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Original Article

Proliferative changes in vascular smooth muscle cells influence the formation of rabbit carotid artery aneurysms

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Abstract: Objective: The rupturing of a cerebral aneurysm is the primary cause of subarachnoid hemorrhage (SAH). SAH often has a high morbidity and mortality rate. During the formation of an aneurysm, the endothelial layer is usually damaged first, followed by an internal elastic lamina breakdown resulting in reduced smooth muscle cell (SMC) degeneration as well as a reduction in the SMC layers. Our study evaluates the proliferative changes of vascular SMCs in aneurysm walls in a rabbit model. Methods: A rabbit carotid artery aneurysm model was established and tissues were isolated from the aneurysms at various time points, such as one week, three weeks and five weeks. The isolated aneurysmal tissues were divided into two parts at each time point. The first part was used for hematoxylin and eosin (H&E) staining, immunohistochemistry, and RT-PCR analysis. The second part was also cultured in 0.5% serum for a week and later used for H&E staining, immunohistochemistry and RT-PCR analysis. Results: We observed that the expressions of SM22α mRNA and the hypertension-related gene 1 (HRG-1) mRNA from the SMCs of the aneurysm wall were reduced remarkably in the specimens from 3-weeks onwards as the size of the aneurysm increases while the expression of platelet-derived growth factor (PDGF) also increases. We also observed a significant proliferation of SMCs in the specimens from 3-weeks onwards in the cultured group. The expressions of SMC marker SM22α mRNA, HRG-1 mRNA, and the cytoskeleton proteins (SM α-actin) were elevated as compared to the expression of PDGF in the cultured specimens from 3-weeks onwards. Conclusion: Aneurysmal growth is related to the proliferative changes of vascular SMC, but further research into the signaling mechanism is still needed.

Keywords: Aneurysm, vascular SMC, SAH, proliferation, rabbit, PDGF

Introduction

The primary cause of subarachnoid hemorrhage (SAH) is the rapturing of a cerebral aneurysm. This event is usually associated with a high morbidity and mortality [1, 2]. The internal carotid artery (ICA) is divided into an endothelium layer, an SMC layer, and the outer membrane layer [3]. The endothelial layer is destroyed first during the formation of the aneurysm, followed by a breakdown of the internal elastic lamina resulting in reduced SMC degeneration and subsequent reduction in the smooth cell layers [3, 4]. Finally, the outer membrane is damaged and the aneurysm ruptures. Studies have shown that the proliferation and apoptosis of SMCs occurs during the formation of aneurysms [3]. Therefore, the plasticity of vascular SMCs is very crucial in the evolution of aneurysms.

Vascular SMCs have distinctive physiological processes such as proliferation, differentiation, and apoptosis, which demonstrate their transformational capabilities as well as their plasticity. The rupturing of an aneurysm becomes inevitable when the number of vascular SMCs undergoing apoptosis gradually increases and the SMC layer is progressively decreased to a certain degree [5, 6]. At present, the mechanism involved in the pathogenesis of aneurysm formation and the subsequent rupture is unclear [6]. It is, however, certain that the formation of an aneurysm takes several years.

Therefore, we speculated that the proliferation activity of SMCs is different over time during the formation of an aneurysm.

Our current study focuses on the pathological changes of vascular SMC layers during aneurysm formation using a rabbit carotid artery aneurysm model. We investigated the proliferation changes during aneurysm formation as well as how these proliferative changes influence the growth of aneurysms. Our study will provide a theoretical basis for clinical aneurysm repair from the perspective of promoting SMC proliferation.

Materials and methods

Establishment of the carotid aneurysm model

New Zealand white rabbits were purchased from Suzhou Huqiao Biotechnology Company and kept at the Animal Center of Jiangsu University, China. All animal experiments were approved by the Administrative Committee of the Experimental Animal Care and Use of Shanghai, and conformed with the National Institute of Health guidelines on the ethical use of animals.

Six healthy New Zealand rabbits (2.5-3.0 kg) were randomly placed into each group, including group A (the control), group B (the one-week group), group C (the three-week group), and group D (the five-week group). The rabbits in group A did not undergo any procedure. Every group consisted of 12 rabbits. For groups B, C and D, the animals were anesthetized with 10% chloral hydrate (3.5 ml/kg) via an intraperitoneal injection. The fur around neck was sheared and the exposed skin was disinfected with iodophor. A 3 cm incision was made midline along the upper neck, and the muscle layers were dissected carefully under the surgical microscope to isolate the ICA and external carotid artery (ECA) bifurcations. The fibrous tissue on the surface of the artery at the start of the bifurcation was carefully removed, and 75 u/ml of 0.3 ml surface elastase (Maikun Bioengineering Ltd., Shanghai, China) was infused. Fifteen minutes later, 500 u of heparin was injected into the auricular vein [5, 7].

The skin was sutured and the animals were returned to their cages to recover. The rabbits in groups B, C, and D were sacrificed 1, 3, and 5 weeks after surgery, respectively. ICAs from the sacrificed rabbits were obtained and divided into two parts. One part of the specimens of the ICA aneurysms were dissected and collected for different procedures, including H&E staining, transmission electron microscopy, RNA preparation, and the isolation of SMCs. Another part was cultured in vitro for a week, and then they were collected for immunocytochemical staining and RNA detection.

