Role of Vascular Endothelial–Cadherin and p120-Catenin in the Formation of Experimental Intracranial Aneurysm in Animals

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■ BACKGROUND: **Dysfunction of endothelial cells (ECs) constitutes a critical factor in the formation of intracranial aneurysms (IAs). However, little is known about the response of ECs to hemodynamic insults and its contribution to IA formation.**

■ METHODS: **IAs models were constructed in both adult female New Zealand white rabbits and male Sprague–Dawley rats. Morphologic changes of vessel wall were detected by hematoxylin and eosin staining. Molecular and cellular changes, including p120-catenin (p120ctn) and vascular endothelial–cadherin, in the median sagittal section of the artery bifurcation were analyzed by fluorescent staining.**

■ RESULTS: **Destructive aneurysmal remodeling and the formation of morphologic IAs were observed at the basilar termini of experimental rabbits and the anterior cerebral artery–olfactory artery bifurcation of rats. The expression of p120ctn colocalized with vascular endothelial–cadherin in ECs decreased. Moreover, the expression of p120ctn colocalized with nucleus of ECs increased. These events suggested that p120ctn was transported from the membrane to the nucleus of ECs.**

■ CONCLUSIONS: **The potential mechanism, that IAs are always localizing in the bifurcation apices, may be that the endothelium injury of vessel wall can be induced by different hemodynamic conditions. Hemodynamic changes in artery bifurcation may initiate the formation of IAs.**

INTRODUCTION

The mechanism of underlying intracranial aneurysm (IA) formation remains unclear. IAs occur more frequently at intracerebral artery bifurcations that typically experience greater hemodynamic shear stress and stronger flow acceleration. This specific hemodynamic condition previously had been shown to promote IA initiation in animals.[1,2] Complex flow patterns, such as a shock flow, are able to injure the vascular endothelium, cause inflammation reactions in endothelial cells (ECs), and induce the reconstruction of the vessel wall[3,4]; such hemodynamic conditions have been considered to be a key factor in the formation of IAs.[5] Jamous et al.[6] suggested that the endothelial layer was a critical structure in the process of IA formation. It also was reported that the early development of irregularly shaped EC at the apical intimal pad of the arterial bifurcation proceeded arterial wall dilation.[7]

In the circulation, ECs are continually exposed to shear stress, compression, and tension induced by blood flow and strain in the extracellular matrix.[8] Adhesion molecules, which participate in EC connections, are essential to maintain cell–cell adhesion, cell–cell communication, and the integrity of vascular tubes, and

Key words
- Endothelial cells
- Hemodynamics
- Intracerebral aneurysm
- p120-catenin
- VE–cadherin

Abbreviations and Acronyms
ACA: Anterior cerebral artery
CCA: Common carotid artery
EC: Endothelial cell
IA: Intracranial aneurysm
OA: Olfactory artery
p120ctn: p120-catenin
SD: Sprague–Dawley
SMC: Smooth muscle cell
VE: Vascular endothelial

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vascular endothelial (VE)−cadherin is identified as a key adhesion molecule of adherens junctional proteins.[9]

As an important regulator of VE−cadherin/catenin complex, p120-catenin (p120ctn) plays a crucial role in regulating VE−cadherin function.[10] However, the effects of VE−cadherin change induced by hemodynamics on the vascular wall remains to be furtherly investigated, and relevance of physical cues needs to be deciphered in relation to the growing understanding of growth factor−driven signaling. Thus, we designed this study to detect the expression of VE−cadherin and p120ctn in different stages of aneurysm and explore the possible mechanism underlying VE−cadherin and p120ctn involvement in the formation of IAs in vivo.

