An Underlying Pathological Mechanism of Meningiomas with Intratumoral Hemorrhage: Undifferentiated Microvessels

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BACKGROUND: **Meningiomas usually present with a gradual onset of symptoms, and their acute presentation with a hemorrhagic event appears to be a rare condition. Although many clinical features of such a condition have been characterized, pathophysiological mechanisms underlying the bleeding remain unclear, and some contradictory results have been reported. The value of tumor vascularity as an index for the bleeding propensity of meningiomas is inconsistent. We sought to identify whether meningiomas have different types of blood vessels, and to explore the association of the different tumor vessels with intratumoral hemorrhage.**

METHODS: **Six patients with meningioma with acute onset due to intratumoral hemorrhage were identified, and 12 nonhemorrhagic meningiomas were matched according to specific clinical data. The characteristics of tumor vessels were examined through immunohistochemical staining of CD31, CD34, and smooth muscle actin (SMA). The number of stained vessels was counted and compared between the 2 groups.**

RESULTS: **Two distinct types of blood vessels were determined in all meningiomas: undifferentiated ($CD31^+$/$CD34^-$) and differentiated ($CD31^+$/$CD34^+$) vessels, and most differentiated vessels were covered by pericytes marked by SMA. However, only the mean number of undifferentiated vessels in hemorrhagic meningiomas was significantly higher than that in controls (15.3 $\pm$ 4.9 vs. 6.4 $\pm$ 3.6; $P < 0.01$). Neither the number of differentiated vessels nor the total number of tumor vessels were significantly different between the 2 groups ($P > 0.05$).**

CONCLUSIONS: **Our results suggest that tumor vasculature in meningiomas is heterogeneous, and that the undifferentiated vessels may play a pivotal role in the spontaneous intratumoral hemorrhage from meningiomas.**

INTRODUCTION

Meningiomas, the most common benign intracranial tumors, were first described by Felix Plater in 1614 and reported in detail by Harvey Cushing in 1938.[1] Although meningiomas encompass a broad spectrum of symptoms and signs, their acute presentation with a hemorrhagic onset appears to be a rare event.[2,3] The rarity of this condition not only makes determining causative factors of the hemorrhage challenging, but also makes the mechanism of spontaneous hemorrhage harder to understand.[2-4] Nonetheless, because the mainstay of treatment is early surgical evacuation, prompt diagnosis of this rare category of intracranial hemorrhage is imperative. Owing to the high morbidity associated with hemorrhagic meningiomas, especially in patients with sudden onset of coma, and their still ambiguous bleeding mechanisms, a more complete understanding of this condition is needed.

According to previously reported cases, the reported incidence of spontaneous meningioma hemorrhage ranges from approximately 0.5% to 2.4%.[2,5] The reason for the low rate of this event in such highly vascularized tumors is not well understood. Fortunately, the body of information on these tumors has been growing, especially over the past several decades. Bosnjak et al.[2] reviewed 145 literature-derived cases and identified 3 main factors associated with an increased propensity for hemorrhage in meningiomas: intraventricular and convexity location, fibrous

Key words
- CD31
- CD34
- Hemorrhage
- Mechanism
- Meningiomas

Abbreviations and Acronyms
CT: Computed tomography
H&E: Hematoxylin and eosin
MRI: Magnetic resonance imaging
SMA: Smooth muscle actin

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histopathology, and age >70 years or <30 years.[2] The pathophysiological mechanisms underlying the bleeding remain poorly defined, however, and the role of intratumoral vasculature in spontaneous hemorrhage from meningiomas is still controversial.[2,3] Some reports have suggested a positive correlation between the intratumoral vasculature and meningioma hemorrhage,[6-8] but other studies have found no significant correlation.[3,9-11]

This apparent discrepancy may be explained in part by the fact that the tumor vasculature is heterogeneous, and different studies have not applied the same vascular marker. Varying characteristics of blood vessels have been identified in prostate cancer, lung cancer, and renal cell carcinoma through various vascular markers.[12-14] For example, 2 distinct types of blood vessels have been identified in renal cell carcinoma: undifferentiated ($CD31^+$/$CD34^-$) and differentiated ($CD31^+$/$CD34^+$) vessels. Most importantly, only the undifferentiated vessels had a significant correlation with higher tumor grades.[14] Although these studies did not examine the relationship between tumor vascularity and spontaneous intratumoral hemorrhage, their results indicate that different types of tumor vessels have distinct implications and significance. Based on these compelling results and our detailed clinical data, we sought to determine whether meningiomas also contain different types of blood vessels in terms of vascular differentiation, and then to explore the associations of these different types of vessels with intratumoral hemorrhage.

