Phosphatase and tensin homolog in cerebral cavernous malformation: a potential role in pathological angiogenesis

Laboratory investigation

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Object. Cerebral cavernous malformations (CCMs) are the most common vascular malformation of the central nervous system and involve dysregulated angiogenesis. However, the underlying mechanism of this disease is poorly understood. Phosphatase and tensin homolog (PTEN) plays a crucial role in regulating angiogenesis. The authors attempted to determine whether PTEN is involved in the pathological angiogenesis of CCM.

Methods. The authors used Western blot analysis and immunohistochemical methods to detect the expression of PTEN, PCNA, and P-Akt in the surgical specimens of CCMs and controls. The function of PTEN in cell proliferation was studied after PTEN silencing in endothelial cultures by using the short interfering RNA technique.

Results. Western blot analysis showed significant reduction of PTEN protein expression in CCMs compared with control brain tissue (p < 0.01). Immunohistochemical analysis confirmed PTEN insufficiency in 33% of vascular endothelia of CCMs, which was significantly higher than that of controls (2%, p < 0.01). Furthermore, PTEN insufficiency occurred more frequently in multiple CCMs (44%) and in small lesions (39%) than in single CCMs (28%, p < 0.05) and large lesions (30%, p < 0.05), respectively, suggesting a potential role of PTEN in the progression of the lesions. Of note, a negative correlation was observed between the expression of PTEN and PCNA in CCM endothelial cells. However, Akt was not constitutively activated in CCMs. Using cultured endothelial cells, the authors demonstrated that PTEN silencing by short interfering RNA increased Akt activation, PCNA expression, and cell proliferation (p < 0.001). Surprisingly, the PTEN silencing-mediated increase in endothelial proliferation was not reversed by the PI3K inhibitor wortmannin.

Conclusions. In this study, the authors report for the first time a significant PTEN insufficiency in CCM vessels associated with endothelial proliferation. The in vitro study provides direct evidence for a pivotal role of PTEN in regulating endothelial proliferation, most likely through a PI3K-independent pathway. The authors suggest that PTEN insufficiency is potentially involved in CCM by stimulating angiogenesis. (DOI: 10.3171/2008.7.17626)

**Key Words** • angiogenesis • cerebral cavernous malformation • endothelial proliferation • PTEN

**Abbreviations used in this paper:** CCM = cerebral cavernous malformation; siRNA = short interfering RNA; VEGF = vascular endothelial growth factor; vWF = von Willebrand factor.

Cerebral cavernous malformation is a neurovascular disease that affects almost 0.5% of the population, and in 20–30% of cases it causes symptoms including headaches, seizures, and neurological deficits due to repeated microhemorrhages. Cerebral cavernous malformations occur either sporadically (in 50–80%) or in a familial pattern. The former usually appears as a single lesion, whereas the latter arises with multiple lesions and a family history of CCMs and/or an autosomal dominant mode of inheritance. Pathologically, CCMs are characterized by enlarged capillary vein structures lined with a single layer of endothelial cells devoid of vascular smooth muscle cells and without intervening brain parenchyma. There is increasing evidence for growth and/or de novo appearance of CCMs over the course of a lifetime, supporting the idea that CCMs are dynamic rather than static lesions. It is therefore important to characterize the underlying mechanism that may lead to CCM progression.

Considering the vascular nature of the lesion, a pathological angiogenesis has been suggested to be involved in this disease. We and others have provided evidence showing endothelial proliferation and increased expression of VEGF and its receptors Flk-1, HIF-1α, FGF2, and integrin in the lesions. In support, Jung et al. reported dynamic de novo formation of lesions in a patient with CCM accompanied by a consistent increase in VEGF in the blood serum. A recent study has shown high angiogenic activity in cultured endothelial cells isolated from surgical specimens of CCMs. However,
the precise mechanism underlying the deregulated angiogenesis is still unclear.

Phosphatase and tensin homolog is a tumor suppressor gene and plays an important role in many cellular processes, including cell growth and differentiation, cell motility, cell size, and apoptosis. The major biological function of PTEN is mediated by its lipid phosphatase property by which PTEN specifically dephosphorylates PIP3 to form PIP2. Phosphatase and tensin homolog is thus a key negative regulator of the PI3K/Akt pathway, which controls multiple cellular functions. Increasing evidence has shown that PTEN plays a crucial role in angiogenesis. Loss of PTEN results in HIF-1 expression and subsequent VEGF expression leading to tumor progression due to high vascularization. Indeed, the N-terminal of PTEN shares close sequence homologous characteristics with the cytoskeletal proteins tensin and auxilin, and is therefore able to inhibit the vascular sprouting and endothelial tube formation induced by VEGF; the dominant mutation of PTEN or PI3K inhibitor abolishes these effects. It is generally accepted that inhibition of angiogenesis is one direct mechanism among others that mediates the tumor suppressive function of PTEN. However, it is unclear whether PTEN regulates pathological angiogenesis in nontumourous disease.