MATERIAL AND METHODS

Experimental IA Model of Rabbits

Adult female New Zealand white rabbits (3−4 kg) were subjected to bilateral common carotid artery (CCA) ligation to increase blood flow to the basilar terminus, as reported in previous studies. The animals were euthanized by an intravenous administration of 100 mg/kg of sodium pentobarbital 6 months after the ligation. The carotid arteries were exposed but not ligated in control rabbits, and the animals were sacrificed 6 months after the surgery.[11]

Experimental IA Model of Rats

An IA was induced in 30 male 6-week-old Sprague−Dawley (SD) rats, who were anesthetized using 4% isoflurane inhalation, by ligating the right CCA and bilateral posterior renal arteries. One week later, 1% NaCl solution was substituted for the drinking water of rats. Ten 6-week-old SD rats whose carotid arteries were exposed but not ligated were used as controls, and the animals were sacrificed 6 months after the surgery.[6] This study complied by the local animal care committees.

Hematoxylin and Eosin Staining

Immediately after sacrifice, the vertebral arteries were perfused with phosphate-buffered saline, and the pressure was fixed in situ at 150 mm Hg using 10% buffered neutral formalin for 30 minutes. The vessels were fixed at this slightly elevated pressure to prevent vasospasm and compensate for the shrinkage that was associated with the fixation process. The brain was removed and fixed in 10% buffered neutral formalin for 24 hours. The basilar bifurcation was excised, embedded in paraffin, and sectioned longitudinally. Adjacent 4-μm-thick sections from the median plane of the bifurcation were used for hematoxylin and eosin staining for signs of destructive aneurysmal remodeling and fluorescent staining for molecular and cellular changes.

Fluorescence Staining

The sections of the bifurcation were incubated on a rotator overnight at 4°C with mouse monoclonal antibodies against VE−cadherin and then for 2 hours with Protein G Sepharose. The immunoprecipitated samples were centrifuged and washed 3 times with IP buffer (50 mM Tris−Cl, 150 mM NaCl, 1 mM ethylenediaminetetraacetic acid, 1% Triton X-100, 6.5 IU/μL aprotinin, 100 μM phenylmethylsulfonyl fluoride, 5 μg/mL leupeptin,1 μg/mL pepstatin A, 300 μM Na_3VO_4, 10 mM NaF, pH 7.5). The washed pellets were resuspended in the sample buffer and boiled for 7 minutes.[12]

RESULTS

Changes of Vessel Wall in IA Models of Rabbits and Rats

In the control group, detected by hematoxylin and eosin staining, it was indicated that the intima of the basilar artery−posterior cerebral artery bifurcation was even and smooth; the ECs were closely linked; the thickness of the middle muscle layer was uniform; and the outer membrane was intact (**Figure 1A**). In the experimental group, an aneurysm-like bulge was observed at the basilar artery−posterior cerebral artery bifurcation (white arrow), and the elastic membrane missed from the normal vascular wall (between the green arrows), and the middle muscle layer was thinner than control group (**Figure 1B**).

In the control group, the inner membrane of the anterior cerebral artery−olfactory artery (ACA-OA) bifurcation vascular wall and the thickness of the middle muscle layer were normal, and the ECs and the outer membrane were intact. In the experimental group, a morphologic aneurysm was observed in the bifurcation of the right ACA-OA; moreover, the elastic membrane of ECs was missing and the middle muscle layer was also thinner than in the control group (**Figure 1C−E**).

Expression and Colocalization of p120ctn and VE−Cadherin on IAs in Rabbits

Changes of the expression and colocalization of p120ctn and VE−cadherin in experimental IAs were examined by using immunofluorescence microscopy and image analysis. In the control group (**Figure 2A, D**, and **G**), p120ctn and VE−cadherin were expressed continuously in the intima, and the middle muscle layer was uniform. The ECs and smooth muscle cells (SMCs) formed 2 continuous layers. Among the 10 experimental rabbits, 4 early aneurysm-like changes (**Figure 2B, E**, and **H**) and 6 IAs formation were detected (**Figure 2C, F**, and **I**) (between white arrows). The expression of p120ctn and VE−cadherin were discontinuity in the region of aneurysm. Thinning and degradation of the smooth muscle layer resulted in a vessel wall protrusion. In the control group, VE−cadherin accumulated in the inner elastic layer of vascular wall and colocalized with p120ctn in the regions of ECs (**Figure 2G**). However, VE−cadherin and p120ctn were distributed continuously and un-colocalized.

