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Original Article

β-catenin in VSMC proliferation induced by fluid shear stress through the non-Wnt signaling pathway

Xin Sheng[1*], Yan Sheng[2*], Yuehua Liu[1], Yingjie Tu[1]

[1]*Department of Biochemistry and Molecular Biology, Zunyi Medical University, Zunyi 563000, Guizhou, China;* [2]*Morphological Laboratory, Zunyi Medical University, Zunyi 563000, Guizhou, China.* [*]*Equal contributors.*

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Abstract: To investigate the relationship between fluid shear stress (FSS), vascular smooth muscle cells (VSMCs) proliferation during atherosclerosis (AS), and β-catenin. A flow-induced AS animal model was established by arterial stenosis in New Zealand white rabbits, in which a physiological level of low shear stress is induced. The animals were randomly assigned to four groups: control (C; n=5), hyperlipidemia (H; n=5), arterial stenosis (S; n=5), and arterial stenosis animals fed a high-fat diet (SH; n=5). 12 weeks after surgery, the arteria carotis was harvested to determine the vasal morphology and ultrastructure, as well as, the expression level and cellular localization of β-catenin. The pathological phenomena that occurred with AS, such as the intracellular connection interruption of endothelial cells, the thickening and severe deformation of endangium, and the proliferation and migration of VSMCs could be observed even in the narrowed arteries (group H and SH). β-catenin, which is localized mainly in the intimal hyperplasia and to a little extent in the tunica elastic and foam cells, also increased remarkably ($P<0.05$). Interestingly, no nuclear translocation of β-catenin was observed. These results suggest that FSS plays a vital role in the early stage of AS by partially increasing the gene expression of β-catenin in the VSMCs. In addition, β-Catenin might function in the proliferation of VSMCs via the intercellular connection with actin rather than the canonical Wnt pathway. This study would facilitate the understanding of the critical role of the Wnt/β-catenin pathway in AS induced by FSS.

Keywords: Fluid shear stress (FSS), atherosclerosis (AS), arteria carotis, β-catenin, smooth muscle cells (SMCs)

Introduction

Presently, atherosclerosis (AS) is a major health threat in China. A majority of cardiovascular and cerebrovascular diseases are caused by AS. It is typically regarded as a chronic vascular inflammation, and several well-identified risk factors (i.e., hypertension, hyperlipidemia, and cigarette smoking) have been implicated in its pathogenesis [1]. For several years, hyperlipidemia and hypertension have been considered the main causes. However, clinical research has indicated that although the whole artery is exposed to a high risk of hypertension and hyperlipidemia, plaques are easily formed, especially in the bend, branch, or stricture of the artery [2]. Moreover, approximately 50% of AS patients do not suffer from hyperlipidemia or hypertension, and some plaques also exist in healthy individuals [3]. Hemodynamic research has provided evidence for a key role of fluid shear stress (FSS) in the process [2, 4]. FSS is a frictional force that results from circulating blood and is parallel to the direction of the flow, acting directly on the endothelial cells. In a healthy and atheroresistant artery, the blood flow produces high laminar shear stress, which is anti-inflammatory and anti-proliferative. However, in the arterial stenosis or curvature region, a disturbed or slow flow produces oscillatory or low shear stress, which is pro-inflammatory and atherogenic [2]. Several studies have demonstrated that vascular endothelial cells and smooth muscle cells (SMCs) differ in morphology and gene expression under diverse FSS [5]. A disturbed flow can induce increased expression of proteins, such as vascular cell adhesion molecule 1 (VCAM-1), E-selectin, and monocyte chemoattractant protein 1 (MCP-1), involved in the process of AS [6].

Vascular smooth muscle cells (VSMCs) localize in the medial layer of the arterial wall, and function in the maintenance and regulation of blood

vessel tone, blood pressure, and blood flow distribution [7]. VSMCs play a crucial role in the vascular lesion formation, including neointima formation and atherosclerosis, by activation and migration from the media into the intima under abnormal environmental conditions [8, 9]. Thus, proliferation and migration of VSMCs contribute to the early lesions in atherosclerosis. Some reports reveal that FSS stimulates the cytokines and signaling molecules in VSMCs [10]. Laminar shear stress could reduce VSMC proliferation, whereas pulsatile or oscillatory shear stress can promote VSMC proliferation and migration [11, 12]. FSS may initiate mechanotransduction pathways in VSMC for functional regulation. Thus, further understanding of VSMC biology, especially in response to FSS, is critical in comprehending the pathogenesis of AS induced by FSS. However, the underlying molecular mechanisms of VSMC response to FSS remain largely unknown.

β-catenin is an evolutionarily highly conserved and functionally diverse protein with two functions. It is a core protein in the canonical Wnt signaling, which targets proteasomal degradation via the adenomatous polyposis coli (APC), axin, and glycogen synthase kinase 3β (GSK3β) complex. However, in the presence of Wnt signaling, β-catenin depolymerizes from the APC-Axin-GSK3β complex, translocates to the nucleus, binds to the lymphoid enhancer factor-1/T-cell factor (LEF-1/TCF) and then stimulates the expression of Wnt target genes, including cell cycle activators and fibronectin [13]. On the other hand, free β-catenin in the cytoplasm is largely unstable, and most of the newly synthesized protein combines with E-cadherin and α-catenin to regulate the actin cytoskeleton, thereby participating in the intracellular connection. Further emerging evidence suggests that the Wnt/β-catenin signaling pathway is unambiguously involved in the pathophysiology of AS and has a pivotal role in the regulation of endothelial inflammation, vascular calcification, and SMCs behavior. Wang et al. showed that VSMC proliferation was promoted via transfection of a degradation-resistant β-catenin transgene into rats [14]. A recent study demonstrated that Wnt/β-catenin signaling occurs in proliferating VSMCs during intimal thickening, and it promotes VSMCs de-differentiation and proliferation through crosstalk with the TGF-β/Smad3 pathway [15-18]. Furthermore, Wnt/β-catenin signaling is also involved in mechanical signal transduction. The protein participates in the process of stress signal transduction in osteocyte cells. Some studies showed that FSS could induce Wnt/β-catenin signaling, and its nuclear translocation can be observed in a variety of cell lines at 16 dynes/cm^2 FSS in osteoblasts [19, 20]. However, reports describing its regulation by FSS in Vascellum are yet lacking. Thus, the correlation between the Wnt/β-catenin signaling pathway and FSS in VSMCs proliferation necessitates further investigation.