Based on the dysregulated angiogenesis in CCM lesions and the important role of PTEN in controlling angiogenesis, we hypothesized that PTEN was involved in the pathological angiogenesis of CCMs. To address this issue, PTEN expression was studied in CCMs on Western blot and immunohistochemical analysis. Furthermore, the endothelial expression patterns of PTEN were correlated with the vascular angiogenesis status in these lesions and with the patients' clinical signs and symptoms. A functional study of PTEN was performed in cultured endothelial cells using the siRNA technique.

Methods

Patient Population

The diagnosis of CCM was based on the specific characteristics of MR imaging and histopathological criteria as described in our previous review. The patients included in the present study underwent microsurgical excision of the CCM at our institution. All patients provided informed consent, and the experiment protocol was approved by the ethics committee of the University Hospital of Marburg (Approval Number AZ67/07).

For Western blot analysis, 15 CCM surgical specimens obtained in adults with supratentorial or brainstem lesions (6 women and 9 men; mean age ± 12 years of age, range 23–60 years) and 7 age-matched normal-brain specimens from epileptic excision (controls) were collected and immediately frozen at −80°C until use.

Samples for immunohistochemistry analysis were obtained in 62 consecutive, symptomatic adult patients with CCMs who underwent treatment at our institution between January 2000 and October 2005. Spinal and cerebellar lesions were excluded due to a low number of samples. The patients included 23 women and 39 men with a mean age of 40 ± 14 years (range 18–65 years). The lesions were supratentorial in 27 patients and in the brainstem in 35. The most common presenting symptoms were seizures in patients with supratentorial lesions and focal neurological deficits in patients with brainstem CCMs. In most patients, at least 1 clinical episode of hemorrhage could be documented. Among 62 patients with CCMs, 3 suffered from familial CCMs. Fourteen patients with CCMs had multiple lesions and the other 48 had single lesions. Twenty-one patients presented with small lesions (lesion diameter ≤ 10 mm) and 41 harbored large lesions (lesion diameter > 10 mm). Sixteen surgical specimens obtained in patients who underwent anterior temporal lobe resection due to temporal lobe epilepsy were also examined. These surgical specimens did not contain vascular lesions and therefore served as control tissues.

Western Blot Analysis

Surgical specimens were sonicated in a lysis buffer containing 10% glycerol, 3% sodium dodecyl sulfate, 0.05 M Tris (pH 6.8), and 0.01% protease inhibitor cocktail (Sigma). The protein assay was performed with a BCA kit (PerbioScience). Samples containing an equal amount of total protein were loaded onto 10% sodium dodecyl sulfate–polyacrylamide gels. After electrophoresis, protein was transferred onto a nitrocellulose membrane. Nonspecific binding was blocked by a buffer containing 0.1% Tween-20, 2% bovine serum albumin, and 5% non-fat dry milk in Tris-buffered saline. The blots were then incubated with primary antibodies at the following dilutions: mouse anti–PTEN (1:1000), rabbit antiphospho-Akt (Ser473, 1:1000, Cell Signaling Technology), rabbit anti-Akt (1:1000, Cell Signaling Technology), or rabbit anti-PCNA (1:500, Dako) overnight at 4°C. Alpha-tubulin (mouse anti–α-tubulin, 1:5000; Sigma) or actin (mouse anti-actin, 1:1000; Sigma) was detected as the housekeeping protein in each blot to control loading. After the secondary antibody reaction, the signal was produced by incubating the blots with enhanced chemiluminescent detection solutions.

Considering that much of the blood in the caverns of CCM vessels could not be washed out completely, the small fraction of total protein extracted from the tissues may be derived from blood. To exclude the detection of blood-sourced PTEN and actin (as a housekeeping protein), PTEN and actin were also examined in blood samples that included blood cells, blood plasma, and whole blood. In addition, SH-SY5Y cell lysate—shown to express PTEN and P-Akt in our previous study—was used as positive control.

