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Spatiotemporal patterns of smooth muscle cell changes in ascending aortic dilatation with bicuspid and tricuspid aortic valve stenosis: Focus on cell–matrix signaling

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Supplemental material is available online.

Objective: The present study examined temporal and spatial patterns of extracellular matrix and smooth muscle cell changes in the ascending aorta with bicuspid and tricuspid aortic valve stenosis.

Methods: Wall specimens were retrieved from both the greater and the lesser curvature ("convexity" and "concavity") of 14 nonaneurysmal and 12 aneurysmal aortas (aortic ratios 1.2 and 1.5, respectively) and from 3 heart donors (normal). Immunochemistry was performed for detection of apoptotic (terminal deoxynucleotidyl transferase-mediated dUTP nick end labelling [TUNEL]-positive) and proliferating (Ki-67-positive) smooth muscle cells and for semiquantification of matrix proteins (collagens, fibronectin, tenascin, laminin). Co-immunoprecipitation assessed the extent of Bcl-2-modifying factor binding to Bcl-2, indicating a matrix-derived cytoskeleton-mediated proapoptotic signaling. Polymerase chain reaction allowed for quantification of messenger RNA expression for Bcl-2.

Results: In both bicuspid and tricuspid aneurysms, fibrillar collagens were reduced, whereas fibronectin and tenascin were increased compared with those in normal conditions. These matrix alterations were already evident in bicuspid nonaneurysmal aortas at the convexity, with significant elevation of apoptotic indexes ($P = .02$ bicuspid vs normal; $P = .48$ tricuspid vs normal). Apoptotic indexes correlated with aortic dimensions only in tricuspid aortas ($P = .01$). No significant increase in Ki-67 was found. Higher levels of Bcl-2-modifying factor-Bcl-2 binding were found in bicuspid nonaneurysmal aorta versus tricuspid ($P = .03$) and normal aortas ($P = .01$). Bcl-2 messenger RNA expression was reduced in the bicuspid aorta versus normal ($P = .08$).

Conclusions: Smooth muscle cell apoptosis with bicuspid aortic valve stenosis occurred before overt aortic dilation, mainly at the convexity, where wall stress is expectedly higher. In this setting, a matrix-dependent proapoptotic signaling was evidenced by increased Bcl-2-modifying factor-Bcl-2 binding. Stress-dependent bicuspid aortic valve matrix changes may trigger early apoptosis by inducing cytoskeletal rearrangement.

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The traditional definition of medial degeneration of the ascending aorta includes the association of smooth muscle cell (SMC) loss, owing to apoptosis,[1,2] and extracellular matrix (ECM) rearrangement.[3,4] Recently, remarkable improvements have been achieved in the knowledge of the mechanisms underlying ascending aortic dilations,[1-4] but without identifying definite pathogenetic sequences. Indeed, medial degeneration can underlie a variety of anatomoclinical conditions (eg, the senile aorta, poststenotic dilations, idiopathic or syndromic aneurysms, dissections), suggesting different possible mechanisms of lesion

Abbreviations and Acronyms

AI = apoptotic index
BAV = bicuspid aortic valve
Bmf = Bcl-2-modifying factor
DAPI = 4′,6-diamidino-2′-phenylindole
ECM = extracellular matrix
GAPDH = glyceraldehyde-3-phosphate dehydrogenase
MMP = matrix metalloproteinases
mRNA = messenger RNA
PBS = phosphate-buffered saline
PCR = polymerase chain reaction
SMC = smooth muscle cell
TAV = tricuspid aortic valve
TUNEL = terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling

development.[1] In particular, aortic dilations associated with congenital bicuspid aortic valve (BAV) are known to differ from those with tricuspid aortic valve (TAV) disease in terms of histopathology,[2] anatomic configuration,[5] and natural history.[6]