Therefore, we hypothesized that β-catenin plays a critical role in FSS-induced AS. In the present study, a flow-induced AS animal model was established by arterial stenosis in New Zealand white rabbits, in which a physiological level of low shear stress is induced to investigate the relationship between FSS, VSMCs proliferation in AS, and β-catenin.

Materials and methods

Animals

Six-month-old male New Zealand white rabbits (2-2.5 kg) were obtained from Experimental Animal Center, Chongqing Medical University (China). All the experimental procedures were performed in accordance with the guidelines of the National Institute of Health and were approved by Zunyi Medical University Animal Care and Use Committee. The animals were maintained at ambient conditions (temperature: 22±2°C, humidity: 30-40%) with free access to food and water.

In vivo alteration of FSS

The animals were randomly assigned to four groups: (1) the control animals (C) (n=5), (2) hyperlipidemia animals (H) (n=5), (3) arterial stenosis animals (S) (n=5), and (4) arterial stenosis animals fed a high-fat diet (SH) (n=5). Groups C and S were fed a control diet (SF06-011, Specialty Feeds, Glen Forrest, Australia) containing 2.63 kcal/g, of which 4.2% of calories were obtained from fat, 18.2% protein, 14.1% crude fiber, and 18% acid detergent fiber. The groups H and SH were fed with a high-fat diet, which was modified standard rabbit chow with 5% pork fat (lard) and 5% soya oil (SF02-005, Specialty Feeds) for 12 weeks.

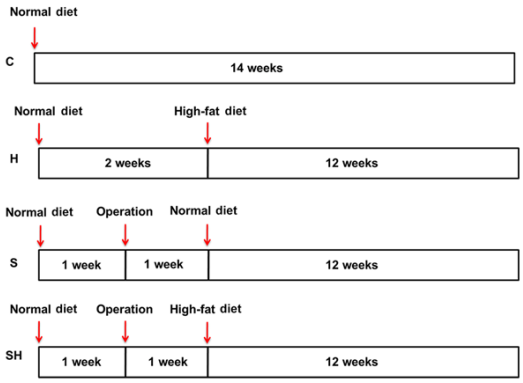


Figure 1. Procedures for the animal operation and feeding.

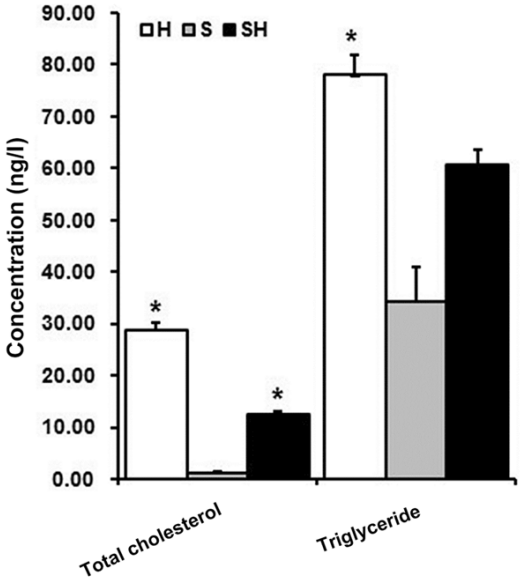


Figure 2. Total cholesterol and triglyceride levels in the serum are increased in groups fed a high-fat diet. The concentrations of total cholesterol and triglycerides were analyzed using ELISA 12 weeks after surgery, and that in groups fed a high-fat diet were remarkably higher. Data were shown as mean ± SEM. n=5, *P<0.05 vs. group S.

Artery stenosis operations were performed according to the method described by Cheng et al. [21]. Briefly, the animals were anesthetized with 30 mg/kg urethane, and a 2-cm-long and 1-mm-diameter silicon rubber tube referred to as the "cast" throughout the study, was sleeved on the left common carotid artery as described previously [3]. The skin was then sutured, and the animals were allowed to recover for 1 week, feeding on a normal diet. 12 weeks after the initial surgery, the rabbits were euthanized with an anesthetic overdose, and the injured left and uninjured right common carotid arteries were harvested for histological, biochemical, or molecular analysis. The experimental procedure is illustrated in **Figure 1**.

Plasma lipid content analysis

Blood was collected after the animals were executed through heart puncture. The blood was centrifuged at 1000 rpm to obtain the supernatant. The cholesterol and triglycerides were determined with an ELISA assay kit (Kesun Biotech, Shanghai, China).