For semiquantification of the results of Western blot analysis, the optical density of the bands representing the specific protein on the blot was measured with commercially available imaging software (Corel Photo-Paint 12). The expression of target protein was normalized by calculating the optical density ratio of the target protein to a housekeeping protein (α-tubulin or actin). Data are presented as the mean optical density ratios ± SDs.

Double Immunofluorescence Analysis

All paraffin-embedded tissue blocks from CCMs (62 samples) and control tissues (16 samples) were used after
routine analysis in the Department of Neuropathology at our hospital. The sections were cut to a thickness of 5 μm and deparaffinized using graded ethanol. The specificity of the PTEN antibody was controlled using Western blot analysis before use. After blocking nonspecific binding, the sections were incubated with mouse anti-PTEN (1:100 dilution, Cell Signaling Technology) and rabbit anti-vWF antibodies (1:200 dilution, Dako) at 4°C overnight. Negative control sections were incubated with mouse and rabbit IgG at the same concentrations as the primary antibodies. After secondary blocking with a biotin blocking system (Dako), biotin-conjugated anti–mouse IgG was added to the sections followed by incubation with fluorescein avidin D (Vector Laboratories). Thereafter, a Texas Red–conjugated anti–rabbit IgG was applied to the sections. The sections were then analyzed under a confocal laser scanning microscope (Zeiss). The specific signal was obtained by setting scanning conditions with respect to laser energy, signal detection (gain and offset), and pinhole size such that no signal was detectable in the negative-staining controls (IgG-incubated section). Immunoreactivity to PTEN was analyzed under background-subtracted conditions and scored as strong (+++), mild (++), weak (+), and negative (−) in the endothelial layer of all vessels as identified on vWF labeling. Specimens that scored weak and negative were considered PTEN insufficient, whereas specimens that scored strong and mild were deemed to represent normal PTEN expression. Data are presented as mean percentage of PTEN-insufficient vessels per patient ± SD and were compared between CCM and control tissues. Within the CCM group, data were compared according to sex, multiple versus single lesions, small versus large lesions, supratentorial versus brainstem lesions, and hemorrhage versus no hemorrhage.

Immunohistochemical Analysis

The deparaffinized adjacent sections were treated with 3% H2O2 for 20 minutes to block endogenous peroxidase activity, and then a blocking buffer containing 2% bovine serum albumin and 10% goat serum in phosphate-buffered saline was used to block nonspecific binding. The sections were then incubated with mouse anti-PTEN (1:50 dilution), rabbit antiphospho-Akt (P-Akt, 1:50 dilution), rabbit anti–PCNA (1:100 dilution, Dako), or rabbit anti–HIF-1α antibodies (1:100 dilution, Biozol) overnight at 4°C. The negative control sections were incubated with mouse or rabbit IgG omitting the primary antibody. After washing, the sections were incubated with horseradish peroxidase–conjugated secondary antibodies (Dako). The substrates 3,3′-diaminobenzidine (Dako) or HistoGreen (LINARIS Biologische Produkte GmbH) solution was applied to the sections according to the manufacturer’s protocol. The sections were finally analyzed by Leica DFC320 microscope and Leica IM50 software.

Human Umbilical Vein Endothelial Cell Cultures

Human umbilical vein endothelial cells were grown in endothelial cell growth medium plus supplement (PromoCell) with 5% CO2 and 95% air in a humid environment. For siRNA experiments, cells (passages 3–6) were seeded at a density of 1.5 × 104/cm2 and the transfection was carried out on the next day. For the proliferation assay, cells were seeded at a density of 1.8–2.0 × 104/cm2 in a 24-well plate, and the assays were performed after different incubation periods.

Silencing of PTEN by siRNA and Cell Treatment

Silencing was achieved by transfection of human umbilical vein endothelial cells with 20 nM of PTEN ShortCut siRNA Mix using GeneSilencer (PeQlab) as described previously. Controls received 20 nM Lit28i Polylinker ShortCut siRNA Mix (both mixes from New England Biolabs).

To establish the time course, cells were cultured for 1, 2, 3, and 4 days after transfection. The time course of PTEN expression was examined on Western blot analysis. Based on this determination of time course, the cell proliferation assay was performed 5 days after transfection. To study whether PI3K/Akt signaling was involved in regulating the proliferation of PTEN, cells were treated twice with wortmannin (100 nM, at 24-hour intervals) alone or on the third day after transfection with PTEN ShortCut siRNA or Lit28i Polylinker ShortCut siRNA Mix.

