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S100A12 Expression in Thoracic Aortic Aneurysm Is Associated With Increased Risk of Dissection and Perioperative Complications

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Objectives The purpose of this study was to determine the relevance of S100A12 expression to human thoracic aortic aneurysms and type A thoracic aortic aneurysm dissection and to study mechanisms of S100A12-mediated dysfunction of aortic smooth muscle cells.

Background Transgenic expression of proinflammatory S100A12 protein in murine aortic smooth muscle causes thoracic aneurysm in genetically modified mice.

Methods Immunohistochemistry of aortic tissue (n = 50) for S100A12, myeloperoxidase, and caspase 3 was examined and S100A12-mediated pathways were studied in cultured primary aortic smooth muscle cells.

Results We found S100A12 protein expressed in all cases of acute thoracic aortic aneurysm dissection and in approximately 25% of clinically stable thoracic aortic aneurysm cases. S100A12 tissue expression was associated with increased length of stay in patients undergoing elective surgical repair for thoracic aortic aneurysm, despite similar preoperative risk as determined by European System for Cardiac Operative Risk Evaluation. Reduction of S100A12 expression in human aortic smooth muscle cells using small hairpin RNA attenuates gene and protein expression of many inflammatory- and apoptosis-regulating factors. Moreover, genetic ablation of the receptor for S100A12, receptor for advanced glycation end products (RAGE), in murine aortic smooth muscle cells abolished cytokine-augmented activation of caspase 3 and smooth muscle cell apoptosis in S100A12-expressing cells.

Conclusions S100A12 is enriched in human thoracic aortic aneurysms and dissections. Reduction of S100A12 or genetic ablation of its cell surface receptor, the receptor for advanced glycation end products (RAGE), in aortic smooth muscle resulted in decreased activation of caspase 3 and in reduced apoptosis. By establishing a link between S100A12 expression and apoptosis of aortic smooth muscle cells, this study identifies novel S100A12 signaling pathways and indicates that S100A12 may be a useful molecular marker and possible target for treatment for human aortic diseases. (J Am Coll Cardiol 2012;60:775–85) © 2012 by the America n College of Cardiology Foundation

Thoracic aortic aneurysms (TAA) encompass a broad range of degenerative, genetic, structural, and acquired disease states and can be complicated by potentially life-threatening type A thoracic aortic aneurysm dissection (TAAD). There is growing evidence of genetic variation in genes known to be critically important in the development of TAA, including *FBN1* encoding fibrillin 1, *TGFBR1/2* encoding transforming growth factor-β receptors, and *ACTA2* and *MHY11*, which encode smooth muscle α-actin and myosin heavy chain, respectively, that predispose humans to aortic diseases (1). We recently reported that transgenic mice engineered to express human S100A12 in the aortic smooth muscle develop thoracic aneurysms (2), similar to mouse models of Marfan syndrome (3). S100A12 is a proinflammatory protein that activates the receptor for advanced glycation end products (RAGE) (4) and accelerates atherosclerosis

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Abbreviations and Acronyms

DNA = deoxyribonucleic acid

EuroSCORE = European System for Cardiac Operative Risk Evaluation

HASMC = human aortic smooth muscle cells

IgG = immunoglobulin G

LPS = lipopolysaccharide

MASMC = murine aortic smooth muscle cells

MPO = myeloperoxidase

RAGE = receptor for advanced glycation end products

SMC = smooth muscle cells

TAA = thoracic aortic aneurysms

TAAD = thoracic aortic aneurysm dissection

TNF = tumor necrosis factor

in apolipoprotein A-deficient mice (5). Moreover, S100A12 gene expression in human peripheral blood cells is one of the most predictive genes for obstructive coronary artery disease (6), and S100A12 is expressed in smooth muscle and inflammatory cells in ruptured coronary artery plaque of victims of sudden cardiac death (7), suggesting a critical role of S100A12 in vascular disease and atherothrombosis. Although the expansion of the aortic wall occurs slowly and often is asymptomatic clinically, dissection of the medial layer with bleeding within and along the aortic wall occurs suddenly and leads to major complications, including rupture, ischemia, and arterial thrombosis with embolization, accounting for at least 16,000 deaths annually in the United States (1,8). The precipitating mechanisms underlying dissection are incompletely understood and include hemodynamic factors, endothelial factors, as well as dysfunctional smooth muscle cells (SMC). Moreover, inflammation and the presence of inflammatory cells within the aortic media recently were emphasized as potential mediator for dissections (8,9). Because S100A12 is a potent proinflammatory and chemoattractant protein, and TAA develops in S100A12 transgenic mice, we wished to examine S100A12 expression in human aortic diseases. We found strong expression of S100A12 in inflammatory cells and in SMC in all cases of thoracic aneurysms with type A dissection and in approximately 25% of cases of clinically stable TAA. Furthermore, studies on cultured human aortic smooth muscle cells (HASMC) obtained from patients with TAA or TAAD showed a direct role of S100A12 in mediating apoptosis. Together, these data demonstrate that S100A12 is up-regulated in TAAD and may contribute to the pathogenesis of TAAD by initiating apoptosis of SMC, at least in part via increased oxidative stress.

