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Original Article

Overexpression of particularly interesting new cys-his rich protein (PINCH) is a risk factor for growth of unruptured intracranial aneurysms

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Abstract: Particularly interesting new cys-his rich protein (PINCH), as an adaptor protein, regulates matrix deposition, cell proliferation, invasion, and metastasis. PINCH plays an important role for tumorigenesis and progression. However, the contributions of PINCH to intracranial aneurysms (IA) remain largely unknown. In our study, we demonstrated that PINCH expression was significantly increased in IA samples compared with healthy controls. The size of IA had a remarkable correlation with PINCH expression. However, PINCH expression had no obvious difference among different Hunt-Hess grades. In addition, the expressions of MMP-2 and MMP-9 were significantly increased in IA tissues compared with healthy controls; moreover, PINCH expression in IA tissues was significantly correlated with MMP-2 and MMP-9 expression. In conclusion, these results suggest that PINCH might play a role similar to MMP-2 and MMP-9 in the pathogenesis of IA. PINCH might be a risk factor for growth of unruptured IA, and this might be a target for diagnosis and therapy of IA.

Keywords: Particularly interesting new cys-his rich protein, intracranial aneurysms, prognosis, MMP-2, MMP-9

Introduction

Intracranial aneurysm (IA) is a relatively common vascular abnormality of the cerebrum ocurring at a 1%-5% rate in the general population [1], of which approximately 0.7%-1.9% will rupture and lead to life-threating subarachnoid hemorrhage (SAH) [2]. Usually, patients with IA are asymptomatic and have mildly pain until the IA ruptures and SAH occurs [3]. Although digital subtraction angiography (DSA) and computed tomography technology have been considered the gold standard for the detection and characterization of IA [4], the pathogenesis of IA has not been fully elaborated. To date, risk factors for IA occurrence include genetic factors, trauma, infection and aging [3]. Therefore, revealing the pathogenesis is urgent for developing diagnosis, treatment and prognosis for IA.

Particularly interesting new cys-his rich protein (PINCH) is composed of 5 LIM domain proteins and is located on chromosome 2q12.2. PINCH is expressed in early embryonic development and adult tissues in a ubiquitous manner, and plays an important role in fundamental physiologic functions, including cell proliferation, differentiation, survival, adhesion and migration [5]. Recent studies have indicated that PINCH plays an important role for the interaction of tumor cells and tumor-associated stroma cells, which regulates the tumorigenesis, progression, and metastasis of tumors [6]. Previous studies demonstrate that PINCH is significantly increased in several types of cancer, such as gastric adenocarcinoma [7], colorectal cancer [8] and breast cancer [9]. Moreover, PINCH-1 contributes to apoptosis resistance through suppression of Bim in HT-1080 fibrosarcoma cells [10]. PINCH, as an adaptor protein, is usually expressed at the invasive front of tumors. Importantly, the expression of PINCH is strongly associated with clinicopathological grades and patient survival [8]. Intriguingly, PINCH silencing in hepatocytes results in histologic abnormalities, sustained proliferation of hepatocytes, increased liver size and development of sponta-

Table 1. Basic characteristics of healthy controls and patients with unruptured intracranial aneurysms

		Age	Gender	IA Location	IA Size (mm)	Hunt-Hess grade	PINCH Staining (IOD)
1	NC	42	F	/	/	/	0.105
2	NC	25	F	/	/	/	0.087
3	NC	10	F	/	/	/	0.124
4	NC	51	M	/	/	/	0.165
5	NC	67	M	/	/	/	0.092
6	NC	55	M	/	/	/	0.143
7	NC	43	M	/	/	/	0.081
8	NC	40	M	/	/	/	0.171
9	NC	35	M	/	/	/	0.141
10	NC	38	M	/	/	/	0.120
11	NC	55	M	/	/	/	0.096
12	NC	62	M	/	/	/	0.153
13	IA	47	F	R-PCA	18	III	0.189
14	IA	34	F	L-PCA	24	IV	0.195
15	IA	57	F	L-ICA	12	I	0.112
16	IA	55	M	L-VA	8	II	0.152
17	IA	37	F	R-MCA	15	II	0.243
18	IA	49	M	R-VA	34	III	0.251
19	IA	54	M	L-PCA	29	III	0.235
20	IA	68	F	L-ICA	6	I	0.153
21	IA	71	M	L-MCA	38	IV	0.282
22	IA	49	M	L-ICA	16	II	0.231
23	IA	61	M	L-PCA	14	I	0.185
24	IA	53	F	R-ICA	30	III	0.237

NC, healthy control volunteers; IA, unruptured intracranial aneurysms; M, male; F, female; R, right; L, left; PCA, posterior cerebral artery; ICA, internal carotid artery; VA, vertebra artery; MCA, middle cerebral artery; PINCH, particularly interesting new cys-his rich protein.