Proliferation Assay

The cells were trypsinized at room temperature, and the resulting 500-μl cell suspension was added to a counting vessel containing 9.5 ml Isoton-II (Beckmann Coulter GmbH). The cells were counted in this mixture using the Coulter Counter (Beckmann Coulter GmbH). The assay was performed in quadruplicate. Cell density was calculated according to the dilution factor.

Statistical Analysis

All data are expressed as means ± SDs. The statistical analysis was performed using the WinSTAT program, and a probability value < 0.05 was considered significant.

Phosphatase and tensin homolog expression on the Western blot study (Fig. 1D) was analyzed with the Student t-test. For the double-immunofluorescence study, PTEN expression was scored as 0, 1, 2, 3, or 4, representing 0–20, 21–40, 41–60, 61–80, or 81–100% PTEN insufficient, respectively. Nonparametric interval statistical analysis (Wilcoxon test) of the difference between 2 groups was performed next (Fig. 2B and Table 1).

The differences among groups in cell culture experiments (Fig. 6A) were analyzed with the analysis of variance followed by the Scheffé test.

Results

Insufficient PTEN Expression in CCMs

To study PTEN expression in CCMs, Western blot and immunofluorescence staining were performed using the surgical specimens. Western blot analysis revealed reduced PTEN expression in CCMs compared with the control samples (Figs. 1A–C). It is important to note that
PTEN and actin were not detectable in blood samples including whole blood, blood cells, and blood plasma on Western blot analysis. This finding ensured that the detected PTEN and actin in the extracted samples were not from the blood, thus allowing us to semiquantitatively evaluate PTEN expression in the surgical tissues. Semiquantification of the optical density in the blots (Figs. 1A–C) showed a 2-fold lower expression of PTEN in the CCM tissue compared with controls (p < 0.01, Fig. 1D). Surprisingly, the level of Akt was also clearly lower in CCM endothelia than in the control tissues, and furthermore, P-Akt was hardly detectable in the CCMs, but was present in the controls (Figs. 1A–C).

To confirm insufficient PTEN expression in CCMs, immunofluorescence staining was performed in sections of CCM and control. All sections were double-stained with PTEN and vWF to permit localization of PTEN immunoreactivity in the endothelial layer. Under background-subtracted conditions, PTEN expression was evaluated in all blood vessels of each sample. Figure 2A shows samples representing normal and insufficient PTEN expression, respectively. In the CCMs, we found that 33.7% (95% CI 27.0–38.8%) of blood vessels showed PTEN insufficiency, which was significantly higher than that of the control tissues (2.8%, 95% CI 0.3–4.2; p < 0.01, Fig. 2B).

Phosphatase and tensin homolog expression was also compared within the CCM group with respect to clinical manifestations (Table 1). Cerebral cavernous malformation samples from female and male patients were very similar in terms of extent of PTEN insufficiency. Interestingly, a significantly higher level of PTEN insufficiency was found in patients with multiple lesions (43.5%, 95% CI 31.1–55.7%) or small lesions (38.9%, 95% CI 28.1–49.7%) in comparison with patients harboring single lesions (28.2%, 95% CI 22.0–34.4%; p < 0.05) or large lesions (29.8%, 95% CI 22.7–36.9%; p < 0.05). A greater tendency toward PTEN insufficiency was noted in brainstem lesions and in patients with seizure and symptomatic hemorrhages than was seen in supratentorial lesions, patients without seizures, and asymptomatic CCMs that had hemorrhaged, respectively.

Activation Status of Akt in CCMs

We demonstrated a downregulated PTEN expression in CCMs on both Western blot analysis and immunostaining. This finding led us to investigate whether Akt is constitutively activated in CCM. Western blot analysis detected Akt expression in CCMs, but this level was lower than that in the control samples (Fig. 1). Surprisingly, a band representing P-Akt was clearly shown in control tissues and SH-SY5Y cells (positive control), but was absent in CCM tissue extracts (Fig. 1). To confirm this result, immunohistochemical analysis of PTEN and P-Akt was performed on the adjacent sections. Negative controls (using IgG instead of the primary antibody) did not show any detectable signal (Fig. 3G and I), whereas clear expression of P-Akt was found in the control section (Fig. 3F) as well as in the glioblastoma section used as a positive control (Fig. 3H). However, P-Akt immunoreactivity was almost missing in CCMs including the endothelial layer of vessels (Fig. 3D and E), regardless of PTEN expression.

