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In vitro characteristics of endothelial cells prepared from human cerebral arteriovenous malformation lesions using a novel method

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ABSTRACT

Background and purpose: The cerebral arteriovenous malformation (cAVM) is a usual and continually unaware reason of heamorrhage and seizure. It contains of feeder arteries, drain veins and abnormal vessel nets. However, pathologic mechanisms of the development of cAVM are unknown. The purpose of this study was to explore a novel protocol to isolate, culture and passage endothelial cells (ECs) from human cAVM lesions.

Methods: We developed a protocol for isolating and growing ECs from eight patients with cAVM. The tissues were microsurgically removed from cAVM lesion and were digested by 0.25% Trypsin-EDTA, and cultured in ECM medium. ECs were selected by FACS and confirmed their EC origin by immunocytochemistry of the basic expression patterns of CD31 and CD34. LDL-uptake and capillary tube formation were used to determine their functional features.

Results: The isolated cAVM-ECs exhibited contact inhibition of growth and appearance of rounded cobblestone. cAVM-ECs were CD31- and CD34-positive. In functional assays, cAVM-ECs were able to uptake LDL and form capillary tubes. cAVM-ECs from younger patients proliferated faster than that from elders, and cAVM-ECs were less stable than normal artery ECs. In addition, cAVM-ECs appeared to more easily transform into mesenchymal cells than normal artery ECs.

Conclusion: Using the protocol, isolated cAVM-ECs are stably established, and retain their endothelial phenotypes. These cAVM-ECs may provide a biological tool to examine molecular phenotypes and mechanisms responsible for human cAVM.

1. Introduction

Cerebral arteriovenous malformation (cAVM) is vascular abnormal nidus composed by arteries and veins, which are directly connected through chaotic webs of abnormal vessels in place of normal capillary networks (da Costa et al., 2009). Symptoms of cAVM are involved in headache, seizure, and intra-cranial hemorrhage. Pathologic mechanisms of cAVM development are unknown. It is important to get a better understanding of the natural history and mechanisms of cAVM. cAVM may develop as a result of irregular organization and changes of cAVM endothelial cells (ECs), which may play a part role of the development of cAVM.

Berg et al. recently established an animal model of cAVM using a well-established *Alk1*-gene and *Endoglin* gene knockout rats, which showed a distinctive angiogenic activity of cAVM in vivo (Berg et al., 2003). However, these animal models cannot completely explain the mechanisms of the occurrence and development of cAVM. ECs are approximately accounted for 1–2% of the total number of cells in the normal vessel (Navone et al., 2013). In addition, ECs are embedded in the inner layer of blood vessels, and tightly connected with other cell types (Baudin et al., 2007). ECs can provide a powerful tool to study different vessel diseases. The development of a method to purify ECs from the patients with cAVM is therefore highly demanding. In the present study, we used a novel method to establish cAVM EC cultures from the patients with cAVM and to explore the mechanisms underlying the development of cAVM.

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Table 1
Clinical data for patients with cerebral arteriovenous malformation (cAVM) who underwent microsurgical cAVM resection in the study.

Patient no.	Age (years)	Sex	Hemorrhage	cAVM size (cm)	Spetzler-Martin grade	Radiosurgery
1	14	Female	Yes	3	3	No
2	32	Male	No	4	4	No
3	51	Female	No	4	4	No
4	29	Female	Yes	5	5	No
5	17	Female	No	4	4	No
6	44	Male	Yes	6	5	No
7	8	Male	Yes	3	3	No
8	42	Male	No	5	4	No

2. Materials and methods

2.1. Patients and tissue specimens

From September 2015 to January 2016, we received cAVM specimens from eight patients with cAVM who underwent surgical resection in the Department of Neurosurgery, Tian Tan Hospital, Capital Medical University, Beijing, China. This expectant research on patients was made up of 4 males and 4 females, aged from 8 to 51 years (average 29.6 years). Patients who involved in this study had not adopted gamma knife irradiation or embolization (Table 1). The Human Subject Review Committee of Tian Tan Hospital approved the experimental protocol. All participants furnished written informed consent before taking part in the study.

Surgically removed AVM specimens were rapidly soaked in ice-cold phosphate-buffered saline (PBS) solution (Life Technologies, Grand Island, USA).