H&E staining

The rabbit ICA aneurysm samples were fixed in 4% paraformaldehyde, dehydrated, embedded in paraffin, sliced into sections, stained with hematoxylin-eosin, and then observed under an Olympus CKX31 inverted lighted microscope (Olympus Corporation, Tokyo, Japan).

Immunocytochemical staining

The rabbit aneurysm paraffin sections were dewaxed, hydrated, blocked with 0.04% hydrogen peroxide in methanol for 15 minutes, and blocked in normal goat serum at room temperature for 20 minutes. The specimens were incubated in mouse anti-platelet-derived growth factor polyclonal antibodies (1:200) at 4°C overnight, goat anti-mouse IgG (1:200) at room temperature for 30 minutes, streptavidin-biotin complex at room temperature for 20 minutes, developed with 3, 3'-diaminobenzidine for 3 minutes, and observed under an Olympus CKX31 inverted lighted microscope (Olympus Corporation, Tokyo, Japan). A 0.01 ml PBS wash was performed three times between each step. The sections were dehydrated, permeabilized in xylene, and mounted. Absorbance values were analyzed using Image-pro plus software (Media Cybernetics, China/Japan) Inc.

RT-PCR

The samples were chopped into pieces and their RNA was prepared using PCR primers synthesized by Shanghai Biological Engineering Ltd. and were sequenced as follows.

Gene	Primer sequence	Length (bp)
SM22α	Upstream: 5'-TTC TGC CTC AAC ATG GCC AAC-3'	252
	Downstream: 5'-CAC CTT CAC TGG CTT GGA TC-3'	
Hypertension-related gene 1	Upstream: 5'-TTG CTG GGC TAC AAT GAT-3'	309
	Downstream: 5'-CTT GCT GGC ACA GAT GAG-3'	

Table 1. Internal carotid artery aneurysm volume after aneurysmal model establishment

Index	Time after lentiviral injection (week)			
	Control	1	3	5
Neck width (mm)	0	6.3±0.3[a]	9.4±0.3[b]	9.8±0.4[c]
Length (mm)	0	10.0±0.2[a]	13.7±0.4[b]	14.1±0.5[c]

The dimensions of the aneurysmal lumen also increased on 3 and 5 weeks. Data are expressed as mean ± SD. There are six rabbits per time point. Least significant difference *t*-test was used for comparison. [a]$P < 0.05$, *vs*. 1 week; [b]$P < 0.05$, *vs*. 3 weeks; [c]$P < 0.05$, *vs*. 5 weeks.

1 μg of RNA was used for the reverse transcription reaction. The PCR reaction was performed under the following conditions: 94°C denaturation for 50 s, 56°C annealing for 50 s, 72°C extension for 1 min, for a total of 32 cycles. The products were visualized by running 1.7% agarose gel electrophoresis, followed by ethidium bromide staining. The intensities of the bands were quantitated using a GIS 2010 automatic image analysis processing system. Each experiment was performed in triplicate.

Statistical analysis

The data are expressed as the mean ± SD. The analysis of variance was conducted using SPSS 16.0 software. The multi-group data were analyzed using a paired comparison and a least significant difference *t*-test. A value of $P < 0.05$ was considered statistically significant.

Results

The establishment of an internal carotid artery aneurysm and the quantitative analysis of the experimental animals

A total of 48 healthy New Zealand rabbits were selected for the ICA aneurysm model establishment. The rabbit models of each group were separately analyzed at 1, 3, and 5 weeks after being infused with elastase. The normal ICAs of six randomly-selected model rabbits were selected as the normal controls. The ICA aneurysm volume was biggest in the 5-week group. The aneurysms of the 3-week group rabbits were a little smaller than the 5-week group rabbits (**Table 1**). It was further noted that the aneurysms grew larger over time.

H&E staining

The rabbits in group A were sacrificed, and the thicknesses of the ICAs on the left and right sides were observed. We noted equal thicknesses in both carotid arteries. Microscopic changes in the aneurysms were further confirmed. In comparison with sections prepared from the control rabbits, vessel media sections prepared from the rabbits with elastase infusions resulting in the development of aneurysms demonstrated a fragmentation of the elastic fiber along the vessel accompanied by a decrease in the number of vascular SMCs as well as the destruction of endothelial cells (**Figure 1**). These changes progressed over time and are consistent with the general idea that the development of aneurysms is associated with the degradation of the extracellular matrix, the activation of proteolytic enzymes, smooth muscle inflammatory response and apoptosis. The degree of repair in the 3-week and 5-week specimens cultured for a week was relatively high, and the most obvious proliferation of SMCs existed in the 3-week specimens.

Immunocytochemical staining

Platelet-derived growth factor (PDGF) expression could not be detected in the ICA vessel walls of the normal rabbits (**Figure 2**). However, the PDGF expressions were high at 3 and 5 weeks (**Figure 2**; **Table 2**). In addition, PDGF was visible in each layer of the aneurysmal wall, and the number of vascular SMCs was decreased at 1 week following the model induction (**Figure 2**). PDGF is a marker that can synthesize vascular SMCs. Thus, the expression of PDGF in the 3-week specimens cultured for a week was higher, demonstrating that the vascular SMCs transformed from the contraction type to the synthesis type, providing greater ability to repair the aneurysmal wall (**Figure 2**). The transformation of the vascular SMCs indicated that there were many vascular SMCs undergoing proliferation.

