Procollagen-Lysine, 2-Oxoglutarate 5-Dioxygenase 2 Expression in Brain Arteriovenous Malformations and its Association with Brain Arteriovenous Malformation Size

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■ BACKGROUND AND OBJECTIVE: **Brain arteriovenous malformations (bAVM) are severe conditions that can cause severe neurologic deficits and mortality. The underlying cellular and molecular mechanisms associated with bAVM growth and rupture remain unclear. The objective of this study was to explore the potential role of PLOD2 (procollagen-lysine, 2-oxoglutarate 5-dioxygenase 2) in the pathophysiology of bAVM.**

■ METHODS: **Expression and localization of PLOD2 were analyzed on tissue microarrays from patients with bAVM ($n = 60$) by immunohistochemistry. Correlations between PLOD2 levels and clinical parameters were examined with a Pearson test or Spearman rank correlation coefficient. Comparison between different clinical parameters was performed using a *t* test or nonparametric Mann-Whitney *U* test. A Fisher exact test was used for categorical data.**

■ RESULTS: **PLOD2 was mainly expressed within the tunica media of the blood vessels. High levels of PLOD2 expression correlated with bAVM size (linear regression, $P = 0.0083$, $R^2 = 0.158$). Small bAVM showed a higher frequency of hemorrhage compared with large ones ($P = 0.001$). Although PLOD2 was not directly associated with bAVM hemorrhage, high PLOD2-expressing bAVM had a lower frequency of hemorrhage compared with low or medium PLOD2-expressing bAVM (25% vs. 63% and 75%, respectively).**

■ CONCLUSIONS: **This study reports for the first time the expression of PLOD2 in bAVM and suggests a potential role of PLOD2 in bAVM pathophysiology. These findings contribute to an better understanding of the microenvironment of bAVM and may foster the development of improved therapeutic strategies for this disease.**

INTRODUCTION

Brain arteriovenous malformations (bAVM) are rare vascular lesions and are considered among the most challenging diseases in neurosurgery. They are characterized by morphologically abnormal vessels that directly shunt blood from the arterial into the venous system without a capillary bed in between. The incidence of bAVM varies between 0.89 and 1.34 cases per 100,000 patient-years.[1-3] The major cause of morbidity and mortality is rupture of bAVM, causing intracerebral hemorrhage. Treatment decisions are based on thorough consideration of the risk of spontaneous hemorrhage in the natural course of bAVM and the risk of multimodal treatment. Known clinical risk factors for hemorrhage include previous history of hemorrhage and arterial hypertension,[4,5] as well as angioarchitectural factors such as intranidal aneurysms, deep venous drainage, high pressure in feeding arteries, deep or periventricular and infratentorial location.[4,6-12] Smaller bAVM were reported to bear an increased risk for hemorrhage, although the underlying pathoanatomic and pathophysiologic reasons remain unclear.[5,9,13] The exact cellular and molecular mechanisms that cause the destabilization and rupture of these lesions are still poorly characterized. The main hypotheses concerning the pathophysiology and clinical course of bAVM focus on inflammatory processes, genetic predispositions, and structural changes in the extracellular matrix (ECM). Although

Key words
- bAVM size
- Brain arteriovenous malformations (bAVM)
- PLOD2

Abbreviations and Acronyms
bAVM: Brain arteriovenous malformations
ECM: Extracellular matrix
PLOD2: Procollagen-lysine, 2-oxoglutarate 5-dioxygenase 2

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Citation: World Neurosurg. (2017) 102:79-84.
http://dx.doi.org/10.1016/j.wneu.2017.02.116

Journal homepage: www.WORLDNEUROSURGERY.org

Available online: www.sciencedirect.com

many recent studies have focused on the role of inflammation in bAVM pathophysiology, the involvement of ECM in this process has also been repeatedly proposed,[14-16] and thus, a recent morphologic analysis of bAVM hypothesized a potential contribution of the collagen system to the pathophysiology of bAVM rupture.[17]