Expression and Colocalization of p120ctn and Nucleus on the Wall of Aneurysm in Rabbits

The expression of p120ctn (**Figure 3A−C**; red) and nucleus (**Figure 3D−F**; blue) was observed using immunofluorescence microscopy and image analysis. In the control group, p120ctn was continuously and normally expressed in the ECs; however, in the experimental group, detected by the fluorescence intensity, the expression of p120ctn in the aneurysm-like changes in the vascular wall (**Figure 3B**) and the aneurysm vascular wall (**Figure 3C**) decreased. No significant changes in the distribution of nucleus was observed in the experimental and control groups, and the distribution was uniform throughout (**Figure 3D−F**). In the control group, the merged images showed that most of the red fluorescence did not

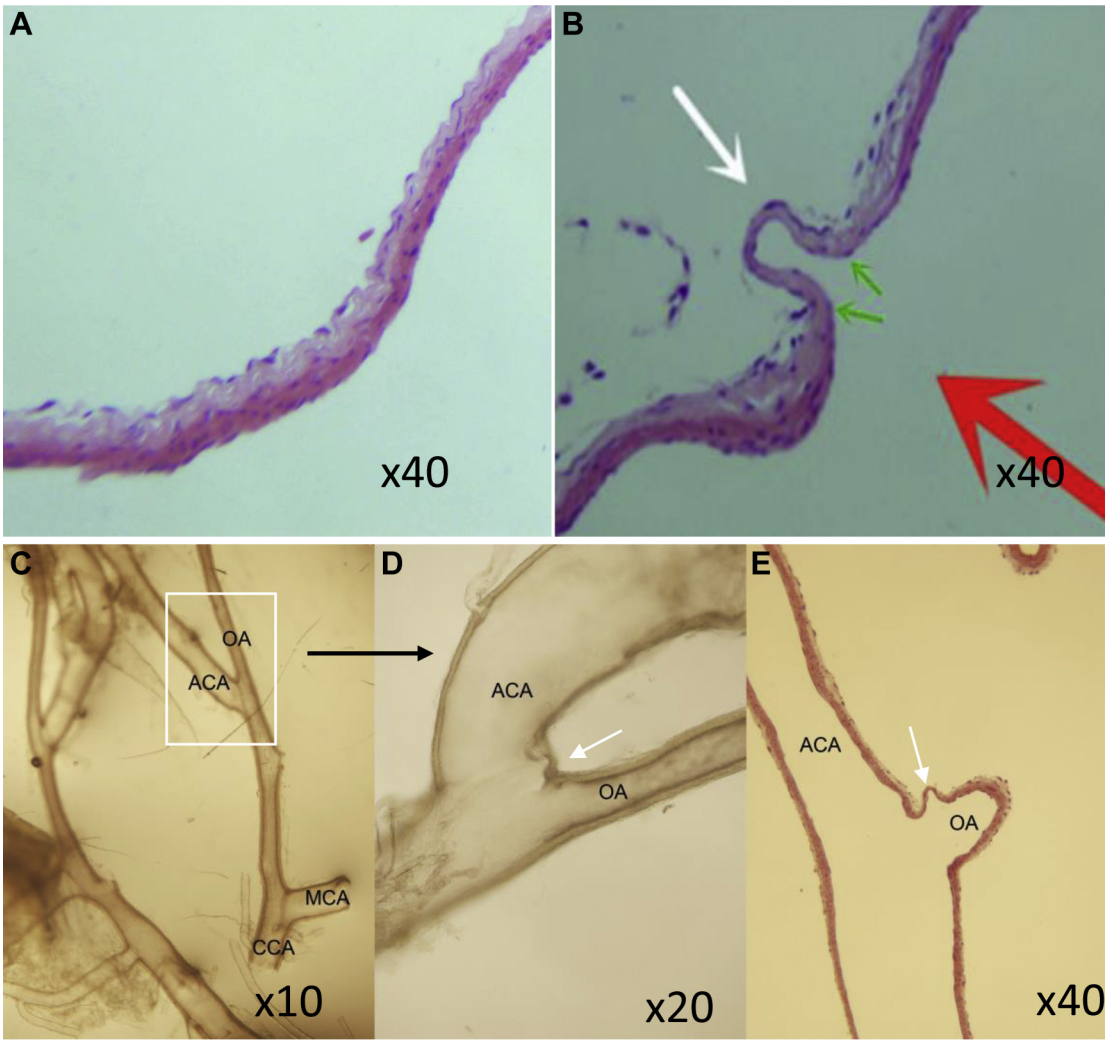

Figure 1. (**A**) Hematoxylin and eosin (HE) staining of the basilar termini after the sham surgery shows dense, continuous internal elastic lamina (IEL) and uniform media. (**B**) After 6 months of bilateral CCA ligation, IEL loss (between *green arrows*), media thinning (between *green arrows*), and bulge formation (*white arrow*) were seen. (**C**, **D**) Optical microscope shows an anterior cerebral artery–olfactory artery (ACA-OA) aneurysm in the experimental group. (**E**) HE staining of the bifurcation of the right ACA-OA after the surgery shows IEL loss, media thinning, and bulge formation (*white arrow*). The *red arrow* indicates the direction of blood flow.

overlap with nucleus, indicating that most of the p120ctn were still located out of the vascular ECs nucleus (Figure 3G). However, in the experimental group, overlapping of p120ctn with nucleus suggested a transfer of p120ctn into the nucleus (Figure 3H,I).

Experimental Rat Results

The expression of VE–cadherin and p120ctn in the IAs of experimental SD rats and the positional relationship between p120ctn, VE–cadherin, and nucleus of ECs were observed by using immunofluorescence microscopy and image analysis. VE–cadherin and p120ctn both were expressed continuously in the intima of control rats by using immunofluorescence. In the experimental group, VE–cadherin and p120ctn discontinuity was expressed, and the colocalization of p120ctn with the nucleus showed a partial transfer of p120ctn into the nucleus of ECs (Figure 4).

DISCUSSION

In this study, by constructing experimental IAs in vivo, we found that well-developed aneurysmal morphology at the basilar termini of rabbits and ACA-OA bifurcation of rats can be induced. Vascular corrosion casts and immunohistochemic staining of serial sections of the bifurcation also were used to examine which arterial layer was critically involved in the formation of saccular