Transmission electron microscopy (TEM)

For electron microscopy, the rabbit tissue was fixed for 4 h in 4% glutaraldehyde and then fixed for 3 h in 2% OsO_4. After dehydration in an alcohol gradient, the samples were embedded in Epon812 and sectioned transversely. Semithin sections were stained with toluidine blue and images captured with a Leica DM microscope (Leica, Germany). The ultra-thin sections were stained with 2% uranyl acetate and lead citrate, visualized, and photographically recorded using an H7650 electron microscope (Hitachi, Japan).

Morphological and immunohistochemical analysis

The normal and injured arteries were fixed for 4 h at 4°C in 4% paraformaldehyde. These fixed tissues were dehydrated by ethanol gradient, clarified in xylene, embedded in paraffin wax, sectioned at 5 μm, and stained with hematoxylin-eosin.

Immunohistochemistry (IHC) was performed on 5-μm-thick paraffin-embedded tissue sections using Histostain-Plus IHC Kit (Thermo Fisher Scientific, USA). Briefly, the slides were deparaffinized and rehydrated. The antigen retrieval was performed at high temperature, the endogenous peroxidase was quenched using 0.5% H_2O_2 for 30 min, and blocked in serum for 30 min. The incubation steps with primary antibody, secondary antibody, ABC reagent, and DAB substrate were performed according to the manufacturer's protocol. The slides were counterstained with hematoxylin. Rabbit anti-β-catenin antibody (1:500; Sigma, USA) was used as the primary antibody. Negative control was

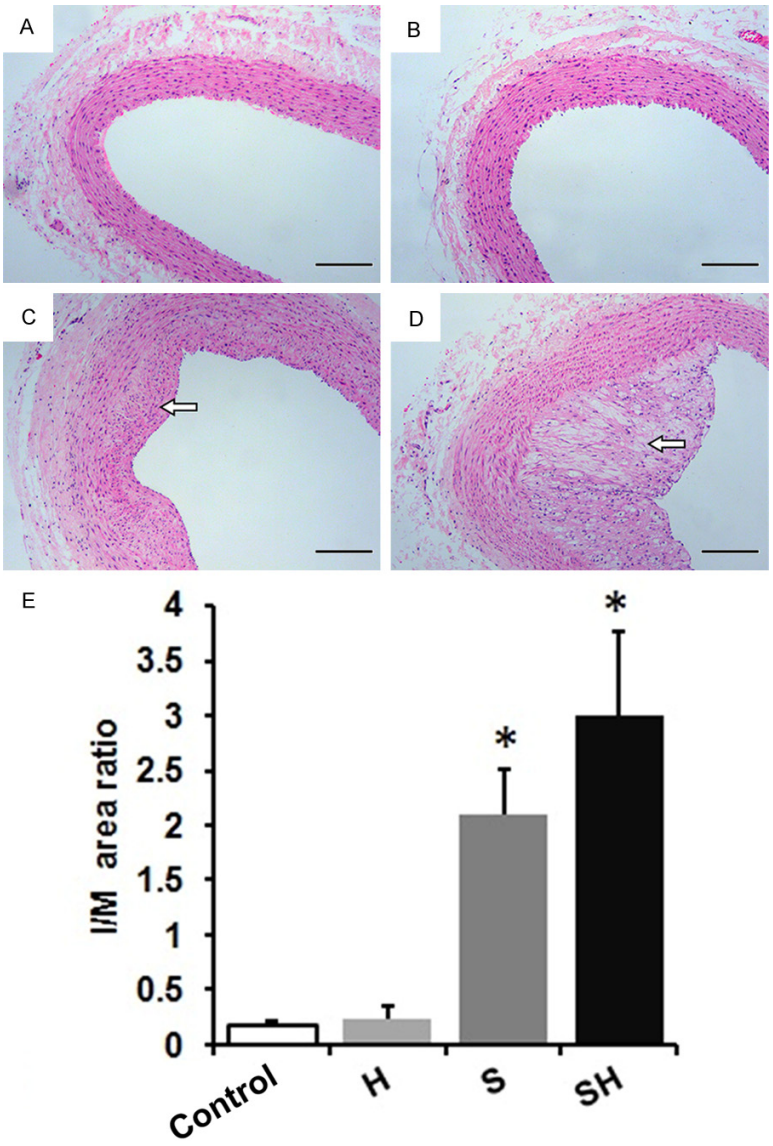


Figure 3. HE staining reveals a clear intima hyperplasia in the arterial stenosis groups. A. Control. B. Group H; the intima hyperplasia is invisible. C, D. Groups S and SH; the intima hyperplasia and proliferation of SMCs are visible (arrows), and the vascular injury in group SH is severer than that in group S. E. The IMR (intima-to-media ratio) between the groups. The IMR in groups S and SH were remarkably higher than that in group H. Scale bars =100 μm. Data were shown as mean ± SEM. n=5, **P*<0.05 vs. group H.

obtained by replacing the primary antibody with PBS. Images were captured by a fluorescent microscope (Olympus IX71, Japan), fitted with a MicroPublisher 5.0 RTV CCD camera. Semi-quantitative analyses were performed using the integrative optical density function in Image-Pro software.

Quantitative PCR (qPCR)

The total RNA from carotid arteries was obtained using the RNAprep Pure Tissue Kit (Tiangen, Beijing, China) and treated with DNase. cDNA was obtained by a reverse transcriptase reaction. mRNA expression levels were analyzed by real-time quantitative polymerase chain reaction (RT-qPCR) using the SYBR PrimeScript™ RT-PCR kit (Takara, Dalian, China). β-actin was used as an internal control to normalize the RNA quantification. The primers used were as follows: β-actin, upstream primer, 5'-CGGGACATCAAGGAGAAGC-3', downstream primer, 5'-AGGAAGGAGGGCTGGAAGA-3'; β-catenin, upstream primer, 5'-TCGTCCTTTAGGAGTAACAATACA-3', downstream primer, 5'-ACAAGCAAGGCTAGGGTTTGA-3'. The reaction was carried out in the CFX96 Touch™ Real-Time PCR Detection system (Bio-Rad, USA). The RNA was quantified using the Livak method, i.e. $2^{-\Delta\Delta Ct}$.