Decreased expressions of SM22α and HRG-1

SM22α is a marker for the contractile mode of vascular SMC. It is usually in the differentiated state. In the experimental rabbits which did not receive an elastase infusion, the vascular SMCs highly expressed SM22α. Also, hypertension-related gene 1 (HRG-1) is associated with the proliferation of the vascular SMC gene. It is highly expressed in normal vascular SMCs. Nevertheless, its expression is usually low in

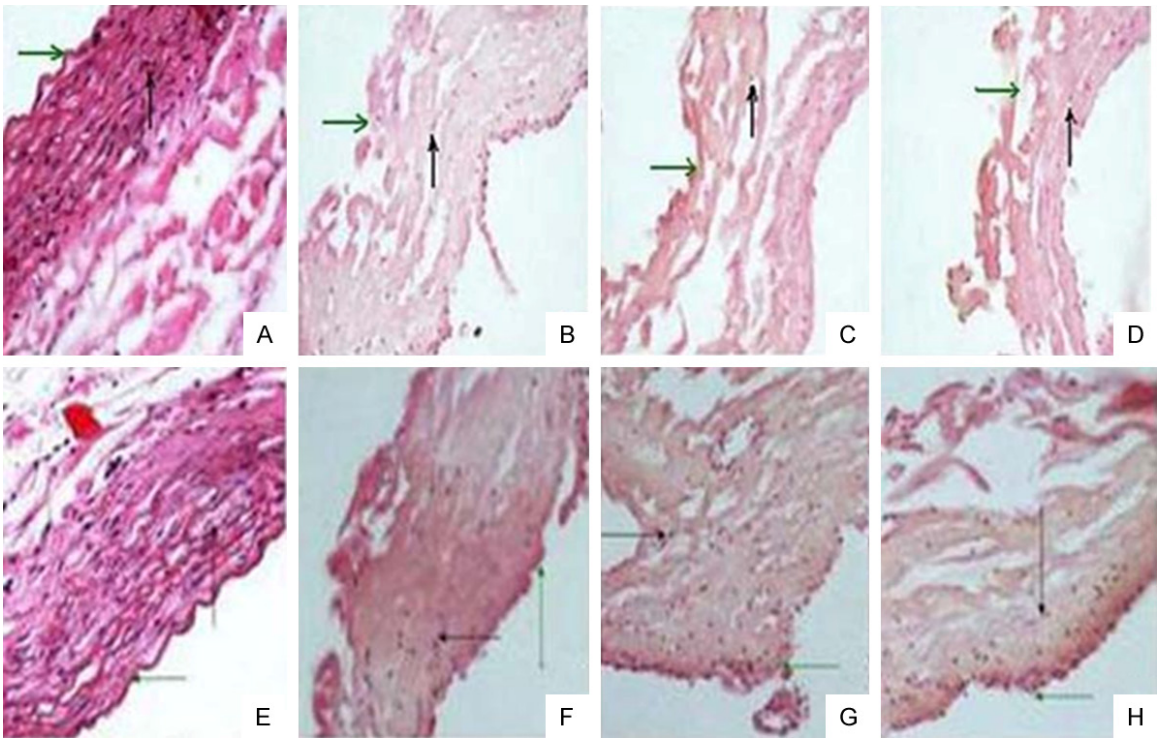

Figure 1. H&E staining to demonstrate the fragmentation of vessel media during the growth of the aneurysms. A-D. From the control group to the 5-week group respectively; E-H. From the control group to the 5-week group cultured in vitro for a week respectively.