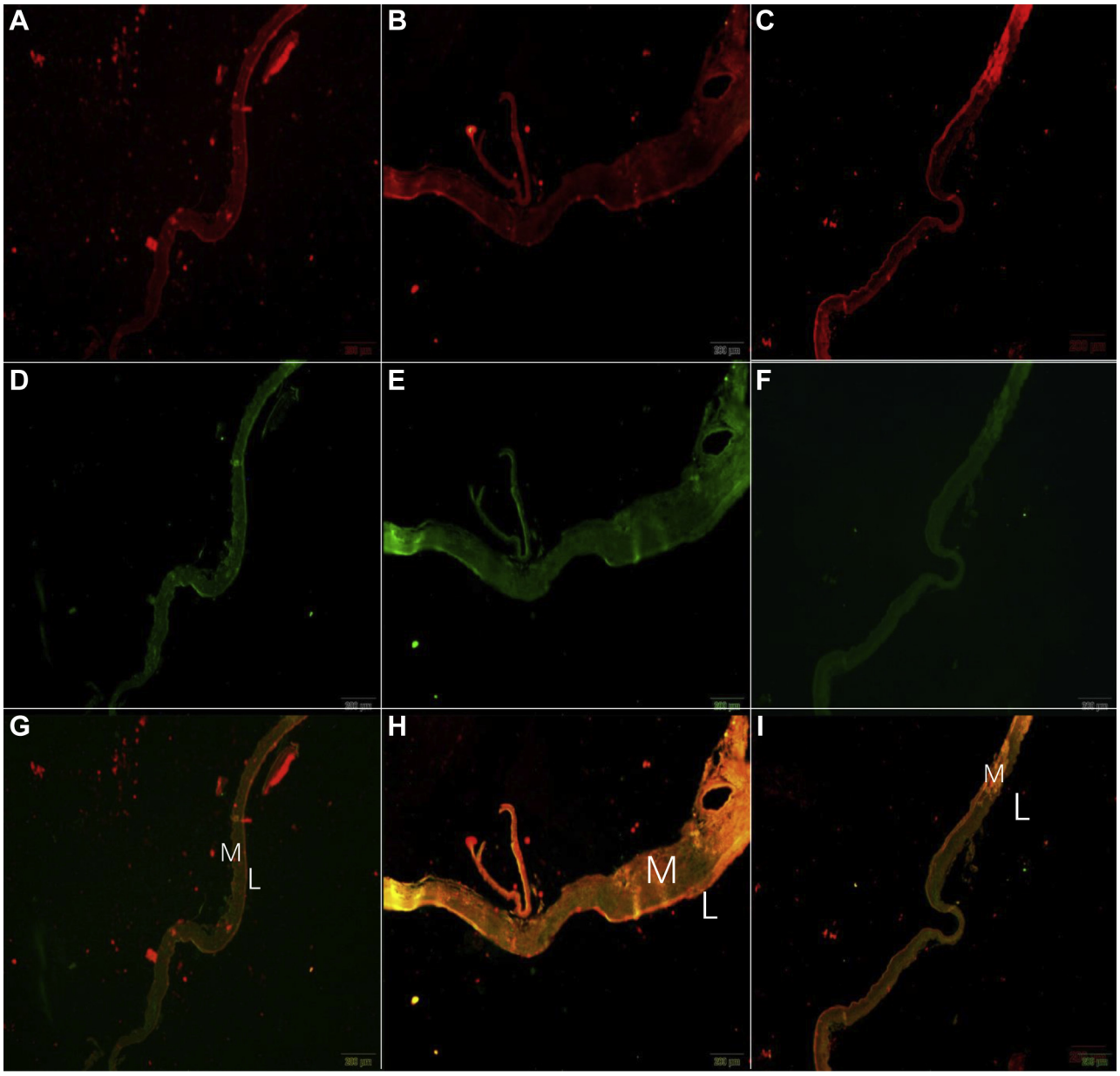

Figure 2. Representative fluorescence images of vascular endothelial (VE)–cadherin and p120-catenin (p120ctn) of the vascular wall: (**A, D,** and **G**) Control groups; (**B, E,** and **H**) aneurysm-like changes; and (**C, F,** and **I**) aneurysm formation. (**D**–**F**) VE–cadherin; (**A**–**C**) p120ctn; and (**G**–**I**) merged images. The region of endothelial cell damage is marked by *white arrows*.

aneurysms and how the aneurysms wall were formed.[13] The findings of the present study indicated that ECs injury, which induced by hemodynamic changes, caused a significant loss of elastic membrane and thinning of middle muscle layer, and thus resulted the formation of a saccular space at the apical intimal pad (see Figure 1). The progression of ECs injury led to a proteolytic destruction of the vessel wall and created a defect in the artery bifurcation situ.[14] This defect, restricted to the area of migrated SMCs, represented the nidus of an IA.[15] ECs injury is considered to be a major factor in the initiation of IAs. Previous studies presented ECs changes of basilar artery bifurcation in response to sustained high blood flow in rabbits and ECs impairment possibly initiates aneurysm formation.[16] Jamous et al.[6] believed that EC injury at the apical intimal pad