Statistical analysis

The data wereexpressed as mean ± SEM. The comparisons among the groups were analyzed by one-way ANOVA. The SNK test was used for post hoc analysis. *P*-values <0.05 were considered statistically significant. The statistical analyses were performed by the SPSS 13.0 software package (SPSS, USA).

Results

Significant increase in the total cholesterol and triglycerides in the serum of groups H and SH

No significant difference was found in the serum of the groups before modeling; however, hyperlipidemia and chylos were visible in groups H and SH 12 weeks after modeling. ELISA was used to assay the concentration of total cholesterol and triglycerides in the serum, which were found to be remarkably higher in groups H and SH than in group S. The concen-

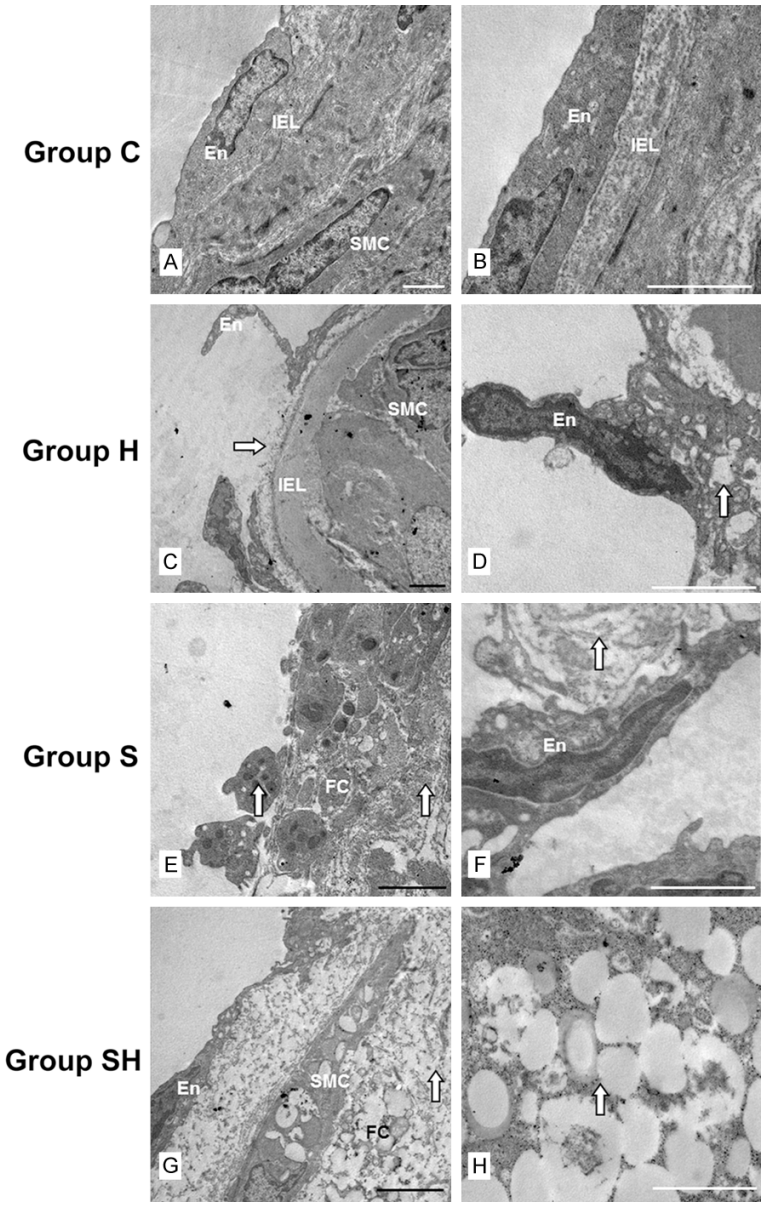
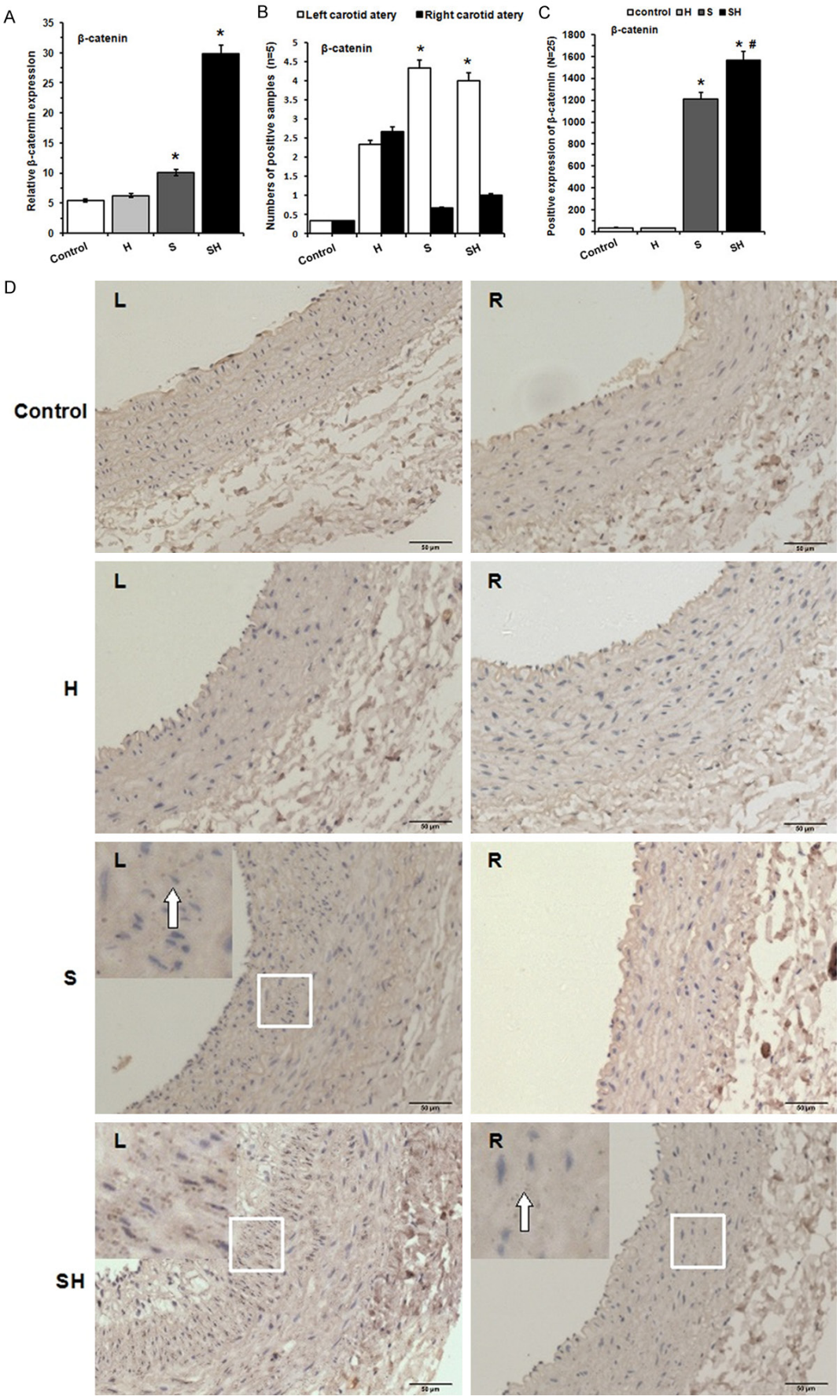