METHODS

Study Population and Tissue Specimens

After obtaining Institutional Ethics Board approval, we retrospectively reviewed the charts of 271 patients with meningiomas who underwent craniotomy for tumor resection at the Li Hui Li Hospital of the Medical Centre of Ningbo between July 2008 and December 2015. Six of these 271 patients presented with an acute spontaneous intratumoral hemorrhage. In this study, intratumoral hemorrhage comprises 2 types of bleeding: solitary intratumoral hemorrhage and combined intra/extratumoral hemorrhage. The extratumoral hemorrhage should be secondary to intratumoral bleeding (**Figure 1**). Twelve patients with nonhemorrhagic meningiomas matched according to sex, histological subtype, and lesion location served as a comparison group. No patient had evidence of bleeding tendency or other predisposing factors for hemorrhage, such as receipt of antiplatelet or anticoagulant medication. Patients without available tissue blocks or slides for analysis were excluded from this study.

Formalin-fixed, paraffin-embedded tissue specimens were consecutively sectioned into 4-μm-thick sections for routine hematoxylin and eosin (H&E) staining and immunohistochemical evaluation. Sections were reviewed by 2 independent pathologists under light microscopy, and the diagnosis was reconfirmed and classified according to histological anaplasia as benign (grade I), atypical with incipient signs of anaplasia (grade II), or overtly anaplastic (grade III). This grading corresponds in essence to the 2007 World Health Organization classification system. However, the meningiomas were not further divided into subgroups by grade in this study. Along with the fact that there was an insufficient number of cases to create subgroups for statistical analysis, most meningiomas are highly vascularized tumors, and they are graded mainly according to the mitotic index, not according to tumor vasculature.

Immunohistochemistry and Quantification of Stained Vessels

Along with routine immunohistochemical examination for the tumor diagnosis, classification, and grading, immunohistochemical staining of CD31, CD34, and SMA was performed to identify the different types of blood vessels related to the vascular differentiation and maturation. We used the following primary antibodies: mouse anti-human endothelial cell CD31 monoclonal antibody (1:200; Dako, Glostrup, Denmark), mouse anti-human endothelial cell CD34 monoclonal antibody (1:100; Dako), and rabbit anti-human pericyte SMA monoclonal antibody (1:200; Abcam, Cambridge, UK). Six consecutive slides of 1 tissue block were used for staining, including 2 slides for CD31, 2 for CD34, and 2 for SMA. Immunohistochemical staining was performed with standard reagents and techniques using a sensitive streptavidin-biotinylated horseradish peroxidase complex system according to the manufacturer's instructions. To control for nonspecific binding of the secondary antibody to endogenous sites within the tissue, immunohistochemical controls were performed in which the primary antibody was omitted.

The technique for counting stained blood vessels was modified from the criteria used in previous studies.[14-16] First, 3 separated tumor regions with the greatest density of positive endothelial cells (hotspots) were circled on 1 slide to identify the tumor vascularity. For evaluation of the discrepant expression of CD31 and CD34 in these consecutive tissue sections, the hotspot was defined by the most highly vascularized area in 1 CD31-stained slide. The same regions were circled on the other consecutive slides to ensure that stained vessels in the same regions on each slide were counted. Then the immunoreactive blood vessels in 1 separated region were counted in 3 consecutive microscopic fields at a magnification of 200×. The mean number of vessels counted in the selected areas of 2 sections stained with the same marker was recorded as the vascular density. Any brown-staining endothelial cell or endothelial cell cluster that was clearly separated from adjacent vessels, tumor cells, and connective elements was considered a single, countable vessel regardless of whether a vessel lumen was seen.

Statistical Analysis

All continuous data are expressed as mean ± standard deviation. Statistical analyses were performed with SAS version 8.1 (SAS Institute, Cary, North Carolina, USA), and Student's t test was used for comparison analysis. Differences were considered significant at $P < 0.05$.

RESULTS

Clinical and Histological Features

Among the 271 patients with meningiomas who underwent surgical treatment, 6 (2.2%) had a significant intratumoral hemorrhage and an accurate diagnosis provided by histopathological examination. The clinical features of these 6 patients are summarized in **Table 1**. All 6 patients experienced a stroke-like episode characterized by the sudden onset of acute headache,