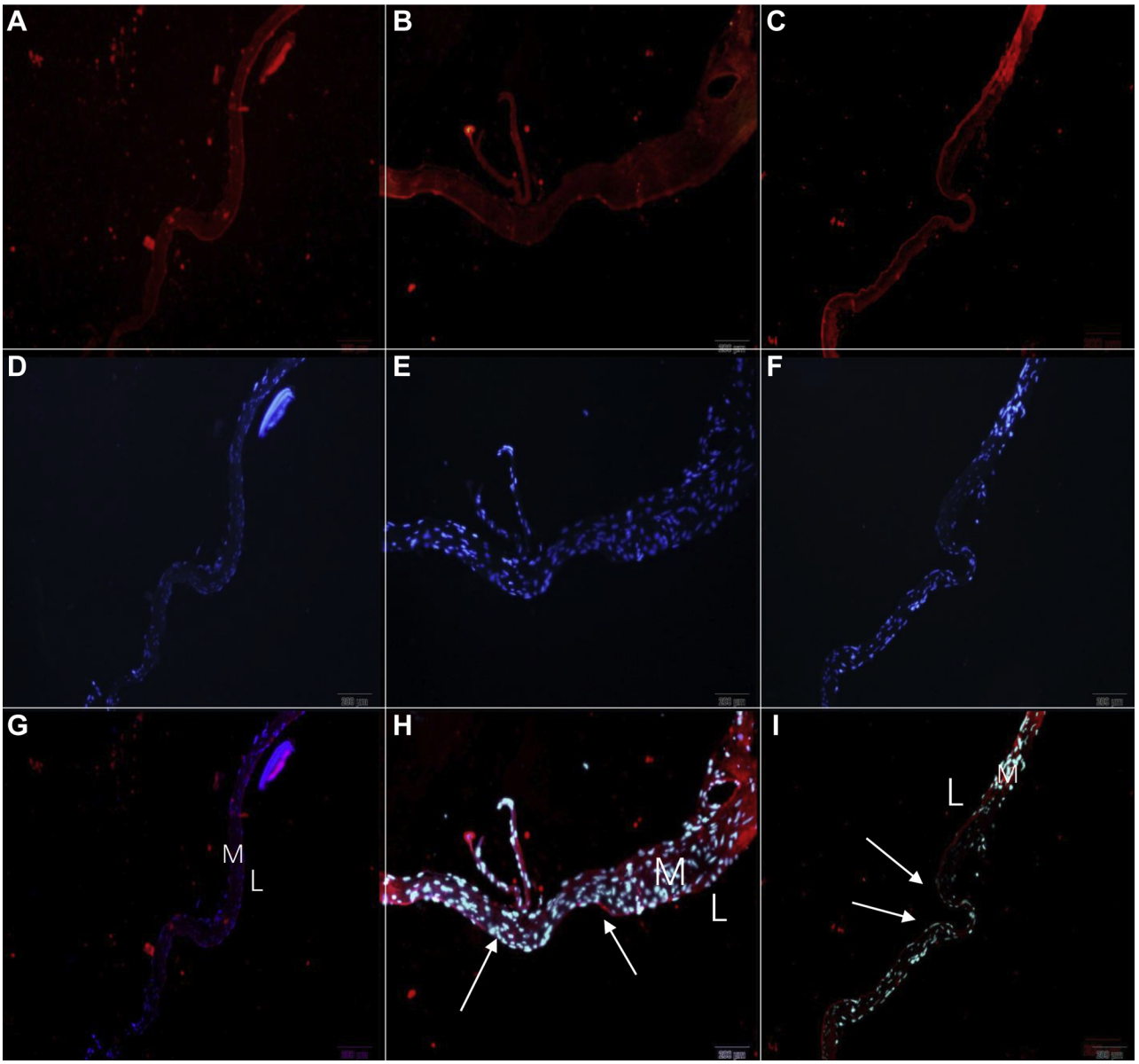

Figure 3. Representative fluorescence images of p120-catenin (p120ctn) and nucleus of vascular wall: (**A, D,** and **G**) Control groups; (**B, E,** and **H**) aneurysm-like changes; and (**C, F,** and **I**) aneurysm formation. (**D**–**F**) Nucleus; (**A**–**C**) p120ctn; and (**G**–**I**) merged images. The region of endothelial cell damage is marked by *white arrows*.

was the basis of the formation of IAs, and subsequently, local inflammation of the vessel wall at the apical intimal pad produced destruction of vessel wall.