Figure 4. Carotid stenosis simultaneously leads to severe vascular injury than hyperlipidemia during AS. A, B. Control; the endothelial cells tightly connect with the basement membrane and are apart from the SMCs by IEL, which are closely arranged parallel to each other. C, D. Group H; the endothelial cells separated from the basement membrane and abscission (arrows in C), and a few liposomes are visible in the endothelial cells (arrows in D). E, F. Group S; the smooth muscle cells migrate inside the IEL and become smaller, which leads to the severe thickening and deformation of the intima (arrows in E), and the vertical or transverse collagen fibers accumulate in IEL (arrow in F). G, H. Group SH; a large number of liposomes and phagocytic vesicles can be observed in the smooth muscle cells (arrows in H), and the foam cells and high-electron-density materials appear in the IEL (arrows in G). (En, endothelium; SMC, smooth muscle cell; IEL, internal elastic lamina; FC, foam cell; scale bars =2 μm).

tration of total cholesterol was 9-20-fold higher, and that of triglycerides was 2-fold higher. Thus, a high-fat diet can significantly increase the concentration of cholesterol and triglycerides in the serum (**Figure 2**, $P<0.05$).

Micro- and ultra-structure of the rabbit carotid artery

HE staining did not show any significant alterations in the morphology of bilateral common carotid arteries in group H (**Figure 3B**). However, in the majority samples of group S, the left carotid artery underwent incrassation. The SMCs migrated from the tunica media to the intima and formed intima hyperplasia (**Figure 3C**). However, distinct intima hyperplasia and foam cells existed because of the cytophagy of lipid by the monokaryon macrophages in the left of group SH (**Figure 3D**). The IMR (intima-to-media ratio) between the groups was analyzed quantitatively and observed as remarkably higher in groups S and SH than that in group H (**Figure 3E**). The ultrastructure displayed additional details of the vascular wall. The linkage interruption of the endothelial cells or some deficiency could also be observed in the carotid arterial tissue of group H. Moreover, the endothelial cells were separated from the basement membrane (**Figure 4C** and **4D**). Noticeably, the vascular damage in group H was mainly in the endothelial cell layer. However, apart from the disruption of intercellular connection, abnormal cell morphology, severe intimal thickening, and deformation were also observed in group S. Furthermore, some vertical or transverse collagen fibers accumulated in the tunica intima (**Figure 4E** and **4F**). In the


