RNA Sequencing Analysis Between Ruptured and Un-Ruptured Brain AVM

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Abstract

Background

Brain arteriovenous malformation (BAVM) arises as congenital vascular abnormalities with a significant risk for intracerebral hemorrhage (ICH). RNA sequencing technology has been recently used to investigate the mechanism of diseases owing to its ability to identify the gene changes on a transcriptome-wide level. In this study, we aimed to gain insights into the potential mechanism involved in BAVM rupture.

Methods

Sixty-five BAVM nidus samples were collected among which 28 were ruptured and 37 were un-ruptured. Then next-generation RNA sequencing were performed on all of them to obtain differential expressed genes (DEGs) between these two groups. In addition, bioinformatics analysis was performed to evaluate the involved biological processes and pathways by GO and KEGG analysis. Finally, univariate Cox regression analysis was utilized to obtain the early rupture-prone DEGs.

Results:

A total of 951 genes were differentially expressed between ruptured and un-ruptured BAVM group, of which 740 genes were up-regulated and 211 genes were down-regulated in ruptured BAVMs. Then bioinformatics analysis showed the biological processes and pathways related to the inflammatory processes and extracellular matrix organization were significantly enriched. Meanwhile, some of down-regulated genes are involved in cell adhesion and genes participating in response to muscle activity, as well as the terms about nervous system development. Finally, one hundred and twenty-five genes, a large number of which were involved in inflammation, were correlated with the early rupture of BAVMs.

Conclusions:

The up-regulated genes in ruptured BAVM group were involved in inflammatory processes and extracellular matrix organization while some of the down-regulated genes were participating in cell adhesion and myofibril assembly, indicating the role of enhanced inflammation and reduced vessel strength in BAVMs rupture.

Introduction

Brain arteriovenous malformations (BAVMs) are a complex tangle of abnormal arteries and veins located in the brain and this vascular abnormality lacks intervening capillary network [1]. The risk of spontaneous intracranial hemorrhage (ICH) caused by the rupture of BAVM was estimated at 2–4% per year and can be as high as 34% under certain conditions, and the ICH could cause catastrophic neurological consequences [2, 3]. Early surgical management can be effective in eliminating the cumulative lifetime risk of ICH, however, the higher rate of permanent neurological defects or death makes the treatment of un-ruptured BAVMs increasingly controversial [4]. To date, there is no specific available medical therapy

for the treatment of BAVMs. The investigation of the mechanism of BAVMs rupture would shed light on developing new medical therapies, but the underlying mechanism of this process remained largely elusive.

It is likely that a number of cellular and molecular events played orchestrally which eventually leads to the rupture of BAVM [5]. Instead of examining individual, or a few interrelated gene products, RNA sequencing technology enables investigation of entire transcriptome simultaneously and profiling the gene signature comprehensively. Recent RNA sequencing studies have been used to explore the pathogenesis and development of BAVM [6, 7]. However, no such studies have looked into the rupture processes of BAVM.

Thus, in this study, we classified the relatively large number of BAVM samples into un-ruptured group and ruptured group, and performed RNA sequencing analysis of these samples to unveil the underlying expression signatures along with the rupture of BAVM.

Materials And Methods

Patients and Samples

All study participants with a defined nidus and evidence of arteriovenous shunting on digital subtraction angiography were recruited and underwent microsurgical resection in Neurosurgical Department of Beijing Tiantan Hospital. BAVMs nidus were collected after the resection. All the specimens were snap-frozen and stored in liquid nitrogen. All the participants provided written informed consent and this study was approved by the institutional review board of Beijing Tiantan Hospital, Capital Medical University (KY2017-035-02). BAVM was dichotomised as ruptured and un-ruptured group based on the radiological examination (CT or MRI) combined with their medical history. After filtering out the patient without rupture information, a total of 65 BAVMs patients were included in this study.

RNA extraction, library preparation and sequencing

Total RNA was extracted from the BAVM nidus using Trizol reagent according to manufacturer's instruction. Then, RNA purity was determined by NanoPhotometer® spectrophotometer (IMPLEN, Germany). The concentration and integrity were examined by Qubit® RNA Assay Kit with a Qubit® 2.0 Fluorometer (Life Technologies, USA) and RNA Nano 6000 Assay Kit in a Bioanalyzer 2100 system (Agilent Technologies, USA). The detailed method, RNA sequencing data and the validation of RNA sequencing using RT-qPCR could be found in our previous studies [7–9]

Differential expressed genes (DEGs) and bioinformatics analysis

For obtaining the DEGs between ruptured and un-ruptured BAVM group, reads containing adaptors or poly-N and low-quality reads was firstly removed from the raw data. Then the clean reads were aligned with both the transcript reference and Hg19 RefSeq (RNA sequences, GRCh37) using STAR v2.2.1. Gene expression was calculated as fragments per kilobase of exon per million mapped fragment values with

RSEM v1.3.0. DEGs were defined as differential expressed transcripts with $P < 0.05$ and foldchange >1.50 or <0.67 .