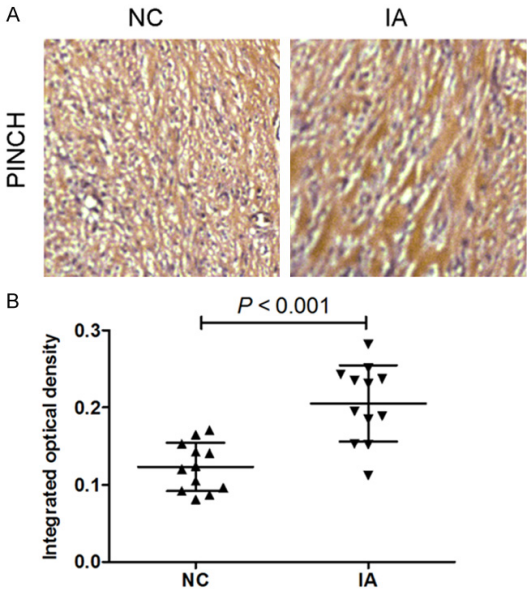

Figure 1. Expression of PINCH was measured by immunohistochemical staining in normal controls (NC) and unruptured intracranial aneurysms (A). The integrated optical density (IOD) was measured for the tumor tissues with positive immunohistochemical staining (B). Values were expressed as mean ± SD, $^{*}P < 0.05$ versus NC group.

neous tumors [11]. However, as far as we know, no literature has been reported showing a correlation between PINCH abnormal expression and IAs. In the present study, we aim to investigate PINCH expression, prognostic significance, and potential molecular mechanisms in the progression of IA.

Materials and methods

Patients and specimens

Twelve IA samples from patients who underwent cerebral aneurysm clipping from June 2009 to December 2014 at the Department of Neurosurgery, Tianjin Huanhu Hospital (Tianjin, China) were examined. Most of the patients were Han nationality residing in Tianjin Provence, China. The patients' characteristics are shown in **Table 1**. All patients recruited in this study were not subjected to preoperative radiotherapy or chemotherapy and were diagnosed with IA based on histopathologic evaluation. Control specimens were twelve intracranial cerebral arteries obtained from surgery patients. All collected tissue samples were immediately fixed in 10% formalin for immunohistochemical staining. Human samples were obtained with written informed consent from all patients. The study was approved by the Ethics Committee of the Tianjin Huanhu Hospital (Tianjin, China).

Immunohistochemical staining

Immunohistochemistry was performed to assess the expression of PINCH (1:500, sc-393133, Santa Cruz Biotechnology, CA, USA), MMP-2 (1:2000, sc-13594, Santa Cruz Biotechnology, CA, USA) and MMP-9 (1:2000, sc-21733, Santa Cruz Biotechnology, CA, USA)