A
β-catenin
Relative β-caternin expression
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Left carotid atery
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H
S
SH
β-catenin
Positive expression of β-caternin (N=25)
D
Control
H
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Figure 5. Expression level of β-catenin is positively correlated with vascular injury. A. The mRNA expression levels of β-catenin in the left carotid artery; the expression levels in the left carotid artery narrowed by cannulation of groups S and SH are remarkably increased. B. Comparison of the positive results of the bilateral carotid artery; the positive numbers in the left carotid artery of groups S and SH are higher than those on the right that were not narrowed. C. The protein expression level of β-catenin in the left carotid artery; the expression levels in groups S and SH are increased and that in group SH is higher than that in group S, which shows that the expression level of this protein is positively correlated with vascular injury during AS. Data were shown as mean ± SEM. n=5, **P*<0.05 vs. group H, #*P*<0.05 vs. group S. D. IHC indicates that β-catenin mainly accumulates in the cytoplasm of proliferative SMCs. (L in the upper left corner of the figures represent the left artery and R the right). In the control and H groups, no localization of β-catenin occurs in the bilateral arteria carotis. In the group S, β-catenin is mainly localized in the cytoplasm of the endometrial hyperplasia and not in the nucleus (arrow) and also on the right side. In the group SH, the expression of β-catenin in the left artery carotid increases compared to that of group S; some locations also exist in the tunica elastic and foam cells (arrow on the left), and slight expression can be found on the right side (arrow on the right). (The insertion is the magnification in the white box. Scale bars =50 μm).

group SH, endothelial cells underwent apoptosis, and the intima severely thickened. The internal elastic fiber membrane was disassembled into irregular elastic fibers in some samples, and the basement membrane was disrupted or disappeared. The foam cells and high electron density particles could be observed in the intima, and generous liposome and phagocytic vesicles appeared in the SMCs (**Figure 4G** and **4H**). Thus, carotid stenosis could damage not only the endothelial cells but also the intima and media simultaneously.

Carotid stenosis leads to an increased expression of β-catenin and the accumulation of protein in the cytoplasm of proliferative smooth muscle cells

The gene expression level of β-catenin was quantified using qPCR and was consistent with the IHC results for each group (**Figure 5A**). The expression level in group S was 2-fold higher than that of the control while that in SH was 5-fold higher. IHC staining revealed that this protein was absent in the control group and the bilateral carotid artery of group H (**Figure 5D**). In groups S and SH, β-catenin was primarily localized in the intima hyperplasia, which is mainly composed of the proliferative SMCs, and a small amount was also located in the tunica media and foam cellular layers. The intracellular β-catenin was present in the cytoplasm rather than in the nucleus (**Figure 5D**). The hypothesis test on the positive expression numbers in the groups indicated that the left side in groups S and SH was remarkably higher than the right side (**Figure 5B**). Image-ProPlus 13.0 was used to quantitatively analyze the optical density of the positive region in the IHC image. Although a positive expression exists in groups S and SH, SH was evidently higher than S (**Figure 5C**). This showed that the expression level of the protein was positively correlated with a vascular injury during AS.

Discussion

For several years, hyperlipidemia and hypertension have been considered the leading causes of AS. Although in recent years, FSS is speculated to be correlated to the occurrence of AS, its relationship with hyperlipidemia is not clear. In the present study, the blood biochemical indexes showed that the lipid concentration in animals fed a high-fat diet is higher than those operated for carotid stenosis. However, micro- and ultramicro-structure results did not show any obvious intima hyperplasia in the former. Nevertheless, injury in representative endothelial cells, the proliferation of SMCs, and the incursion of macrophages are visible in the latter. In addition, necrosis of the intima and tunica media tissue could be observed in the same carotid stenosis animals that formed an irreversible pathophysiological cycle [22]. Therefore, the results of the present study suggested that carotid stenosis via cannulation tends to cause vascular inflammation and initiate stable AS rather than the high-fat diet. Notably, vascular injury is more severe in the carotid stenosis animals fed a high-fat diet than in the carotid stenosis only. A large number of foam cells accumulate in the intima, which further leads to the plaque formation. Some reports confirmed that continued low FSS could precipitate an increase in endothelial cell permeability and MCP-1 expression. This is beneficial to recruit macrophages and assist the low-density lipoproteins located in the endothelial cells [23, 24]. Also, hyperlipidemia provides more lipids to infiltrate the injured vessels caused by artery stenosis. Thus, our data also indicate that FSS

plays a primary role in the early stage of AS, and hyperlipidemia promotes pathological development.

Multiple other studies have converged on β-catenin activation as a key component of arteriosclerotic physiology. In endothelial cells, it interacts with stress fibers to maintain the integrity and regulate the permeability, which is related to the inflammatory reaction of endothelial cells [25, 26]. In SMCs, β-catenin participates in the proliferation and migration [18, 27]. However, we observed for the first time that the protein expression increases markedly in flow-induced AS animals rather than the hyperlipidemia-induced AS animals. In addition, a positive correlation between vascular injury and the expression level of this protein was noted in each group. Although recent studies have shown that β-catenin may play a role in VSMC proliferation induced by hyperlipidemia through the Wnt signaling pathway [27], during the flow-induced AS, it may be initially activated by FSS. Accumulating evidence shows that activation of the β-catenin signaling pathway is a critical downstream event in response to mechanical loading [28-30]. Accordingly, the results of the present study indicate that β-catenin participates in the flow-induced vascular injury.