GO and KEGG analysis

For further analyzing the common DEGs, gene ontology (GO) analysis and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis were conducted on DAVID (The Database for Annotation, Visualization and Integrated Discovery, <https://david.ncifcrf.gov/>). A FDR-value of 0.05 was set as the cutoff criterion. Gene expression data were analyzed in R v3.5.1.

Cox regression analysis

The univariate Cox regression analysis was applied to select for BAVM early rupture-prone prognostic DEGs. DEGs associated with overall survival (OS) with $P < 0.05$ were considered statistically significant. The univariate analysis was performed using the R packages “survival” and “survminer” to identify OS-related DEGs.

Results

The clinical baseline of the 65 BAVM patients group were summarized in Table 1, among which 28 were ruptured BAVMs and 37 were un-ruptured BAVMs. All of these patients were Chinese Han ethnicity.

Table 1 Baseline Characteristics of the BAVM patients	
Characteristic	
Sex, female	26 (40%)
Age, y	28 ± 15 (range 6–68)
Clinical presentation	
Ruptured BAVMs	28 (43%)
Un-ruptured BAVMs	37 (67%)
Main location	
Frontal	27 (42%)
Temporal	23 (35%)
Parietal	10 (15%)
Occipital	3 (5%)
Cerebellar	2 (3%)

Differential expressed genes analysis

A total of 951 genes were differentially expressed between ruptured and un-ruptured BAVM group ($P < 0.05$; Foldchange > 1.5 or < 0.67), of which 740 (77.8%) were up-regulated and 211 (22.1%) genes were down-regulated in ruptured BAVMs (Fig. 1). The top 5 up-regulated and down-regulated DEGs (based on the foldchanges) were summarized in Table 2. Notably, all of the 5 up-regulated DEGs (HMOX1, PLA2G7, FUCA1, ACP5 and IFI30) were related to inflammatory process or oxidative stress injury. Meanwhile, among the top 5 down-regulated genes, TNFSF18 and CCL4L1 belongs to cytokines; TPH1 encodes a member of the aromatic amino acid hydroxylase family which plays crucial roles in biosynthesis of serotonin[10]; PKHD1L1 could encode a receptor with inducible T lymphocyte expression[11] and TMEM235 encodes a membrane protein which is over-expressed in brain.

Table 2
The top 5 up-regulated and down-regulated genes between the ruptured BAVM group and un-ruptured BAVM group

Genes	Up- or down-regulation	Foldchange	P value
HMOX1	Up-regulation	10.700	0.004
PLA2G7	Up-regulation	7.869	0.004
FUCA1	Up-regulation	7.618	0.026
ACP5	Up-regulation	6.471	0.006
IFI30	Up-regulation	6.071	0.004
PKHD1L1	Down-regulation	0.361	0.002
TNFSF18	Down-regulation	0.368	0.000
TMEM235	Down-regulation	0.385	0.001
TPH1	Down-regulation	0.394	0.010
CCL4L1	Down-regulation	0.401	0.008

Functional Analysis of The DEGs

Functional analysis of the up-regulated genes in ruptured BAVM showed the biological processes and pathways related to the inflammatory processes were significantly enriched (Fig. 2, Table S1 and 2). GO analysis demonstrated that several inflammatory terms including innate immune response and chemotaxis were highly enriched. In addition, GO terms about extracellular matrix organization was also highly enriched, comprising of integrin-mediated signaling pathway and collagen catabolic process, indicating their important roles in BAVM rupture. KEGG analysis showed inflammation-related diseases and signaling pathways, such as systemic lupus erythematosus, Toll-like receptor signaling pathway, NF- κ B signaling pathway, complement and coagulation cascades were enriched (FDR-adjusted $P < 0.05$). Meanwhile, some of the down-regulated genes were involved in cell adhesion (DSCAM, CNTN2, CERCAM, ITGBL1 and LAMA3 etc.) and genes participating in myofibril assembly (CAPN3, MYOZ1 and MYOZ2).

GO and KEGG analysis showed the down-regulated genes were also enriched in nervous system development, possibly indicating an important parenchymal impact of BAVM rupture (Table S3 and 4).

Early rupture-prone DEGs by univariate Cox regression analysis

One hundred and twenty-five genes were correlated with the early rupture of BAVMs, among which 96 were up-regulated and 29 were down-regulated. GO analysis showed that inflammatory response and innate immune response were significantly enriched (Table S5). There were not KEGG terms from up-regulating genes, as well as GO and KEGG terms from down-regulating genes significantly enriched.