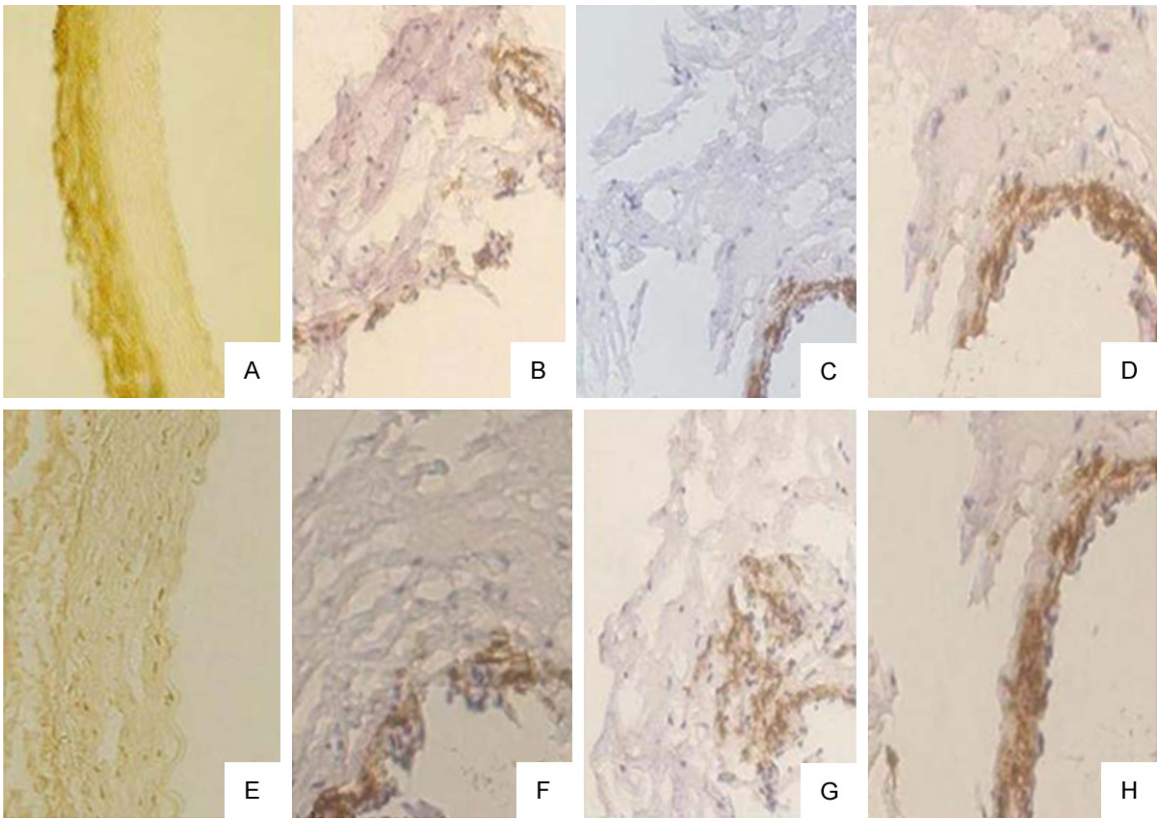

Figure 2. Immunostaining to demonstrate the protein expression of PDGF in aneurysms. A-D. From the control group to the 5-week group respectively; E-H. From the control group to the 5-week group cultured in vitro for a week respectively.

Table 2. Internal carotid artery aneurysm volume after aneurysmal model establishment

Index	Time after lentiviral injection (week)			
	Control	1	3	5
Neck width (mm)	0	6.3±0.3[a]	9.4±0.3[b]	9.8±0.4[c]
Length (mm)	0	10.0±0.2[a]	13.7±0.4[b]	14.1±0.5[c]

The PDGF expression was high at 3 and 5 weeks. The dimensions of the aneurysmal lumen also increased on 3 and 5 weeks. Data are expressed as mean ± SD. There are six rabbits per time point. Least significant difference *t*-test was used for comparison. [a]$P < 0.05$, *vs*. 1 week; [b]$P < 0.05$, *vs*. 3 weeks; [c]$P < 0.05$, *vs*. 5 weeks.

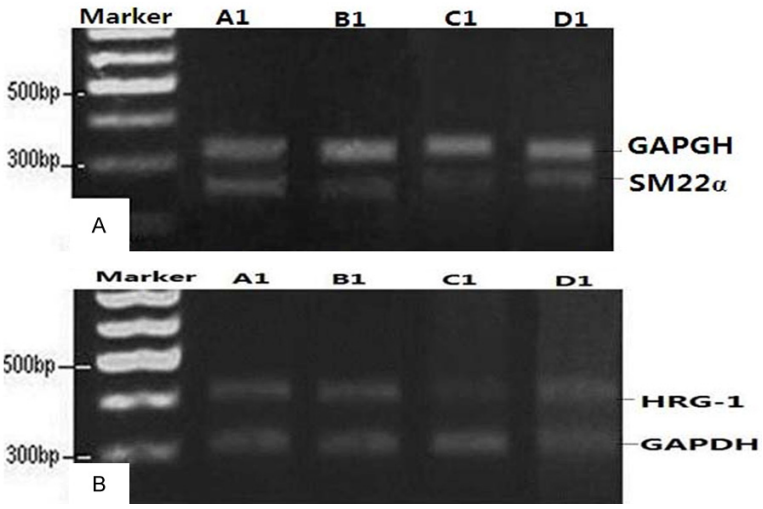

Figure 3. RT-PCR analysis to demonstrate the transcript level of VSMC marker SM22α and HRG-1 in rabbits with aneurysms. A1-D1, from the control group to the 5-week group respectively.

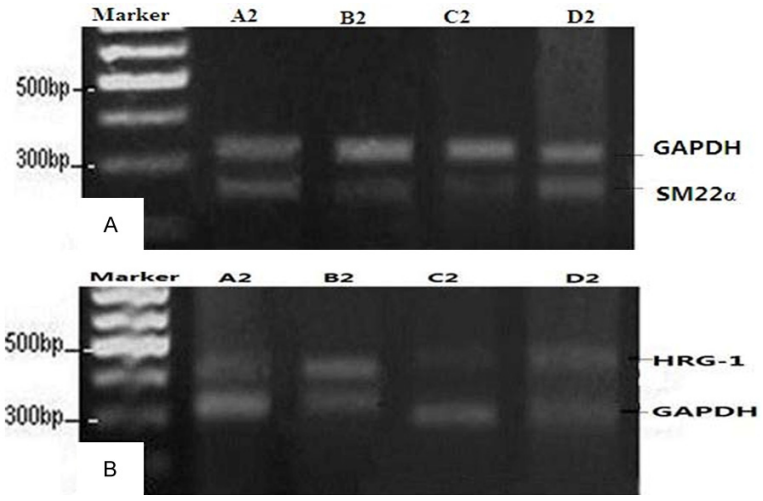

Figure 4. RT-PCR analysis to demonstrate the transcript level of VSMC marker SM22α and HRG-1 in rabbits with aneurysms. A2-D2, from the control group to the 5-week group cultured in vitro for a week respectively.

the synthetic phenotype of SMCs. However, in rabbits with aneurysms, the expressions of SM22α and HRG-1 were decreased in a time-dependent manner (**Figures 3**, **4**; **Tables 3**, **4**). Their expression was lowest in the 3-week specimens. Also, the expressions of SM22α and HRG-1 were lowest in the 3-week specimens cultured for a week.