Previous studies showed that activated Wnt/β-catenin signaling pathway led to VSMC proliferation. Friedrich et al. reported that it can mediate the cell matrix connection in the VSMCs [31], and some reports also posit that the activation of the Wnt/β-catenin signal pathway can promote proliferation of VSMCs and neointima formation [17, 32, 33]. In the present study, we observed that a large amount of β-catenin was localized to the intima hyperplasia, which was due to the proliferation and migration of VSMCs in the flow-induced group. Thus, β-catenin is putatively involved in the proliferation and migration of SMCs during flow-induced AS. Interestingly, the IHC results showed that this protein accumulated in the cytoplasm of VSMCs rather than undergoing a nuclear translocation. Although β-catenin is the key element in Wnt signaling pathway, it also binds to the cytoplasmic region of cadherins, a superfamily of calcium-dependent cell-cell adhesion proteins associated with adherent junctions [34]. β-catenin dissociates from cadherin and translocates to the nucleus while cadherin is modulated by metalloproteinase-mediated proteolysis [35]. This indicates that FSS may not induce VSMCs proliferation through the canonical Wnt signaling pathway. Some reports deem that E-cadherin/β-catenin/Tcf signaling exhibits a pivotal role and participates in AS development, as well as, the β-catenin signal induced by FSS is initiated by adherent junction signaling [36, 37]. We infer that β-catenin may be involved in the flow-induced VSMCs proliferation and migration via the intercellular connection with actin. Nevertheless, the loading of FSS, the relationship between the β-catenin, actin, and cadherin during VSMCs proliferation remains to be studied further. In addition, considering that the protein has been examined for a long time after the surgery, supplementary investigations are essential to determine the FSS regulation of the function of VSMCs through the Wnt/β-catenin pathway in the early stage.

Low shear stress increases the expression of β-catenin and its accumulation in the cytoplasm of proliferative VSMCs rather than its nuclear localization. Thus, we show that β-catenin is an important signaling molecule in shear stress-induced AS. In addition, β-catenin may have some role in the proliferation of VSMCs via the intercellular connection with actin rather than the canonical Wnt signaling pathway.

Acknowledgements

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Disclosure of conflict of interest

None.

Address correspondence to: Xin Sheng, Department of Biochemistry and Molecular Biology, Zunyi Medical University, Zunyi 563000, Guizhou, China. Tel: +86-18212136401; Fax: +86-21-57643271; E-mail: xshengbio@163.com

References

[1] Shaaban AM and Duerinckx AJ. Wall shear stress and early atherosclerosis: a review. AJR Am J Roentgenol 2000; 174: 1657-1665.

[2] Hahn C and Schwartz MA. Mechanotransduction in vascular physiology and atherogenesis. Nat Rev Mol Cell Biol 2009; 10: 53-62.
[3] Conway DE, Williams MR, Eskin SG and McIntire LV. Endothelial cell responses to atheroprone flow are driven by two separate flow components: low time-average shear stress and fluid flow reversal. Am J Physiol Heart Circ Physiol 2010; 298: H367-374.
[4] Chien S. Mechanotransduction and endothelial cell homeostasis: the wisdom of the cell. Am J Physiol Heart Circ Physiol 2007; 292: H1209-1224.
[5] Zaragoza C, Marquez S and Saura M. Endothelial mechanosensors of shear stress as regulators of atherogenesis. Curr Opin Lipidol 2012; 23: 446-452.
[6] Brooks AR, Lelkes PI and Rubanyi GM. Gene expression profiling of human aortic endothelial cells exposed to disturbed flow and steady laminar flow. Physiol Genomics 2002; 9: 27-41.
[7] Owens GK. Regulation of differentiation of vascular smooth muscle cells. Physiol Rev 1995; 75: 487-517.
[8] Owens GK, Kumar MS and Wamhoff BR. Molecular regulation of vascular smooth muscle cell differentiation in development and disease. Physiol Rev 2004; 84: 767-801.
[9] Rudijanto A. The role of vascular smooth muscle cells on the pathogenesis of atherosclerosis. Acta Med Indones 2007; 39: 86-93.
[10] Shi ZD and Tarbell JM. Fluid flow mechanotransduction in vascular smooth muscle cells and fibroblasts. Ann Biomed Eng 2011; 39: 1608-1619.
[11] Asada H, Paszkowiak J, Teso D, Alvi K, Thorisson A, Frattini JC, Kudo FA, Sumpio BE and Dardik A. Sustained orbital shear stress stimulates smooth muscle cell proliferation via the extracellular signal-regulated protein kinase 1/2 pathway. J Vasc Surg 2005; 42: 772-780.
[12] Goldman J, Zhong L and Liu SQ. Negative regulation of vascular smooth muscle cell migration by blood shear stress. Am J Physiol Heart Circ Physiol 2007; 292: H928-938.
[13] Valenta T, Hausmann G and Basler K. The many faces and functions of beta-catenin. EMBO J 2012; 31: 2714-2736.
[14] Wang X, Xiao Y, Mou Y, Zhao Y, Blankesteijn WM and Hall JL. A role for the beta-catenin/T-cell factor signaling cascade in vascular remodeling. Circ Res 2002; 90: 340-347.
[15] Marinou K, Christodoulides C, Antoniades C and Koutsilieris M. Wnt signaling in cardiovascular physiology. Trends Endocrinol Metab 2012; 23: 628-636.
[16] Mill C and George SJ. Wnt signalling in smooth muscle cells and its role in cardiovascular disorders. Cardiovasc Res 2012; 95: 233-240.
[17] Tsaousi A, Williams H, Lyon CA, Taylor V, Swain A, Johnson JL and George SJ. Wnt4/beta-catenin signaling induces VSMC proliferation and is associated with intimal thickening. Circ Res 2011; 108: 427-436.
[18] Chaudhary M, Direnzo D, Shi XD, Franco S, Zent J, Little C, Wang K, Liu B, Guo LW and Kent KC. TGF-β/Smad3 promotes smooth muscle cell de-differentiation and proliferation through crosstalk with the Wnt/β-Catenin pathway. Arteriosclerosis, Thrombosis, and Vascular Biology 2015; 35: A227-A227.
[19] Kamel MA, Picconi JL, Lara-Castillo N and Johnson ML. Activation of beta-catenin signaling in MLO-Y4 osteocytic cells versus 2T3 osteoblastic cells by fluid flow shear stress and PGE2: implications for the study of mechanosensation in bone. Bone 2010; 47: 872-881.
[20] Gelfand BD, Meller J, Pryor AW, Kahn M, Bortz PD, Wamhoff BR and Blackman BR. Hemodynamic activation of beta-catenin and T-cell-specific transcription factor signaling in vascular endothelium regulates fibronectin expression. Arterioscler Thromb Vasc Biol 2011; 31: 1625-1633.
[21] Cheng C, van Haperen R, de Waard M, van Damme LC, Tempel D, Hanemaaijer L, van Cappellen GW, Bos J, Slager CJ, Duncker DJ, van der Steen AF, de Crom R and Krams R. Shear stress affects the intracellular distribution of eNOS: direct demonstration by a novel in vivo technique. Blood 2005; 106: 3691-3698.
[22] Soulis J, Giannoglou G, Dimitrakopoulou M, Papaioannou V, Logothetides S and Mikhailidis D. Influence of oscillating flow on LDL transport and wall shear stress in the normal aortic arch. Open Cardiovasc Med J 2009; 3: 128-142.
[23] von der Thusen JH, van Berkel TJ and Biessen EA. Induction of rapid atherogenesis by perivascular carotid collar placement in apolipoprotein E-deficient and low-density lipoprotein receptor-deficient mice. Circulation 2001; 103: 1164-1170.
[24] Ding SF, Ni M, Liu XL, Qi LH, Zhang M, Liu CX, Wang Y, Lv HX and Zhang Y. A causal relationship between shear stress and atherosclerotic lesions in apolipoprotein E knockout mice assessed by ultrasound biomicroscopy. Am J Physiol Heart Circ Physiol 2010; 298: H2121-2129.
[25] Millan J, Cain RJ, Reglero-Real N, Bigarella C, Marcos-Ramiro B, Fernandez-Martin L, Correas I and Ridley AJ. Adherens junctions con-