Discussion

In the ruptured BAVM group, a large cohort of up-regulated genes were involved in inflammatory processes including recruited leukocytes, enhanced cytokines, leukocyte migration, all of which contribute to vessel wall damage and weakening[12]. Notably, the innate immune responses were highly up-regulated in ruptured BAVMs, indicating the crucial roles of innate immune system in BAVMs rupture processes. The cell components of innate immune system mainly include monocytes, macrophages and highly phagocytic neutrophils[13], among which macrophage load in BAVM nidus was correlated to higher hemorrhage risk by using ferumoxytol-enhanced MRI [14]. Another study found a higher density of M2 macrophages in the perinidal dilated capillary network (PDCN) in the ruptured BAVM and postulated M2 macrophages could lead to an inappropriate BBB which was prone to bleed[15]. TLRs (toll-like receptors) have crucial roles in activating the innate immune system[16]. As a member of TLR family, TLR4 is mainly expressed on the cell surface of inflammatory cells, endothelial cells and smooth muscle cells[17, 18]. TLR4 can bind to MyD88 and the binding between these two proteins lead to downstream expression of pro-inflammatory cytokines [19]. It has been shown that pathway involves TLR4 and MyD88 promotes tissue remodeling and rupture of brain aneurysm[20]. In this study, TLR4 signaling pathway and MyD88-dependent toll-like receptor signaling pathway were up-regulated in ruptured BAVMs. Except for innate immune responses, several other inflammation-related pathways were also increased in ruptured BAVM group. For instance, NF- κ B is a transcription factor which could functionally regulate several pro-inflammatory genes. NF- κ B p65, a subunit of NF- κ B, was found a higher level in the ruptured BAVM group compared to the un-ruptured BAVM group [21]. Consistently, in our study, NF- κ B signaling pathway was up-regulated in ruptured BAVMs.

Moreover, extracellular matrix organization was also activated in ruptured BAVMs. The genes involved in this process includes COL15A1, COL11A1, COL1A1, COL3A1, COL1A2, COL5A1, COL5A2, COL6A1 and COL6A3, all of which encode subtypes of collagen that constitute the ECM basement membrane and interstitial matrix [22]. Matrix metalloproteinases (MMPs) are capable of degrading all kinds of extracellular matrix proteins and also can process a number of bioactive molecules. MMP9 have been reported causing damage to the BAVM vessel walls, leading to rupture of the nidus[23]. In our study, MMP2, instead of MMP9, was increased in ruptured BAVMs, possibly indicating its important role in

BAVM rupture. In addition, several genes encoding the activities of central nervous system, such as MOG, ZBTB16, CNTN2 and MAL etc., were down-regulated in ruptured BAVMs. Owing to the abnormal vasculature of blood vessels in BAVM, the perinidal brain parenchyma which mainly include the glial cells, astrocytes and neurons are highly affected [24]. We hypothesized that destabilizing BAVM which had increased hemorrhage risk would affect more on the brain parenchyma and result in the abnormal gene expressions and cell functions.

In conclusion, the up-regulated genes in ruptured BAVM group were involved in inflammatory processes and extracellular matrix organization while some of the down-regulated genes were participating in cell adhesion and myofibril assembly, indicating the role of enhanced inflammation and reduced vessel strength in BAVMs rupture.

There are some limitations to the present study. First, despite we removed the surrounding tissue of BAVM nidus as clean as possible, there were still remnants which may partly affect the DEGs. Second, a larger number of BAVMs were still needed to validate the DEGs and the further *in vitro* and *in vivo* experiments would make our results more confirmative.

Conclusions

In ruptured AVM, upregulated genes were primarily involved in inflammatory processes and extracellular matrix organization, while genes related to cell adhesion and myofibril assembly were down-regulated, suggesting that inflammation and reduced vessel strength contribute to AVM rupture. These results provide new ideas for the clinical treatment of AVM in the future.

Abbreviations

BAVM

Brain arteriovenous malformation

ICH

intracerebral hemorrhage

DEGs

differential expressed genes

GO

gene ontology analysis

KEGG

Kyoto Encyclopedia of Genes and Genomes

DAVID

The Database for Annotation, Visualization and Integrated Discovery

OS

overall survival

PDCN

perinidal dilated capillary network

TLRs

toll-like receptors

MMPs

Matrix metalloproteinases

Declarations

Ethics approval and consent to participate

This study was approved by the institutional review board of Beijing Tiantan Hospital, Capital Medical University (KY2017-035-02).

Consent for publication

All the participants provided written informed consent.

Availability of data and materials

Please contact author for data requests.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

Hao Li was responsible for the design of the experiment and the analysis of the results and data. Zihan Yan, Ran Huo, Xiaolong Ya, Jiancong Weng, Hongyuan Xu, Jie Wang wrote the article and drew the relevant charts. Shuo Wang and Yong Cao provided administrative permission for the study and supervised the progress of the study. All authors supervised and approved the final version of the manuscript.

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Not applicable.

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Figures

Figure 1

Volcano map represents differentially expressed genes between ruptured BAVM group and un-ruptured BAVM group. DW, down-regulated; UP, up-regulated; N, not changed.

Figure 2

The top 15 GO and KEGG terms up-regulated in ruptured BAVMs compared with un-ruptured BAVMs.

Supplementary Files

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