In our preliminary investigation, we[4] defined medial ECM changes in BAV-associated dilations, observing a decrease in collagen content versus normal aorta and an increase in fibronectin and the expression of tenascin. However, no comparison with TAV-associated dilations and no distinction between early changes and mature lesions were performed. The present study was undertaken as a more in-depth continuation of the previous one,[4] with the aim to assess spatial and temporal patterns of ECM protein and SMC changes in BAV- and TAV-associated aortic dilations. Moreover, inasmuch as changes in the ECM composition, including those previously observed in BAV aortopathy,[3,4] are known to possibly influence SMC phenotype, proliferation, survival, and synthetic activity,[7,8] we focused on the hypothesis that SMC apoptosis in ascending dilations could be provoked by matrix-derived signaling. *Anoikis* (the ancient Greek word for homelessness) is the term indicating apoptosis caused by the loss of normal adhesion of cells to a normally organized ECM, occurring via altered cell "tensegrity," the tensional integrity of the cytoskeleton.[9] Matrix disarray and/or cell detachment can induce loss of cytoskeletal integrity, cell shape changes, and eventually cell death[9,10]: thus, "amorphosis" is considered as a typical feature/modality of anoikis.[10,11] Proapoptotic cues are known to be transmitted to cells by the ECM in both the settings of vascular physiology and disease.[9] In the present study, we hypothesized that matrix-derived proapoptotic signals could be involved in the development of BAV aortopathy.

Patients and Methods

Study Patients

The study included 26 patients with aortic valve stenosis (mean age 59 ± 15 years, 65% male) undergoing aortic valve replacement and/or ascending aorta replacement. Valve morphology (BAV/TAV) was defined on the basis of concordant echocardiographic diagnosis, surgical inspection, and pathologic examination. In case of discordances, patients were considered noneligible. Twelve patients had degenerative stenosis of a TAV, and 14 had a stenotic congenital BAV. Respective TAV and BAV subgroups were comparable for clinical and echocardiographic data (Table E1). No patient had inherited connective tissue abnormalities, atherosclerotic aneurysm, or associated aortic regurgitation (more than mild). To define temporal patterns of medial change progression, we distinguished subgroups according to aortic dimensions: nonaneurysmal aortas (mild dilations) were defined as an aortic ratio (between echocardiographically measured maximal diameter and expected diameter, based on age and body surface area) less than 1.4 (corresponding, on average, to a diameter of 4.5 cm), and aneurysms were defined as a ratio greater than 1.4.

Sample Retrieval Protocol

At surgical inspection, all BAV-associated aortic aneurysms appeared asymmetric, with predominant bulging of the right anterolateral side of the vessel. This configuration was found also in 3 TAV aneurysms (60%), whereas the other 2 showed a fusiform, symmetric dilation at both the outer (convexity) and inner curves (concavity). The pattern of cusp fusion was right-left coronary in 12 BAV patients and right-noncoronary in the other 2. To define spatial patterns of lesion expression, according to a previously introduced protocol,[4] soon after the aortotomy was performed, we retrieved 2 full-thickness aortic wall samples distal to the sinotubular junction (maximal dilation level) in each patient: one at the convexity and the other at the concavity (from the two ends of the transverse aortotomy in patients without indication to concomitant ascending aorta replacement). Wall specimens were retrieved at the same levels also from 3 normotensive heart donors during multiorgan procurement (2 men, 1 woman; mean age 40 ± 3 years; mean diameter 3.4 ± 0.15 cm). Thus, 58 specimens overall were available for examination. The study was approved by our institution's ethical committee and patients gave informed consent.

Immunohistochemistry of Matrix Proteins

Specimens (n = 52) were fixed in buffered 10% formalin, embedded in paraffin, and sectioned. Serial 4 μm-thick sections of aortic specimens were deparaffinized, covered with primary monoclonal antibodies against type I collagen, tenascin-C, and laminin or polyclonal antibody for fibronectin (Sigma-Aldrich, St Louis, Mo), and type III collagen (Santa Cruz Biotechnology Inc., Santa Cruz, Calif), and incubated in a moist chamber for 1 hour at 37°C. After washings in phosphate-buffered saline (PBS), they were covered with fluoresceinated secondary antibodies (Sigma-Aldrich) and reincubated. Nuclei were stained with propidium iodide, and then sections were mounted in Vectashield mounting medium (Vector Laboratories, Burlingame, Calif) and observed with Leica DMLB fluorescence microscope (Leica Microsystems, Inc, Bannockburn, Ill). Moreover, a Zeiss LSM-510 confocal microscope (Karl Zeiss