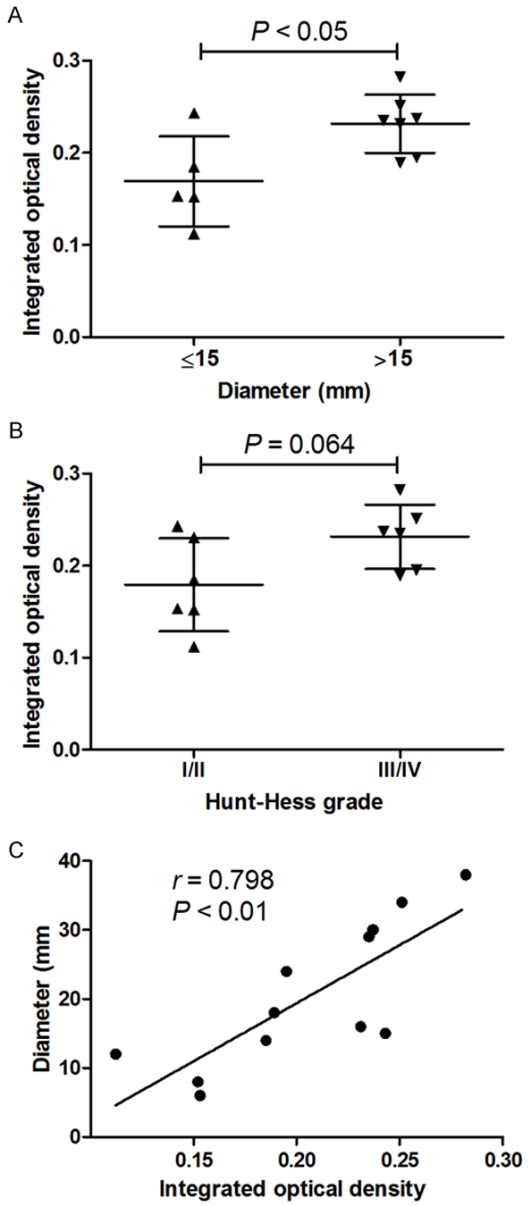

Figure 2. Expression of PINCH in different sizes of unruptured intracranial aneurysms tissues (A) and Hunt-Hess grade (B). The correlation between PINCH and the diameter of IA tissues was measured (C). Values were expressed as mean ± SD, $^{*}P < 0.05$ versus NC group.

protein in IA tissues. Paraffin embedded tissues were cut into 3-5 μm sections, mounted on glass slides and stained using indirect immunoperoxidase. The paraffin sections were baked in an oven at 65°C for 24 h, then dewaxed to water, and rinsed with PBS three times (10 min per time). Well-washed sections were placed in the EDTA buffer for microwave antigen retrieval, boiled on high heat, then continued boiling on low heat for an interval of 10 min. After natural cooling, the sections were washed with PBS 3 times. The sections were put into 3% hydrogen peroxide solution and incubated at room temperature for 10 minto block endogenous peroxidase, then washed with PBS 3 times, and blocked with 5% bovine serum albumin (BSA) for 20 min after drying. After removal of BSA liquid, each section received 50 μl diluted primary antibody overnight at 4°C, then was washed with PBS 3 times. After the removal from PBS, each slice received 50-100 μl secondary antibody and was incubated at 4°C for 50 min. Sections were washed with PBS 3 times; each slice received 50-100 μl freshly prepared DAB solution and color change was observed under the microscope. After washing, sections were counterstained with hematoxylin, rinsed with tap water, dehydrated, and mounted. Three randomly selected and non-overlapping areas (×200) were observed and photographed (Leica DM 2500). The Image Pro-Plus 6 software was used for analysis. The integrated optical density (IOD) was respectively measured for the IA tissues of immunohistochemical positive stain.

Statistical analysis

Data were reported as mean ± standard deviation (SD) for each group. All statistical analyses were performed by using PRISM version 6.0 (GraphPad). The significance of differences between groups was estimated by unpaired t-test. Spearman rank correlation analysis was used to analyze the correlations between the size of IA tissues, PINCH, MMP-2 and MMP-9, and $P < 0.05$ was considered statistically significant.

Results

Expression of PINCH in intracranial aneurysms

First, the immunohistochemical staining of PINCH was performed in normal controls (n = 12) and unruptured IA (n = 12). These results demonstrated that PINCH levels were different in the analyzed samples. Normal control arteries exhibited weak positive staining for PINCH; however, unruptured IA stained extensively for PINCH (**Figure 1A** and **1B**). In addition, the clinical characteristics of healthy controls and patients with unruptured IA are recorded as shown in **Table 1**, and the location and size of

