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Mechanisms of aortic dissection smooth muscle cell phenotype switch

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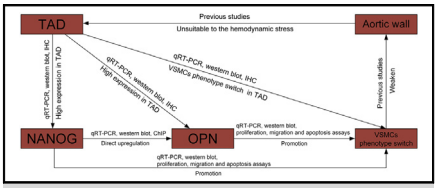
ABSTRACT

Objective: To investigate the expression of Nanog homeobox (NANOG) in thoracic aortic dissection (TAD) and the role of NANOG in regulating human aortic vascular smooth muscle cells (VSMCs) phenotype switch.

Methods: Aortic specimens were collected from 20 patients undergoing TAD and 10 controls. VSMCs were isolated by adherent cultivation approach. The expression of NANOG, osteopontin (OPN), and VSMCs phenotype markers were determined by quantitative real-time polymerase chain reaction, Western blot, immunohistochemistry, and immunofluorescence. Cell counting, scratch wound-healing assay, Transwell migration, and apoptosis assays were used for cell function assessment. Deoxyribonucleic acid–protein binding detection was performed by chromatin immunoprecipitation.

Results: Our experiment results showed that NANOG and OPN were highly expressed in TAD aortic wall and VSMCs, both accompanying VSMCs phenotype switch. Overexpression of NANOG induced the up-regulation of VSMCs synthetic marker matrix metalloproteinase 2 and the down-regulation of VSMCs contractile markers α-smooth muscle actin and smooth muscle 22α. Overexpression of NANOG also enhanced the proliferation, migration, and antiapoptosis capabilities of VSMCs. The results also showed that these functions of NANOG was via OPN and NANOG directly up-regulated OPN by binding to its promoter region.

Conclusions: Our study suggests that NANOG is highly expressed in TAD aortic wall and VSMCs. Increased NANOG promotes VSMCs phenotype switch by directly up-regulating OPN through binding to its promoter region. (J Thorac Cardiovasc Surg 2017;154:1511-21)



Expression of Nanog homeobox (NANOG) and OPN was increased in TAD accompanying vascular smooth muscle cells (VSMCs) phenotype switch. Overexpression of NANOG promoted VSMCs phenotype switch by directly up-regulating OPN.

Central Message

Nanog homeobox is highly expressed in thoracic aortic dissection and promotes the vascular smooth muscle cells phenotype switch by directly up-regulating osteopontin through binding to its promoter region.

Perspective

Thoracic aortic dissection (TAD) is a life-threating disease. Although the treatment strategy of it has been improved continuously, the mortality of TAD is still very high and its pathogenesis has not been clarified fully. This study aimed to clarify the mechanism responsible for the occurrence of TAD.

See Editorial Commentary page 1522.

Thoracic aortic dissection (TAD) is a life-threating medical emergency with very high mortality.[1,2] Some connective tissue diseases can result in TAD, but only approximately 10% of TAD can be attributed to connective tissue diseases, and the pathogenesis of the others is still unclear.[3] It is known that the abnormity of aortic media is the pathogenetic base of TAD.[4,5] Vascular smooth muscle cells (VSMCs) are the main cell type of aortic media and can transform between contractile and synthetic phenotypes.[6] Compared with contractile VSMCs, synthetic VSMCs show the enhanced proliferation, migration, and expression of extracellular matrix components like matrix metalloproteinase 2 (MMP2) and matrix metalloproteinase 9, but synthetic VSMCs express decreased levels of contractile markers such as α-smooth muscle actin (α-SMA) and smooth muscle 22α (SM22α).[7] It was reported the VSMCs phenotype switch might be involved

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Scanning this QR code will take you to supplemental figures, video, and appendix for this article

Abbreviations and Acronyms

Ad-NANOG	= NANOG overexpression adenovirus
α-SMA	= α-smooth muscle actin
ChIP	= chromatin immunoprecipitation
MMP2	= matrix metalloproteinase 2
NANOG	= Nanog homeobox
OPN	= osteopontin
qRT-PCR	= quantitative real-time polymerase chain reaction
si-NANOG	= NANOG-specific small interfering RNA
si-NC	= scrambled negative control siRNA
si-OPN	= OPN-specific small interfering RNA adenovirus
SM22α	= smooth muscle 22α
TAD	= thoracic aortic dissection
TUNEL	= terminal deoxynucleotidyl transferase dUTP nick end labeling
UV	= ultraviolet
VSMC	= vascular smooth muscle cell

in the pathophysiologic processes of TAD.[8-11] However, little is known about the molecular mechanism of the VSMCs phenotype switch in TAD.