Cytoskeleton proteins in aneurysms

To further confirm the phenotypic change of vascular SMC, we also checked the expressions of cytoskeleton proteins such as SM α-actin. In rabbits with elastase-induced aneurysms at the right internal carotid artery, the expressions of SM α-actin along vascular SMCs were significantly decreased in a time-dependent manner (**Figure 5**; **Table 5**), indicating that the decreased expression of cytoskeleton proteins was associated with development of aneurysms. Their expressions were low in the 3-week specimens. Also, the expression of α-actin was low in the 3-week specimens cultured for a week.

Discussion

Aneurysm expansion may occur due to many reasons, but as a common clinical disease, its pathogenesis is still unclear [5, 6]. Aneurysms occur as a result of vessel dissection with changes in the composition of blood vessel media [6]. Studies have shown that SMCs are proficient in undergoing phenotypic and func-

Table 3. The results of image analysis of SM22α by PCR

Group	A1	B1	C1	D1
GAPDH	28.32±1.27	17.36±1.07	11.82±1.34	6.19±0.84
SM22α	98418±129	43773±143	56835±157	34636±141
Mean Ratio	1.15	0.99	0.57	0.33

The expression of SM22α was decreased in a time-dependent fashion in rabbits with aneurysm. The samples were grouped into A1, B1, C1 and D1. Data are expressed as mean ± SD. There was a total of 24 rabbits in each GAPDH & SA22α groupings, six per each group (A1, B1, C1 & D1). Least significant difference *t*-test was used for comparison. $^{GAPDH\ \&\ SA22\alpha}P < 0.05$ *for* A1; $^{GAPDH\ \&\ SA22\alpha}P < 0.05$ *for* B1; $^{GAPDH\ \&\ SA22\alpha}P < 0.05$ *for C1*; $^{GAPDH\ \&\ SA22\alpha}P < 0.05$ *for D1. Mean ratios for A1, B1, C1 & D1 were 1.15, 0.99, 057 and 0.33 respectively.*

Table 4. The results of image analysis of HRG-1 by PCR

Group	A1	B1	C1	D1
GAPDH	84612±134	74241±112	87617±118	72321±115
SM22α	93450±113	46236±159	00824±036	63564±127
Mean Ratio	1.19	0.62	0.01	0.87

The expression of HRG-1 was decreased in a time-dependent fashion in rabbits with aneurysm. The samples were grouped into A1, B1, C1 and D1. Data are expressed as mean ± SD. There was a total of 24 rabbits in each GAPDH & HRG-1 grouping, six per each group (A1, B1, C1 & D1). Least significant difference *t*-test was used for comparison. $^{GAPDH\ \&\ SA22\alpha}P < 0.05$, *for* A1; $^{GAPDH\ \&\ SA22\alpha}P < 0.05$, *for* B1; $^{GAPDH\ \&\ SA22\alpha}P < 0.05$, *for C1*; $^{GAPDH\ \&\ SA22\alpha}P < 0.05$, *for D1. Mean ratios for A1, B1, C1 & D1 were 1.15, 0.62, 0.01 and 0.87 respectively.*

tional modifications leading to a proliferative, inflammatory phenotype [8-11]. This modification potential is often depicted with a reduced expression of SMC contractile proteins as well as the augmented production of proinflammatory cytokines. This phenomenon has been observed during the pathological formation of neointima [8, 9, 12]. It is confirmed that mature SMCs contributed enormously to neointima formation [8].

Studies have demonstrated that precise SMC fate-mapping methodology in the location of restenosis confirms that the majority of proliferating intimal cells originated from well-developed SMCs [8, 13]. Reporter-positive, but SMC marker-negative, SMC-derived cells in the arterial adventitia signify that mature SMCs partake in both intimal and adventitial modification [8]. Studies have demonstrated that an extreme proportion of SMCs in atherosclerotic lesions are clearly deficient in the expression of conformist SMC markers but demonstrate a macrophage-like phenotype [8, 14, 15]. Our results, using the rabbit aneurysm model, demonstrated a disease progress related to changes in vascular SMC. Therefore, it is possible that the maintenance of the proliferative activity of vascular SMC and related cytoskeleton proteins might help prevent the dissection of vessel media. This therapeutic hypothesis needs to be further tested.

The ICA is divided into the endothelium, the smooth muscle cell layer, and the outer membrane. Studies have shown that SMC proliferation and apoptosis occur and are crucial during aneurysm formation [6, 16]. Aneurysms usually rupture as a result of shear stress and inflammation. Furthermore, the proliferative activity of SMC might affect aneurysm formation as well as rupture [6]. SMCs have different physiological processes such as proliferation, differentiation, and apoptosis, as well as transformational abilities during the formation of intracranial aneurysms. SMCs also exhibit a very high plasticity during the pathogenesis of aneurysms. Studies have shown that the rupturing of aneurysms occurs when the amount of SMC apoptosis gradually increases, resulting in a decrease in the smooth muscle layer [17-19]. Our study, therefore, focuses on the proliferation activity of vascular SMCs in the walls of aneurysms in New Zealand rabbits. This study provides the hypothetical basis of vascular SMC proliferation and intracranial aneurysm wall repair.