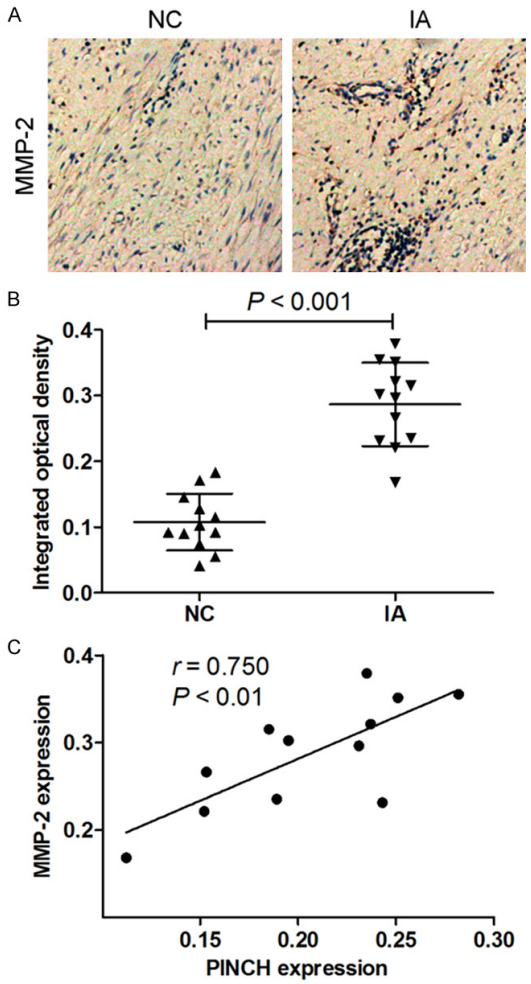

Figure 3. Expression of MMP-2 was measured by immunohistochemical staining in NC and unruptured intracranial aneurysms (A). The integrated optical density (IOD) was measured for the tumor tissues with positive immunohistochemical staining (B). The correlation between PINCH and MMP-2 expression was measured (C). Values were expressed as mean ± SD, $^{*}P < 0.05$ versus NC group.

IA were distinguished by digital subtraction angiography (DSA).

The unruptured IA samples were divided into two groups according to size. We analyzed the differential expression of PINCH in different-sized unruptured IA samples. As shown in **Figure 2A**, the expression of PINCH was significantly increased in the > 15 mm diameter group compared with the < 15 mm diameter group. These results suggest that the expression of PINCH might be related to the size of unruptured IA. As expected. a statistical correlation between PINCH expression and the size of IA was found ($r = 0.798$, $P < 0.01$; **Figure 2C**). However, the expression of PINCH had no obvious difference in different Hunt-Hess grades (**Figure 2B**).

PINCH expression is related to MMP-2 and MMP-9 expression

A previous study showed that MMPs may be involved in the formation and development of aneurysms [12]. Particularly, MMP-9 polymorphism is associated with the pathogenesis of intracranial aneurysms [13], and MMP-9 expression is significantly increased in intracranial aneurysms tissues [12]. Moreover, MMP-2 expression in intracranial aneurysm wall tissue is significantly higher than in the normal intracranial arterial tissues [14]. In our study, the expression of MMP-2 and MMP-9 were measured, and the correlation of PINCH with MMP-2 and MMP-9 was performed by Spearman rank correlation analysis. As shown in **Figure 3A** and **3B**, the expression of MMP-2 was up-regulated approximately 3 times compared to normal controls. We tested whether there was a relationship between PINCH and MMP-2 levels, measured in the same individuals. As shown in **Figure 3C**, measurements obtained from the same individuals were strongly correlated between PINCH and MMP-2 ($r = 0.750$, $P < 0.01$). In addition, the immunohistochemical staining intensity of MMP-9 was markedly enhanced in the unruptured IA samples compared with the control group (**Figure 4A** and **4B**). A statistical correlation between PINCH expression and MMP-9 expression was significantly positive ($r = 0.788$, $P < 0.01$; **Figure 4C**). In general, these results suggested that PINCH, MMP-2 and MMP-9 tended to have stronger expression in unruptured IA.

Discussion

The objective of this study was to investigate the functional significance of PINCH in the progression of IA. Our results demonstrated that PINCH expression was significantly increased in IA samples compared with normal controls. The size of IA had a remarkable correlation with the expression of PINCH. In addition, we found that MMP-2 and MMP-9 were markedly up-regulated in IA tissues, and disclosed a positive correlation between MMP-2/-9 and PINCH expression.