Osteopontin (OPN), also known as secreted phosphoprotein 1, is a multifunction protein that is highly expressed in synthetic VSMCs.[9] OPN is related to some important biological behaviors of VSMCs, and high-level OPN promotes the proliferation and migration of VSMCs.[12] In addition, OPN also contributes to the apoptotic resistance of VSMCs.[13] It was reported OPN up-regulates the expression of MMP2 and down-regulates the expression of α-SMA in VSMCs.[14-16] Moreover, Wang and colleagues[8] reported OPN was highly expressed in TAD aortic media accompanying the VSMCs phenotype switch. All these findings indicate that OPN may induce the VSMCs phenotype switch in the pathophysiologic processes of TAD. However, there are no reports on the exact mechanism of OPN up-regulation in TAD.

Nanog homeobox (NANOG) is a homeodomain protein and has a vital function in the transcriptional network of pluripotency and proliferation in embryonic stem cells. The expression of NANOG maintains the pluripotency and self-renew of embryonic stem cells and induces cell dedifferentiation.[17-19] In contrast, deficiency of NANOG induces cell differentiation.[20] The function of NANOG suggests it may be related with the phenotype switch of VSMCs in TAD, which is regarded as a dedifferentiation process.[11,21] More importantly, it was reported NANOG might regulate the expression of OPN in human embryonic stem cells.[22] All these indicate that the up-regulation of OPN in TAD may be regulated by NANOG.

In this study, by a series of histology and molecular biology experiments, we sought to determine whether NANOG promoted the VSMCs phenotype switch by directly up-regulating OPN in the pathophysiologic processes of TAD.

METHODS

Specimen Collection

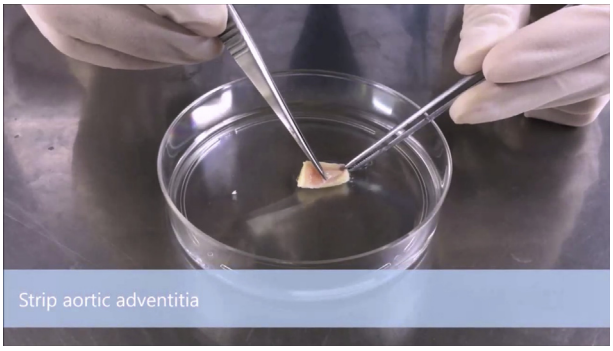
Specimens used in this study were acquired at the Department of Cardiovascular Surgery, Changhai Hospital, from April to June, 2015. Dissected aortic specimens near the crevasse were obtained from the rupture area in 20 continuous patients who consented to undergo TAD repair surgery. Patients with traumatic aortic injury, inflammatory aortic disease, Ehlers-Danlos syndrome, Marfan syndrome, and other connective tissue disorders were excluded. Control aortic specimens were obtained from 10 continuous patients who underwent aortic valve replacement and who had no vascular diseases. Table E1 summarizes patient details. Specimens were divided into pieces and preserved under different conditions for cell culture, histologic, and biochemical analysis. This study conformed to the principles outlined in the Declaration of Helsinki, and informed consent was obtained from all patients or their direct relatives. This study was approved by the Institutional Review Board of Changhai Hospital.

Cell Culture

Primary VSMCs were isolated from aortic media by an adherent cultivation approach (Video 1 and Appendix E1).

Genes Overexpression and Silencing in VSMCs

NANOG overexpression adenovirus (Ad-NANOG) with green fluorescent protein tag and green fluorescent protein control adenovirus were purchased from Hanbio Biotechnology (Shanghai, China). NANOG-specific small interfering RNA (si-NANOG) and the scrambled



VIDEO 1. Isolation of aortic vascular smooth muscle cells by adherent cultivation approach. First, aortic adventitia and intima were stripped from specimens. Then, the rest were cut into about 4-mm^2 tissue blocks and each block was inoculated in a 24-well culture plate. The plate was inversed and incubated at 37°C with 5.0% carbon dioxide for 1 hour. After that, 200 μL of Medium 231 with smooth muscle growth supplements (Cascade Biologics, Waltham, Mass) was added into each well. At last, the plate was incubated at 37°C with 5.0% carbon dioxide and the culture medium was replaced every 72 hours. Video available at: http://www.jtcvsonline.org/article/S0022-5223(17)31119-4/addons.