Studies have demonstrated that SM22α mRNA, a marker gene of the vascular SMC phenotype, is only secreted by contractive vascular SMCs [20, 21]. Further studies have shown that HRG-1 is a negative regulatory gene associated with vascular SMCs [21, 22]. We demonstrated in our early research that early and persistent lentivirus trans-infection could augment synthetic vascular SMC numbers by restraining cell differentiation as well as repairing damaged aneurysmal walls [21]. We observed that, SM22α mRNA and HRG-1 mRNA expressions were high in the aneurysmal wall 1- and 2-week old rats after lentiviral injection immediately following the aneurysmal model formation [21]. In our current experiment, we observed that

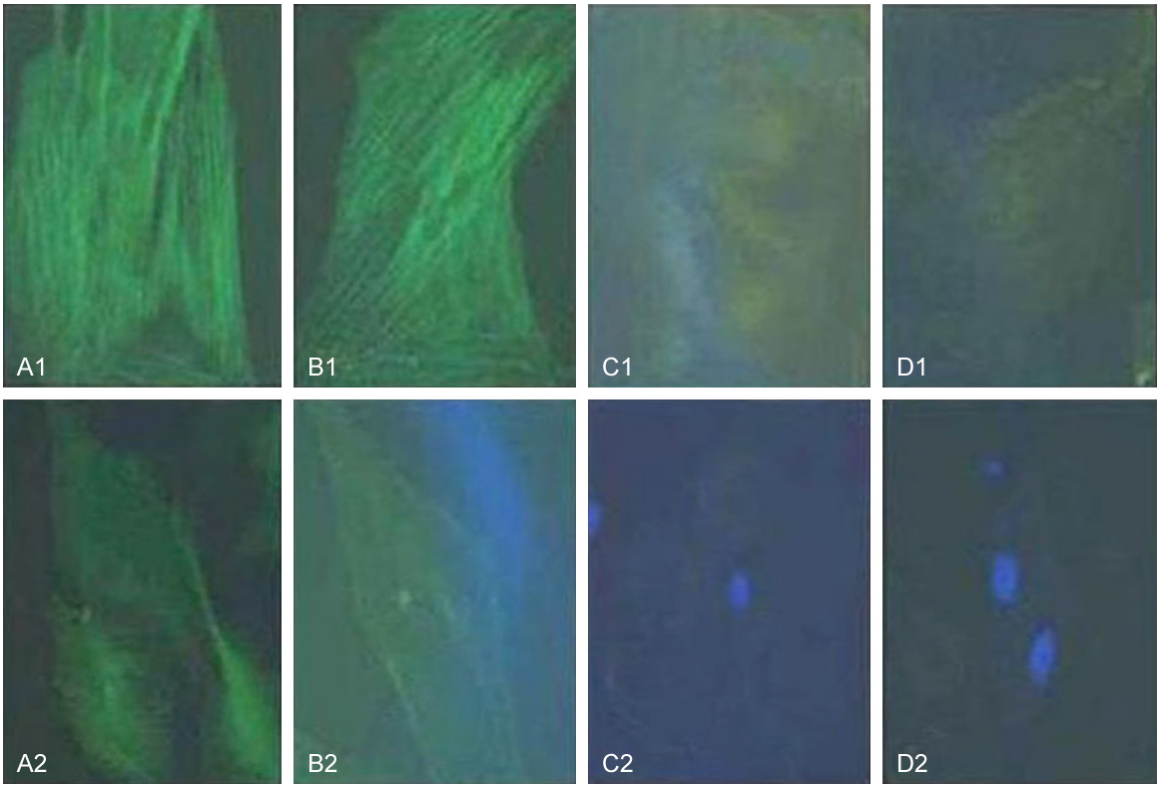

Figure 5. Immunostaining to demonstrate the protein expression of cytoskeleton proteins in aneurysms. A1-D1. From the control group to the 5-week group respectively; A2-D2. From the control group to the 5-week group cultured in vitro for a week respectively.

Table 5. Internal carotid artery aneurysm volume after aneurysmal model establishment

Group 1	A1	B1	C1	D1
α-actin	28.32±1.27	17.36±1.07	11.82±1.34	6.19±0.84
Group 2	A2	B2	C2	D2
α-actin	25.35±1.15	15.22±1.03	3.82±0.74	1.19±0.38

Cytoskeleton protein SM α-actin was use to confirm the phenotypic change of VSMC. The samples were grouped into two (1 & 2). Group 1 (A, B, C & D) were valves ICA before the aneurysmal model establishment while Group 2 (A, B, C & D) where valves for the ICA aneurysm volume after aneurysmal model establishment. Data are expressed as mean ± SD. There a total of 24 rabbits in both grouping, six rabbits in each group (A1, B1, C1 & D1) & (A2, B2, C2 & D2). Least significant difference *t*-test was used for comparison. $^{\alpha\text{-actin}}P < 0.05$, *for* both groups (1 & 2).