2.2. Hematoxylin and eosin (HE) staining of cAVM tissues

Thin slices of cAVM tissue for whole cases were fixed with 4% formaldehyde solution (PFA) (pH 7.0) for 72 h, and then dehydrated with 30% sucrose for 48 h. The tissues were processed entirely by OCT embedding and stored in − 80 °C freezer, and 30 mm-thick sections were incised and placed on glass slides. Tissues were stained with hematoxylin and eosin (HE), and the pathologists in our hospital determined the histological types.

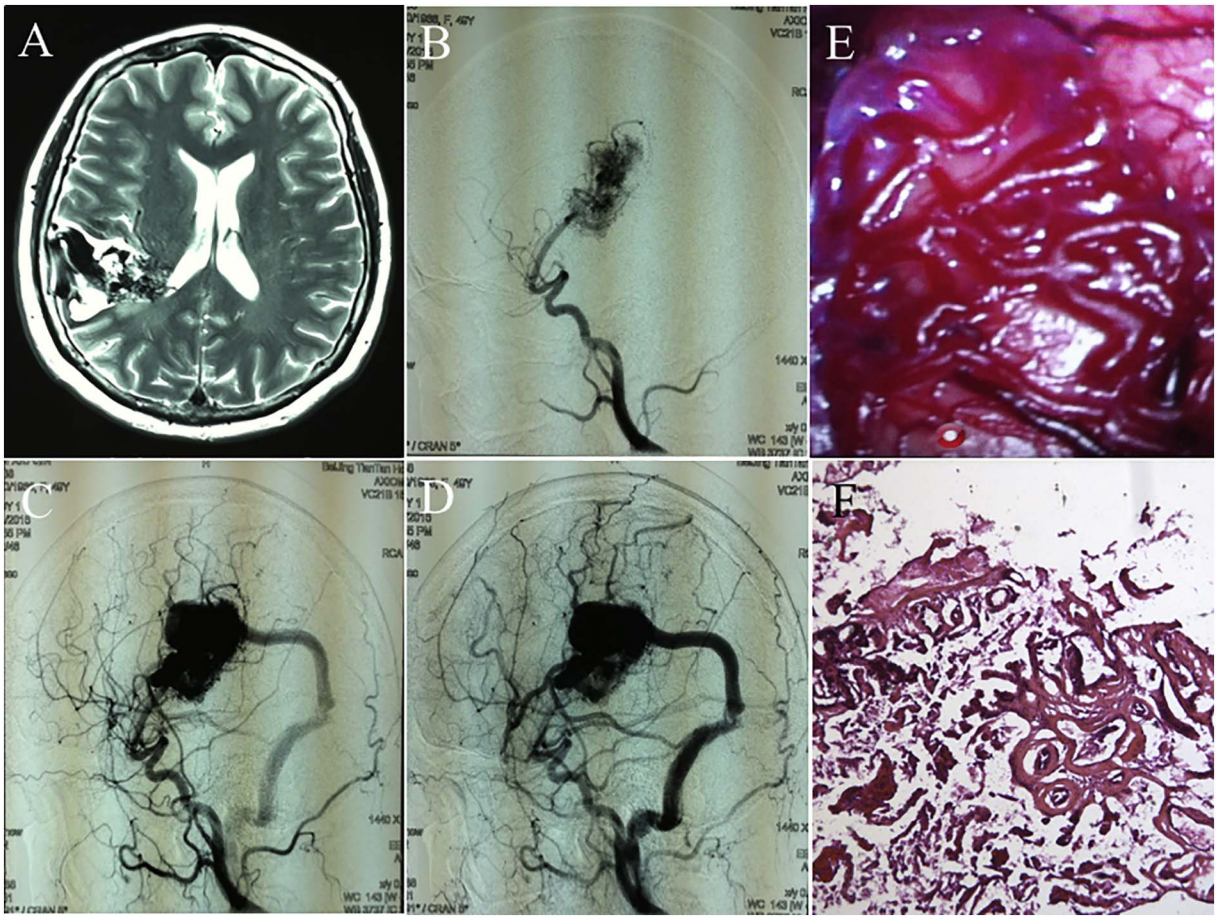

Fig. 1. The characteristics of imaging and histology of cerebral arteriovenous malformation (cAVM). (A) T2WI of MRI imaging; (B–D) digital subtraction angiography (DSA) showed cerebral middle artery as feeder artery to provide blood for the cAVM nidus, which connected to the sigmoid sinus through thick draining vessel; (E) morphology of cAVM under the operational microscope; (F). photomicrograph of cAVM with deformed vascular wall from the nidus of the AVM had many collagen fibers, lacked smooth muscle and elastic fibers with incomplete wall.