Methods

The University of Chicago Medical Center Pathology Tissue Bank was searched between 2007 and 2010, and 20 cases of elective TAA repair were chosen randomly. To validate our findings from the initial 20 randomly chosen patients, 30 additional patients with TAA or TAAD were studied with either emergent surgery for type A aortic dissections or elective surgery for large aneurysms (>5.5 cm). All patients had no pre-operative diagnosis or known coronary artery diseases or peripheral artery disease. Retrospective data were collected by reviewing patient medical records, and operative risk before surgery was calculated using the additive and the logistic European System for Cardiac Operative Risk Evaluation (EuroSCORE). Normal control aortic tissue was obtained from heart donors (n = 3, 2 men, age: 44 ± 12 years), aortic valve replacement for stenotic tricuspid aortic valve with normal aorta (n = 2, 2 men, age: 48 ± 8 years), and elective left ventricular assist device (n = 1, woman, age: 32 years). Serial histological sections were prepared from paraffin-embedded aortic tissue blocks and were stained with hematoxylin and eosin, Verhoeff Van Giessen, and Movat, and immunochemistry analyses were carried out with staining with αS100A12 immunoglobulin G (IgG, Abcam 37657, Abcam Cambridge, United Kingdom), α-myeloperoxidase (MPO) IgG (Abcam 9535, Abcam, Cambridge, Massachusetts), and α-smooth muscle actin (Sigma-Aldrich). Staining for S100A12 was semiquantified by 2 blinded investigators (D.D., M.A.H.B.) as absent (0), mild (1+), moderate (2+), or intense (3+) in cells that demonstrated either positive or negative results for MPO staining. Loss of aortic medial elastic fibers was graded as 1 (trace), 2 (mild), 3 (moderate), and 4 (full-thickness loss), as previously reported by Roberts et al. (10,11).

Aortic SMC. HASMCs were cultured from operatively excised aortic tissue (bicuspid aortic valve: n = 3, *TGFBR1* mutation: n = 2, *FBN1* mutation: n = 2, unknown cause: n = 3), as previously described (2). Control HASMC were obtained from heart donors (n = 3), aortic valve replacement for stenotic tricuspid aortic valve with normal aorta (n = 2), and elective left ventricular assist device (n = 1). Murine aortic smooth muscle cells (MASMC) were obtained from hemizygous S100A12 transgenic mice of the C57BL6/J strain previously generated (2) and from mice mated with C57BL6/J mice lacking RAGE ($RAGE^{-/-}$), supplied by Dr. Ann Marie Schmidt (New York University, New York, New York). Only cell cultures demonstrating negative results for contaminating leukocytes (α-CD45.2 antibody from Pharmingen no. 559985 and α-CD68 antibody from Pharmingen no. 556059, BD Biosciences Pharmingen, San Diego, California) were propagated. SMC characterized by staining for smooth muscle actin (Sigma-Aldrich) from passage 4–7 were used for experiments. If indicated, soluble RAGE, the extra cellular ligand binding domain of RAGE (20 μg/ml, R&D Systems), bovine serum albumin (20 μg/ml, Sigma-Aldrich), α lipoic acid (1 μM, 10 μM, and 100 μM, AstraZeneca), or diphenyleneiodonium (DPI) (1 and 10 μM, Sigma-Aldrich) was added before stimulation with lipopolysaccharide (LPS) (100 ng/ml, Sigma-Aldrich), tumor necrosis factor alpha (TNFα) (10 ng/ml, Pierce, Rockford, Illinois), or αFas IgG (CH 11, EMD Millipore). Where indicated, HASMC were transfected with small hairpin RNA (shRNA) encoding for S100A12 and control shRNA (SABiosciences) using the lipofectamine method (Clontech). Transfected HASMC were selected 3 days later by fluorescence-