SM22α mRNA and HRG-1 mRNA expressions from the aneurysm wall were remarkably reduced in the 3-week specimens with the constant growth of the aneurysm while PDGF expression was increased in our rabbit aneurysm models.

It is confirmed that the growth factor PDGF-BB is a critical regulator during initial vascular formation. It is intensively secreted by the endothelial cells. It is also a prerequisite for the early conscription as well as the successive proliferation of pericytes and SMCs within the maturing vasculature [23, 24]. Nevertheless, the introduction of differentiated SMC to PDGF-BB triggered the SMC phenotypic modulation by increasing SMC proliferation and migration, and by down-regulating the SMC differentiation marker gene secretion [23]. We also noted a significant proliferation of SMCs in the 3-week cultured specimens. The expression of smooth muscle markers SM22α mRNA, HRG-1 mRNA, and the cytoskeleton proteins (SM α-actin) where decreased while the expression of PDGF was low in the 3-week specimens. This suggests that the aneurysm continual growth is related to active transformations of vascular SMC.

The remodeling of SMC from a contractile to a synthetic mode has been observed in damage repair and tissue healing [16, 25] which corre-

sponds to a specific type of cytoskeleton protein under various conditions [17, 26]. The signals associated with the induction of SMC remodeling are yet to be understood, although changes in the phenotype might represent a compensatory mechanism. For example, the decrease in the expression of the cytoskeleton protein may indicate an increase in cell mobility during cell division, associated with an increased remodeling of SMCs from the differentiated contractile mode to the less differentiated synthetic mode with the ability to proliferate. In our study, we observed a reduced expression of cytoskeleton proteins.

Mechanical stretching and shear stress may induce changes in gene expression through which SMC remodeling could be modulated [22, 27]. Conditions such as atherosclerosis can result in the destruction and/or changes in the structural components of blood vessel walls resulting in a change in the mechanical and/or shear stress of blood flow to induce aneurysms. It will be worth studying the pattern of gene expression under conditions of mechanical stretching and/or shear stress. In addition to the change in the SMCs, changes in collagen and the extracellular matrix (ECM) are also important for the formation of aneurysms. Further studies to explore the relationship between changes in ECM and the changes in SMC during the development of aneurysms will be of help in understanding the pathophysiological processes of this disease.

Overall, using the rabbit aneurysm model, we established that the changes in gene expression in the vessel media occur during the development of aneurysms. These changes indicate the proliferation activity of vascular SMC remodeling from contractile vascular SMC of a more differentiated state to the synthetic vascular SMC of a less differentiated state, accompanied by a decreased expression of the cytoskeleton protein SM α-actin [28, 29].

Conclusions

The growth of the aneurysm wall is closely related to the proliferation activity of vascular SMC. This finding might provide an experimental hypothesis to help establish whether the maintenance of contractile vascular SMC may help prevent the formation and progress of aneurysms.

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Disclosure of conflict of interest

None.

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References

[1] Lantigua H, Ortega-Gutierrez S, Schmidt JM, Lee K, Badjatia N, Agarwal S, Claassen J, Connolly ES and Mayer SA. Subarachnoid hemorrhage: who dies, and why? Crit Care 2015; 19: 309.

[2] Schievink W, Wijdicks E, Parisi JE, Piepgras D and Whisnant J. Sudden death from aneurysmal subarachnoid hemorrhage. Neurology 1995; 45: 871-874.

[3] Kothapalli CR and Ramamurthi A. Induced elastin regeneration by chronically activated smooth muscle cells for targeted aneurysm repair. Acta Biomater 2010; 6: 170-178.

[4] Padró T, Mesters RM, Dankbar B, Hintelmann H, Bieker R, Kiehl M, Berdel WE and Kienast J. The catalytic domain of endogenous urokinase-type plasminogen activator is required for the mitogenic activity of platelet-derived and basic fibroblast growth factors in human vascular smooth muscle cells. J Cell Sci 2002; 115: 1961-1971.

[5] Chalouhi N, Ali MS, Jabbour PM, Tjoumakaris SI, Gonzalez LF, Rosenwasser RH, Koch WJ and Dumont AS. Biology of intracranial aneurysms: role of inflammation. J Cereb Blood Flow Metab 2012; 32: 1659-1676.

[6] Penn DL, Witte SR, Komotar RJ and Connolly ES Jr. The role of vascular remodeling and inflammation in the pathogenesis of intracranial aneurysms. J Clin Neurosci 2014; 21: 28-32.

[7] Altes TA, Cloft HJ, Short JG, DeGast A, Do HM, Helm GA and Kallmes DF. Creation of saccular aneurysms in the rabbit: a model suitable for testing endovascular devices. AJR Am J Roentgenol 2000; 174: 349-354.

