low group showed significantly better prognosis than any other group subdivided based on KIBRA and aPKCλ/ι expression. It raises the possibility that the KIBRA-negative/aPKCλ/ι-low group are consistent with relatively earlier stage of cancer cases than KIBRA-positive/aPKCλ/ι-low cases. We examined the impact of KIBRA expression on prognostic value in stage-matched populations; the aPKC-low patients group was divided into two group; stage I/II and stage III/IV, and then we examined whether or not the KIBRA-negative group had a favorable prognosis in these stages. There was a tendency that the KIBRA-negative/aPKC-low patients group showed better prognosis than the KIBRA-positive/aPKC-low group, independently of stage (data not shown). Furthermore, there was no significant difference in clinical stages between the KIBRA-negative/aPKCλ/ι-low group and the KIBRA-positive/aPKCλ/ι-low group (Table 3). These findings suggest that the KIBRA-negative/aPKCλ/ι-low group are not earlier stage of cancer cases than KIBRA-positive/aPKCλ/ι-low cases.

Nuclear accumulation of YAP, a transcription co-activator that is suppressed by the Hippo pathway, is associated with poor prognosis of gastric cancer.[37] KIBRA activates the Hippo pathway,[29–31] thus nuclear accumulation of YAP might be accelerated to cause poor prognosis in KIBRA-negative cancer compared with KIBRA-positive cancer. In this study, however, KIBRA expression alone did not correlate with prognosis (Fig. 2). *Drosophila* KIBRA (Kibra) acts together with FERM proteins Merlin and Expanded, and loss of Kibra alone showed mild overgrowth phenotype.[29–31] Combined analysis of NF2 (human Merlin homolog) and FERMD6 (human Expanded homolog) will be required to conclude whether Hippo pathway regulation by KIBRA is important for gastric cancer progression.

Our study showed that the expression level of KIBRA can be a prognostic factor in the low aPKCλ/ι-expressing group (Fig. 3). Note that the KIBRA expression by itself is not a prognostic factor (Fig. 2). One possible explanation is that both overexpression and loss of KIBRA can affect cell polarity. Loss of KIBRA can increase the activity of aPKC, which leads to its overactivation and subsequent poor prognosis in gastric cancer. In contrast, overexpression of KIBRA can lead to a loss of polarity through inhibition of aPKC, as discussed above. It is quite possible that the expression level of KIBRA is precisely regulated in gastric tissue. As mentioned above, high expression of KIBRA only in low aPKCλ/ι-expressing tumors correlated with poor prognostic factors (Fig. 3). One possibility is that gastric cancer expresses other aPKC such as aPKCζ, and KIBRA expression alone could not suppress total aPKC activity if all aPKC family members are expressed. Unfortunately, there is no aPKCζ specific antibody available in immunohistochemistry, thus it is difficult to determine the expression level of aPKCζ in the gastric cancer tissues used in this study. The expression of aPKCζ is required for further investigation. The importance of this result is that aPKCλ/ι-low tumors have been associated with better prognoses. Combined with the expression level of KIBRA, we observed worse prognoses for the aPKCλ/ι-low groups. The combined analysis of aPKCλ/ι and KIBRA expression might be a more precise predictor of prognosis in gastric cancer.

In conclusion, our findings provide evidence that supports the importance of a KIBRA-aPKC connection in the progression of gastric cancer. We found that the KIBRA high/aPKCλ/ι low expression could indicate poor prognosis, which would have been missed if diagnosed with aPKCλ/ι expression only.

Disclosure Statement

The authors have no conflict of interest.

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