Fig. 1. Reduced PTEN expression in the surgical specimens of CCM patients is shown. A–C: Expression of PTEN on Western blot analysis. Total protein was extracted from surgical specimens of CCM and from normal brain tissue (control). Expression of PTEN, P-Akt, Akt, and actin were detected on Western blot analysis using specific antibodies. Lanes I–VIII and lanes 1–15 represented 7 controls and 15 CCM cases, respectively. Phosphatase and tensin homolog and actin were not detectable in whole blood (Bf), blood cells (Bc), or blood plasma (Bp) samples. The SH-SY5Y cells were used as positive controls. D. Graph showing semiquantification of PTEN expression. The optical density (OD) of the bands represent the level of PTEN and actin expression in each sample measured. The expression of PTEN in each case was normalized by calculation of the OD ratio of PTEN to actin, and presented as the means of OD ratios ± SDs. ** p < 0.01 compared with the control.

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The expression of proliferation marker PCNA in endothelial cells has been considered an indication of the angiogenetic bioactivity of a vessel. We found a negative correlation of PCNA and PTEN expression in CCM endothelial cells. Figure 4 shows 2 sample fields in which PCNA was expressed in PTEN-negative endothelial cells (Fig. 4A and B) but missed in PTEN-positive cells (Fig. 4D and E). Of note, HIF-1α was consistently detected in the endothelial cells of these vessels (Fig. 4C).

**Role of PTEN in Endothelial Proliferation**

To study the role of PTEN in regulating angiogenesis, PTEN was silenced by siRNA in cultured endothelial cells. As shown in Fig. 5A, PTEN was efficiently downregulated throughout Days 1–4 after transfection with PTEN siRNA, whereas the control siRNA did not influence PTEN expression. Phosphatase and tensin homolog silencing was confirmed by the activation of Akt, a downstream kinase in PTEN/PI3K signaling, detected in the same blots (Fig. 5A). Under this silencing condition,"
PCNA expression in endothelial cells increased by 35, 21, and 22% on the 2nd, 3rd, and 4th days after transfection, respectively (Fig. 5B).

Next we attempted to discover whether PTEN silencing would influence the proliferation of endothelial cells, a key step in angiogenesis. As shown in Fig. 6A, PTEN silencing resulted in a 38% increase in cell density 5 days after transfection (p < 0.001), whereas the control siRNA did not alter the proliferation rate of the endothelial cells. The specificity of this function of PTEN was further proven by significant downregulation of PTEN expression in the sister cultures (Fig. 6B). Interestingly, neither wortmannin alone nor its combination with PTEN siRNA significantly altered endothelial proliferation compared with control and the cells with PTEN transfection alone (Fig. 6A).

Discussion

In the present study, we investigated PTEN expression in CCMs and corroborated the role of PTEN in angiogenesis after PTEN gene silencing in cultured endothelial cells. We report here for the first time significant PTEN insufficiency in CCM, which was negatively correlated with angiogenic bioactivity and positively associated with multiple clinical manifestations including multiplicity of lesions and lesion size. Our studies using cultured endothelial cells demonstrated that PTEN silencing upregulated PCNA expression and resulted in endothelial proliferation, most likely through a PI3K-independent mechanism. These findings suggest that PTEN is involved in the pathological angiogenesis of CCMs.

Potential Role of PTEN Insufficiency in CCMs

Increasing evidence supports the notion that CCMs are dynamically developing lesions involving angiogenesis. Therefore, much attention has been paid to identifying the key molecules involved in the pathomechanisms of angiogenesis in CCM. Other authors have documented PTEN’s role in the governance of multiple steps in the angiogenesis processes in normal vascular development, as well as under pathological conditions such as tumors and hypoxia-induced angiogenesis. However, it remains unclear whether PTEN plays a role in the angio-
genesis of CCM. In the present study, we demonstrated a significant PTEN insufficiency in CCMs compared with controls on both Western blot analysis and immunostaining. Furthermore, it is known that the detection of PCNA-expressing cells permits an assessment of the proliferative state of a tissue. We have shown a negative correlation between PTEN and PCNA expression in the endothelial cells of CCM vessels, which suggests a potential role for PTEN in the angiogenesis of CCMs.

Analysis of the association of PTEN expression with the clinical manifestations of CCMs revealed a more pronounced PTEN insufficiency in multiple lesions than in single lesions, indicating PTEN involvement in the de novo formation of CCMs. The significantly higher rate of PTEN insufficiency in smaller lesions (diameter ≤ 10 mm) may also imply a critical role of PTEN in the early development of these lesions.