[8] Majesky MW, Horita H, Ostriker A, Lu S, Regan JN, Bagchi A, Dong XR, Poczobutt J, Nemenoff RA and Weiser-Evans MC. Differentiated smooth muscle cells generate a subpopulation of resident vascular progenitor cells in the ad-

ventitia regulated by Klf4. Circ Res 2017; 120: 296-311.
[9] Clowes AW, Reidy M and Clowes M. Kinetics of cellular proliferation after arterial injury. I. Smooth muscle growth in the absence of endothelium. Lab Invest 1983; 49: 327-333.
[10] Mitra A and Agrawal DK. In stent restenosis: bane of the stent era. J Clin Pathol 2006; 59: 232-239.
[11] Ackers-Johnson M, Talasila A, Sage AP, Long X, Bot I, Morrell NW, Bennett MR, Miano JM and Sinha S. Myocardin regulates vascular smooth muscle cell inflammatory activation and disease. Arterioscler Thromb Vasc Biol 2015; 35: 817-828.
[12] Glass CK and Witztum JL. Atherosclerosis: the road ahead. Cell 2001; 104: 503-516.
[13] Nemenoff RA, Horita H, Ostriker AC, Furgeson SB, Simpson PA, VanPutten V, Crossno J, Offermanns S and Weiser-Evans MC. SDF-1α induction in mature smooth muscle cells by inactivation of PTEN is a critical mediator of exacerbated injury-induced neointima formation. Arterioscler Thromb Vasc Biol 2011; 31: 1300-1308.
[14] Feil S, Fehrenbacher B, Lukowski R, Essmann F, Schulze-Osthoff K, Schaller M and Feil R. Transdifferentiation of vascular smooth muscle cells to macrophage-like cells during atherogenesis. Circ Res 2014; 115: 662-667.
[15] Vengrenyuk Y, Nishi H, Long X, Ouimet M, Savji N, Martinez FO, Cassella CP, Moore KJ, Ramsey SA and Miano JM. Cholesterol loading reprograms the microRNA-143/145-myocardin axis to convert aortic smooth muscle cells to a dysfunctional macrophage-like phenotype. Arterioscler Thromb Vasc Biol 2015; 35: 535-546.
[16] Ailawadi G, Moehle CW, Pei H, Walton SP, Yang Z, Kron IL, Lau CL and Owens GK. Smooth muscle phenotypic modulation is an early event in aortic aneurysms. J Thorac Cardiovasc Surg 2009; 138: 1392-1399.
[17] Neuman NA, Ma S, Schnitzler GR, Zhu Y, Lagna G and Hata A. The four-and-a-half LIM domain protein 2 regulates vascular smooth muscle phenotype and vascular tone. J Biol Chem 2009; 284: 13202-13212.
[18] Prakash SK, LeMaire SA, Guo DC, Russell L, Regalado ES, Golabbakhsh H, Johnson RJ, Safi HJ, Estrera AL and Coselli JS. Rare copy number variants disrupt genes regulating vascular smooth muscle cell adhesion and contractility in sporadic thoracic aortic aneurysms and dissections. Am J Hum Genet 2010; 87: 743-756.
[19] Prakash S, LeMaire S and Guo D. Rare copy number variants disrupt genes regulating vascular smooth muscle cell adhesion and contractility in sporadic thoracic aortic aneurysms and dissections. J Vasc Surg 2011; 54: 281.
[20] Cheng Y, Han M and Wen J. SM22α: a maker of vascular smooth muscle cell differentiation. Xibao Shengwu Xue Zazhi 2004; 26: 281-284.
[21] Jiao L, Jiang M, Fang J, Deng Y, Chen Z and Wu M. Basic fibroblast growth factor gene transfection in repair of internal carotid artery aneurysm wall. Neural Regen Res 2012; 7: 2915-21.
[22] Jiao L, Wang MC, Yang YA, Chen EQ, Xu HT, Wu KY and Zhang SM. Norepinephrine reversibly regulates the proliferation and phenotypic transformation of vascular smooth muscle cells. Exp Mol Pathol 2008; 85: 196-200.
[23] Mack CP. Signaling mechanisms that regulate smooth muscle cell differentiation. Arterioscler Thromb Vasc Biol 2011; 31: 1495-1505.
[24] Betsholtz C, Lindblom P, Bjarnegard M, Enge M, Gerhardt H and Lindahl P. Role of platelet-derived growth factor in mesangium development and vasculopathies: lessons from platelet-derived growth factor and platelet-derived growth factor receptor mutations in mice. Curr Opin Nephrol Hypertens 2004; 13: 45-52.
[25] Jiao L and Zhang SM. Study of relationship between formation of internal carotid artery aneurysm and changes of vascular smooth muscle cells in rabbits. Zhonghua Shenjing Waike Zazhi 2010; 26: 900-903.
[26] Xiong J, Wang SM, Chen LH, Lin Y, Zhu YF and Ye CS. Elastic fibers reconstructed using adenovirus-mediated expression of tropoelastin and tested in the elastase model of abdominal aortic aneurysm in rats. J Vasc Surg 2008; 48: 965-973.
[27] Wang XW and Liu WG. Experimental study on bFGF gene lentiviral vector transfecting BMSCs. Zhongguo Xiandai Yisheng 2010; 48: 13-16.
[28] Orlandi A, Hao H, Ferlosio A, Clément S, Hirota S, Spagnoli LG, Gabbiani G and Chaponnier C. Alpha actin isoforms expression in human and rat adult cardiac conduction system. Differentiation 2009; 77: 360-368.
[29] Mangrum W, Farassati F, Kadirvel R, Kolbert C, Raghavakaimal S, Dai D, Ding Y, Grill D, Khurana V and Kallmes DF. mRNA expression in rabbit experimental aneurysms: a study using gene chip microarrays. AJNR Am J Neuroradiol 2007; 28: 864-869.