By using immunofluorescence microscopy, decreased expression of p120ctn and VE–cadherin were detected in IAs samples (Figures 2 and 4). Based on the number of ECs detected in the well-developed aneurysmal region, it was indicated that high blood flow aggravated EC injury. EC function as mediators of fluid homeostasis, hormone distribution, immune cell trafficking, and nutrient distribution. Within the plumbed and integrated vasculature, the cellular adhesion of ECs was crucial for all these major functions.[17] A principal adhesion protein establishing cell-to-cell contacts in the adherens junction between ECs was VE–cadherin. Although it was clear that these domains were essential for cell–cell adhesion, it was not understood how this occurred at a structural level. Intercellular signals at cell-to-cell contacts could be transferred by VE–cadherin to the intracellular domain via transmembrane and cytoplasmic binding partners.[18]

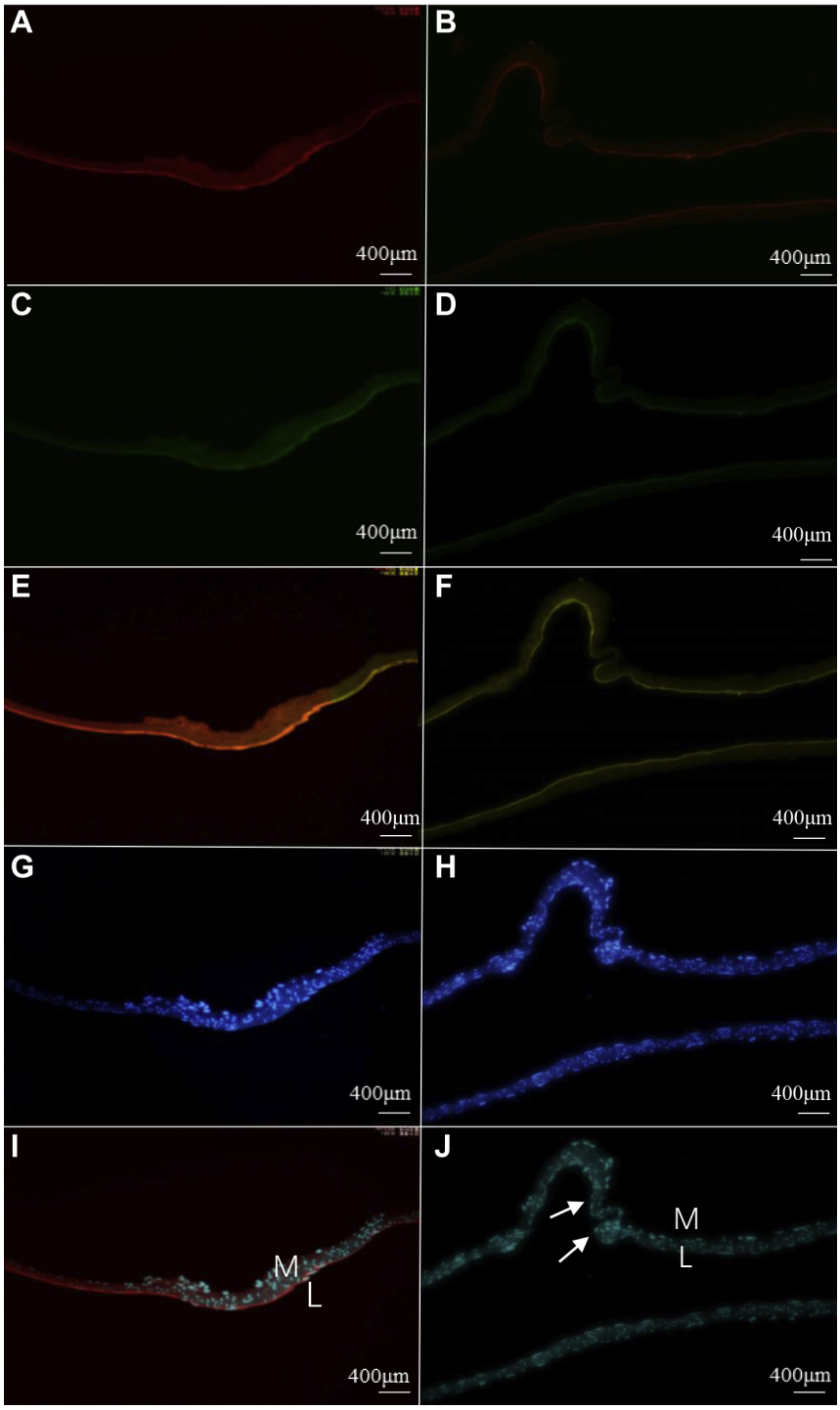

Figure 4. Representative fluorescence images of vascular endothelial (VE)–cadherin and p120-catenin (p120ctn) of the vascular wall: (**A, C,** and **E**) Control groups; and (**B, D,** and **F**) aneurysm formation. (**C, D**) VE–cadherin; (**A, B**) p120ctn; and (**E, F**) merged images. Representative fluorescence images of p120ctn and nucleus of vascular wall: (**G** and **I**) Control groups, (**H** and **J**) aneurysm formation, (**G** and **H**) nucleus, (**A** and **B**) p120ctn, and (**I** and **J**) merged images.

The VE−cadherin/catenin complex is the backbone of adherens-type junctions in endothelium and is similar to the E-cadherin/catenin complex in the epithelium.[19] The endothelial-specific VE−cadherin/catenin complex acts in close interaction with actin filaments and actin/myosin-mediated contractility to fulfill the junction demands. The functional connections between the cadherin/catenin complex and actin filaments might be either directly through α-catenin, p120ctn, α-actin, or EPLIN.[20] p120ctn is a component catenin of the VE−cadherin/catenin complex and plays a crucial role in the adhesion and attachment of vascular ECs.[21] First, p120ctn is involved in the regulation of angiogenesis, microtubule model formation, vascular integrity, and EC proliferation. Second, p120ctn is linked to VE−cadherin in the juxtamembrane region, allowing vascular endothelium to stably express on the cell membrane surface and preventing it from endocytosing and migrating. p120ctn can enter the nucleus of ECs and bind to the transcriptional regulator Kaiso to form the p120ctn−Kaiso complex as a regulator of Wnt signaling pathway.