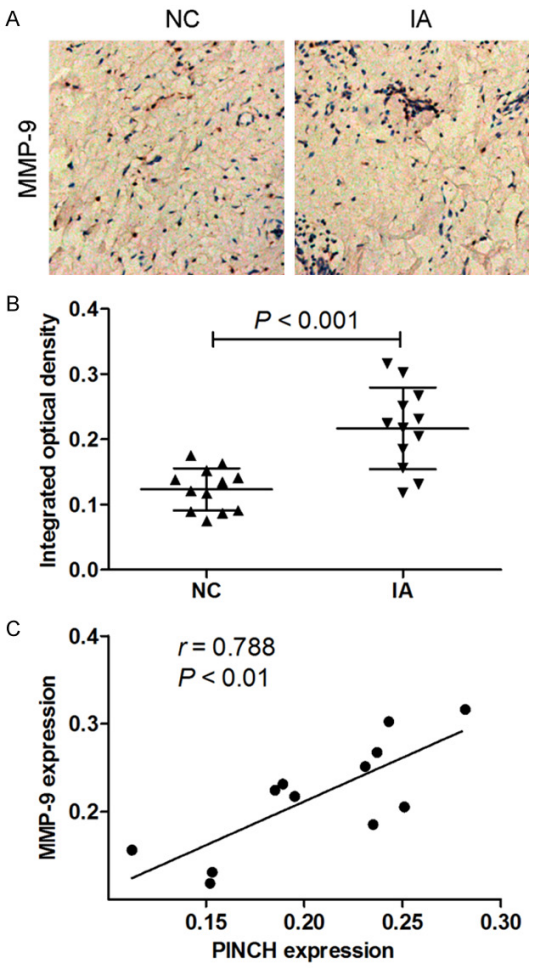

Figure 4. Expression of MMP-9 was measured by immunohistochemical staining in NC and unruptured intracranial aneurysms (A). The integrated optical density (IOD) was measured for tumor tissues with positive immunohistochemical staining (B). The correlation between PINCH and MMP-9 expression was measured (C). Values were expressed as mean ± SD, $^{*}P < 0.05$ versus NC group.

A previous study has shown that Kinase-PINCH-Parvin (IPP) complex plays an important role in regulating the size of the liver [11]. PINCH1 regulates cell-matrix and cell-cell adhesion, cell polarity, and cell survival during the periimplantation stage [15]. PINCH loss-of-function causes distinct defects, which are characterized by abnormal muscle attachment and embryonic lethality [16]. Recent studies have indicated that the interaction of tumor cells and tumor-associated stromal cells can regulate tumorigenesis, progression, and metastasis [7]. Variations in this interaction between the tumor and surrounding tissues may facilitate the metastasis and invasion of tumor cells [17]. PINCH, as an adaptor protein, is related to poorly differentiated glioma and oral squamous cell carcinoma with lymph node metastasis and can independently predict unfavorable prognosis of colorectal cancer patients [7, 8]. Depletion of PINCH1 in HeLa cells by RNA interference can significantly induce cell apoptosis [18]. In the present study, we showed that PINCH was increased in IA tissues as compared to a normal control group, and these results confirmed the presence of PINCH protein in IA tissues. We also showed a significant positive correlation between the size of IA and PINCH expression.

A previous study showed that PINCH forms a complex with integrin-linked kinase (ILK) regulation of fibronectin matrix deposition and cell proliferation [19]. In intestinal and mammary epithelial cells, ILK can stimulate MMP-9 expression via GSK-3β and AP-1 transcription factor [20]. MMPs can degrade biologic macromolecules in the extracellular matrix. Studies have confirmed that MMP activity is increased in IA patients, and MMPs may be involved in the formation and development of aneurysms [12, 21]. In the present study, the expression of MMP-2 and MMP-9 were significantly increased in IA tissues compared with healthy controls; moreover, PINCH expression in IA tissues was significantly correlated with MMP-2 and MMP-9 expression. MMPs are by far the proteases that are most closely related to the pathogenesis of intracranial aneurysms [12].

Taken together, these results suggest that PINCH might play a similar role as MMP-2 and MMP-9 in the pathogenesis of IA. PINCH might be a risk factor for growth of unruptured IA, and might be a target for diagnosis and therapy of IA.

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Disclosure of conflict of interest

None.

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