Procollagen-lysine,2-oxoglutarate 5-dioxygenase 2 (PLOD2, also known as lysylhydroxylase 2) is involved in the posttranscriptional modification of collagen molecules and considered as a key mediator of collagen cross-linking and thus an important factor for the stability of the ECM.[18] PLOD2 is a ubiquitously expressed enzyme located in the rough endoplasmic reticulum.[19] Alterations in PLOD2 expression have often been shown to enhance the rigidity of tumors in various malignancies.[20-23] Besides its role in cancer, PLOD2 is known to be associated with several connective tissue diseases, such as Ehlers-Danlos syndrome type VI A,[24] systemic sclerosis,[18] and Bruck syndrome.[25]

The role of PLOD2 in the pathophysiology of bAVM is unknown. The objectives of this study were to determine whether PLOD2 1) is expressed in bAVM tissues and 2) associates with relevant clinical parameters in bAVM.

METHODS

Study Participants

After obtaining the approval of the local ethics committee, we collected the data of all patients with bAVM who were treated in our neurosurgical department between 1995 and 2014. In 60 cases, paraffin-embedded bAVM tissue was available. Five patients had to be excluded because of technical failure, 11 because of low total blood vessels count (<3 vessels). In 1 patient, clinical data concerning hemorrhage were not available; in another patient, the bAVM size was not determinable. The characteristics of the remaining 44 patients are shown in Table 1. These patients had a mean age of 37 years and a mean AVM size of 24 mm.

Table 1. Patient Characteristics Divided by Gender

	Female (n = 22)		Male (n = 22)	
	n	%	n	%
Spetzler-Martin Score				
1	7	31.80	5	22.70
2	5	22.70	7	31.80
3	9	40.90	6	27.30
4	1	4.50	2	9.10
5	0	0.00	0	0.00
n.d.	0	0.00	2	9.10
Preoperative modified Rankin Scale score				
0–2	9	40.90	12	54.50
3	2	9.10	1	4.50
4–6	11	50.00	9	40.90
Hemorrhage				
Yes	7	31.80	9	40.90
No	14	63.60	13	59.10
n.d.	1	4.50	0	0.00
Embolization preoperative				
Yes	2	9.10	3	13.60
No	20	90.90	19	86.40
Localization				
Supratentorial	21	95.50	20	90.90
Infratentorial	1	4.50	2	9.10

n.d., not determinable.

Tissue Microarrays: Construction and Immunohistochemistry

Tissue microarray blocks were built using the Arraymold kit E (Arraymold, Riverton, Utah, USA). Tissue cores were extracted from formalin-fixed/paraffin-embedded bAVM tissues using a 3-mm biopsy punch. The cores were brought into recipient blocks and cut into 2-µm sections. The sections were deparaffinized and the antigens were retrieved by HIER (heat-induced epitope retrieval) in citrate buffer pH 6.0 (Thermo Scientific, Freemont, California, USA). Samples were stained with 0.2 ng/mL rabbit antihuman PLOD2 (ProteintechEurope, Manchester, United Kingdom) overnight at 4°C. Secondary and calorimetric reactions were performed using the UltraVision LP Detection System according to the manufacturer's instructions (Thermo Scientific). Nuclei were counterstained with hematoxylin (Carl Roth, Karlsruhe, Germany) and the sections were covered with Mountex (Medite, Burgdorf, Germany). The samples were digitalized with an Aperio AT2 high-resolution whole-slide scanner and the digital images were viewed with the AperioImageScope software (both from Leica Biosystems, Nussloch, Germany).

PLOD2 in bAVM: Expression and Scoring System

Expression of PLOD2 was determined by immunohistochemistry using tissue microarrays from patients with bAVM. The strongest expression was shown within the tunica media of vessel walls (Figure 1). Next, we assessed the total number of vessels in each sample. The expression of PLOD2 within the tunica media of the determined bAVM vessels was scored as PLOD2 negative and PLOD2 positive; using a modified previously described scoring method[26,27] (representative examples are shown in Figure 2). The samples were scored independently by B.N., L.T., and C.A.D.

Statistical Analysis

Analysis was performed using SPSS for Windows version18.0 (SPSS Inc., Chicago, Illinois, USA) and GraphPad Prism 5 (GraphPad Inc., San Diego, California, USA). Deviation from normal distribution was tested using the Kolmogorov-Smirnov test. Correlation analysis between continuous data was performed using a Pearson test or Spearman ρ for not normally distributed data. Linear regression was performed for the analysis