Symptomatic hemorrhage is often the indication for surgical treatment of CCMs; however, the true biological basis of the underlying hemorrhage remains unclear. The present study revealed a higher tendency toward PTEN insufficiency in CCMs with recent hemorrhaging. Whether PTEN insufficiency is a feature that predisposes to hemorrhage or whether it is a biological consequence of hemorrhage needs to be studied. Further studies of the PTEN expression pattern in other vascular malformations that carry a high risk of hemorrhage, such as arteriovenous malformations and fistulas, may yield some additional evidence in this regard.

Role of PTEN in Angiogenesis: Regulation of Endothelial Proliferation in a PI3K/Akt-Independent Manner

Angiogenesis is a complex biological process that requires the precise coordination of multiple steps including vessel destabilization and matrix degradation, endothelial cell proliferation and migration, lumen formation, and vessel stabilization, all regulated by the delicate balance of various pro- and proangiogenic factors. Stimulation of endothelial proliferation, a putative marker of angiogenesis, has been suggested to outweigh the effect of angiostatic factors during angiogenesis. Here, we provided evidence that PTEN silencing resulted in the activation of Akt, upregulation of PCNA, and a significant increase in endothelial cell proliferation. Together with the negative correlation of PTEN and PCNA expression observed in CCM lesions, we suggest that PTEN is an important molecule in the regulation of endothelial proliferation.

It was surprising that the increase in endothelial proliferation following PTEN silencing was not altered by wortmannin, a specific PI3K inhibitor, which was added to cells at a sufficient concentration and frequency to block Akt activation. This finding suggests the existence of a PI3K/Akt-independent pathway involved in this process, which is consistent with the observation that P-Akt was hardly detectable on either immunohistochemical analysis of CCM sections or on Western blot analysis of CCM tissue extracts. The PI3K/Akt signaling pathway is therefore unlikely to be constitutively activated in CCMs; the reason for this could be, at least partially, the lower expression of Akt as shown by Western blot (Fig. 1).

Potential Pathways of PTEN Insufficiency–Mediated Angiogenesis in CCMs

Cross-talk between PTEN and p53 may shed some light on the PI3K-independent functions of PTEN. Tumor suppressor factor p53 can inhibit angiogenesis in the
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brain via multiple mechanisms, for example by repressing VEGF expression and promoting the expression of p21. The authors of some recent studies have identified a positive feedback mechanism for the regulation of PTEN and p53 protein levels, which occurs even in a PI3K-independent manner.\(^3,21\) We therefore propose that PTEN silencing (or PTEN insufficiency in CCMs) leads to the loss of p53, which subsequently leads to an increase in VEGF levels and downregulation of p21 expression (a potent inhibitor of cyclin-dependent kinase\(^22\)), and eventually to angiogenesis.

Interestingly, Plummer et al.\(^14\) recently reported typical vascular defects mimicking CCM lesions in \(ccm1^{−/−}\) and \(p53^{−/−}\) double mutant mice, but not in \(ccm1^{−/−}\) mice. The \(ccm1\) gene is frequently mutated in patients with familial CCMs. These results suggest that p53 loss results in the sensitization of CCM. It is unclear whether p53 knockout affected PTEN expression in these mutant mice due to the positive feedback regulatory mechanism. If so, PTEN may function as a potential cofactor in the sensitization of CCMs.

Integrin/FAK signaling has recently been suggested to be implicated in the pathogenesis of CCMs.\(^{16,24,25}\) Through its dual specificity protein phosphatase property, PTEN is able to directly inhibit the tyrosine phosphorylation of FAK, a downstream kinase of integrin, thereby
suppressing integrin-mediated cell invasion, migration, and focal adhesion, which are all essential for angiogenesis.\textsuperscript{5,20} Based on these findings, we assume that PTEN insufficiency may cause increased activation of integrin/FAK signaling, thereby promoting the formation and/or progression of CCMs.

**Conclusions**

Frequent PTEN insufficiency in CCM vessels and the crucial role of PTEN in angiogenesis implicate PTEN as a pivotal molecule in the pathogenesis of CCMs. Whether PTEN insufficiency is a direct causative factor in the formation of CCMs, however, and which mechanisms lead to PTEN insufficiency in these lesions requires further investigation. Elucidation of the detailed mechanisms underlying PTEN insufficiency and the downstream signaling affected by PTEN insufficiency in CCMs may advance our understanding and lead to the development of novel approaches for the treatment of this disease.

**Disclosure**

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