nect stress fibres between adjacent endothelial cells. BMC Biol 2010; 8: 11.
[26] de Bruin RG, van der Veer EP, Prins J, Lee DH, Dane MJ, Zhang H, Roeten MK, Bijkerk R, de Boer HC, Rabelink TJ, van Zonneveld AJ and van Gils JM. The RNA-binding protein quaking maintains endothelial barrier function and affects VE-cadherin and beta-catenin protein expression. Sci Rep 2016; 6: 21643.
[27] Zhuang Y, Mao JQ, Yu M, Dong LY, Fan YL, Lv ZQ, Xiao MD and Yuan ZX. Hyperlipidemia induces vascular smooth muscle cell proliferation involving Wnt/beta-catenin signaling. Cell Biol Int 2016; 40: 121-130.
[28] Robinson JA, Chatterjee-Kishore M, Yaworsky PJ, Cullen DM, Zhao W, Li C, Kharode Y, Sauter L, Babij P, Brown EL, Hill AA, Akhter MP, Johnson ML, Recker RR, Komm BS and Bex FJ. Wnt/beta-catenin signaling is a normal physiological response to mechanical loading in bone. J Biol Chem 2006; 281: 31720-31728.
[29] Yonemura S. A mechanism of mechanotransduction at the cell-cell interface: emergence of alpha-catenin as the center of a force-balancing mechanism for morphogenesis in multicellular organisms. Bioessays 2011; 33: 732-736.
[30] Lara-Castillo N, Kim-Weroha NA, Kamel MA, Javaheri B, Ellies DL, Krumlauf RE, Thiagarajan G and Johnson ML. In vivo mechanical loading rapidly activates beta-catenin signaling in osteocytes through a prostaglandin mediated mechanism. Bone 2015; 76: 58-66.
[31] Friedrich EB, Clever YP, Wassmann S, Werner N, Bohm M and Nickenig G. Role of integrin-linked kinase in vascular smooth muscle cells: regulation by statins and angiotensin II. Biochem Biophys Res Commun 2006; 349: 883-889.
[32] Slater SC, Koutsouki E, Jackson CL, Bush RC, Angelini GD, Newby AC and George SJ. R-cadherin:beta-catenin complex and its association with vascular smooth muscle cell proliferation. Arterioscler Thromb Vasc Biol 2004; 24: 1204-1210.
[33] Yu XM, Wang L, Li JF, Liu J, Li J, Wang W, Wang J and Wang C. Wnt5a inhibits hypoxia-induced pulmonary arterial smooth muscle cell proliferation by downregulation of beta-catenin. Am J Physiol Lung Cell Mol Physiol 2013; 304: L103-111.
[34] Oda H and Takeichi M. Evolution: structural and functional diversity of cadherin at the adherens junction. J Cell Biol 2011; 193: 1137-1146.
[35] Uglow EB, Slater S, Sala-Newby GB, Aguilera-Garcia CM, Angelini GD, Newby AC and George SJ. Dismantling of cadherin-mediated cell-cell contacts modulates smooth muscle cell proliferation. Circ Res 2003; 92: 1314-1321.
[36] Bedel A, Negre-Salvayre A, Heeneman S, Grazide MH, Thiers JC, Salvayre R and Maupas-Schwalm F. E-cadherin/beta-catenin/T-cell factor pathway is involved in smooth muscle cell proliferation elicited by oxidized low-density lipoprotein. Circ Res 2008; 103: 694-701.
[37] Arnsdorf EJ, Tummala P and Jacobs CR. Non-canonical Wnt signaling and N-cadherin related beta-catenin signaling play a role in mechanically induced osteogenic cell fate. PLoS One 2009; 4: e5388.