[22] In this study, the expression of p120ctn and VE−cadherin was discontinuity and decreased in the region of IAs. Thinning and degradation of SML created a defect and latterly produced vessel wall protrusion. In the control group, VE−cadherin accumulated in inner membrane and colocalized with p120ctn in the regions of ECs (**Figures 2** and **4**). However, the VE−cadherin and p120ctn were also distributed continuously and un-colocalized at the aneurysm. In the present study, the formation of aneurysm was observed when blood flow increased after CCA ligation, which was followed by significant EC injury and bulge formation.[16] Thus, these morphologic changes could be triggered by the separation of 120-ctn and VE−cadherin under increased flow rate.

In the ECs of IAs, the inner membrane showed that colocalization of p120ctn with the nucleus revealed partially transforming of p120ctn into the nucleus of ECs[23] (**Figures 2** and **4**). Our results suggested that VE−cadherin and p120ctn underwent similar changes of β-catenin during the formation of IAs; however, the exact mechanism involved in the aneurysm formation remains to be studied further. The effects of p120ctn on vascular endothelium was not only manifested in adhesion function but also expressed in the inflammatory response induced by vascular endothelium. p120ctn might be one of most crucial factors in the formation of IAs. Phosphorylation of VE−cadherin, which regulated partial shifting of p120ctn into the nucleus, also played a key role in vascular inflammation and angiogenesis.

In summary, hemodynamic changes at the basilar termini of rabbits or ACA-OA bifurcation of SD rats induced arterial wall destructive remodeling. Specifically, this condition stimulated EC damage, including adherents injury of ECs, intercellular gap widening, events that may contribute to thinning and weakening of the vessel wall, and IA development. These events were initiated when endothelium was present without localization and thus could be attributed to the local dysfunction of the endothelium and/or SMCs. Hemodynamic induction of destructive cascades in the vessel wall would help explain why IAs often localize to bifurcation apices and how hemodynamics can contribute to aneurysm initiation in conjunction with other risk factors. However, we will elucidate the relationship between additional factors, inflammatory markers, and intimal injury in further research; this is a limitation of our current research.

CONCLUSIONS

The potential mechanism that is IAs are always localizing in the bifurcation apices maybe that the endothelium injury of the vessel wall can be induced by different hemodynamic conditions. Hemodynamic changes in artery bifurcation may initiate the formation of IAs.

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