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Distinct structural and molecular features of the myocardial extracellular matrix remodeling in compensated and decompensated cardiac hypertrophy due to aortic stenosis

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ABSTRACT

Objectives: We used immuhistochemistry and Western blot to study fibrillar and non-fibrillar collagens, collagen metabolism, matricellular proteins and regulatory factors of the ECM remodeling in left ventricular (LV) septum biopsies from 3 groups of patients with aortic valve stenosis (AS): (AS-1,n = 9): ejection fraction (EF) > 50%; AS-2,(n = 12): EF 30%–50%; AS-3,(n = 9): EF < 30%). Samples from 8 hearts with normal LV function served as controls.

Results: In comparison with controls, fibrillar collagens I and III were progressively upregulated from compensated (AS-1) toward decompensated hypertrophy (AS-3). The collagenIII/collagen I ratio decreased 2-fold in the AS-2 and AS-3 groups as compared with AS-1 and controls. Non-fibrillar collagen IV was upregulated only in AS-3 patients, whereas collagen VI progressively increased from AS-1 to AS-3 group. Collagen synthesis in AS-3 was shifted to collagen I, while the maturation/degradation level was shifted to collagen III. RECK was downregulated only in AS-3 patients. Matricellular proteins tenascin and osteopontin were increased in all AS patients. However, thrombospondin 1, 4 and CTGF were increased only in AS-3. Only AS-3 patients were characterized by increased levels of TGFβ1 and downregulation of TGFβ3, TGFβ-activated kinase1 and Smad7. In contrast, Smad3 gradually increased from AS-1 toward AS-3. Similar trend of changes was observed for TNFα-R1 and TNFα-R2, whereas TNFα was diminished only in AS-2 and AS-3.

Conclusions: Distinct changes in fibrillar collagen turnover, non-fibrillar collagens, matricellular proteins and the key regulatory profibrotic and anti-fibrotic factors of the myocardial ECM remodeling are involved in the transition from compensated to decompensated LV hypertrophy and HF in human patients with AS.

Abbreviations: AS, aortic valve stenosis; BMP1, bone morphogenetic protein 1; CNN, Cyr61, connective tissue growth factor, Nov; CTGF, connective tissue growth factor; EF, ejection fraction; ECM, extracellular matrix; HF, heart failure; IHC, immunohistochemistry; ICTP, carboxyterminal telopeptide (degradation product of cross-linked collagen I); IIINP, aminoterminal telopeptide (cross-linked mature collagen III); MMP, matrix metalloproteinase; NYHA, New York Heart Association; LV, left ventricle; LVEDP, left ventricular end-diastolic pressure; PCP, procollagen c-proteinase; PINP, aminoterminal propeptide of type-I procollagen (newly synthesized collagen I); PIIINP, N-terminal type III collagen peptide (newly synthesized collagen III); QIF, quantitative immunofluorescence; RECK, reversion-inducing cysteine-rich protein with Kazal morifs; Smad, small mother against decapentaplegic; TAK1, transforming growth factor β activated kinase 1; TBST, Tris buffer saline Tween-20; TGFβ, transforming growth factor β; TIMP, tissue inhibitor of matrix metalloproteinases; TNFα, Tumor necrosis factor α; TNFα-R1, Tumor necrosis factor α receptor 1; TNFα-R2, Tumor necrosis factor α receptor 2; TSP, thrombospondin; WB, Western blot.

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Table 1
Clinical data.

	Control (n = 8)	AS-1 (n = 9)	AS-2 (n = 12)	AS-3 (n = 9)
Age, years	55.8 ± 9.8	64.3 ± 8.5	66.1 ± 8.2	67.2 ± 9.9
Men/women	6/2	5/4	5/7	6/3
NYHA (classes III–IV)	0	0	3	7 (77.8%)
EF, %	>60%	59.5 ± 4.8	40.6 ± 6.4	24.3 ± 4.8
LV mass/m^2	92.7 ± 12.2	130.5 ± 20.2	138.3 ± 11.7	145.2 ± 14.4
IVS (mm)	11 ± 0.8	15.1 ± 4.2	17.4 ± 4.6	19.8 ± 6.2
ΔP mean (mm Hg)	–	65.2 ± 11.5	54.3 ± 14.9	42.8 ± 17.7
LVEDP	8 ± 1	14 ± 4	18 ± 5	23 ± 7
LVESP	130 ± 12	171 ± 17	165 ± 14	152 ± 15
LVEDD	49 ± 2	47 ± 6	48 ± 6	56 ± 6
LVESD	33 ± 3	30 ± 7	36 ± 6	42 ± 10
Diuretics, (n/%)	–	0	3 (25%)	7 (77.8%)
Digitals, (n/%)	–	0	1 (8.3%)	7 (77.8%)
ACE inhibitors, (n/%)	–	2 (22.2%)	2 (16.7%)	2 (22.2%)
β-Blockers, (n/%)	–	1	3 (25%)	3 (33.3%)

LVEDP, LV end-diastolic pressure; LVESP, LV end-systolic pressure; IVS, interventricular septum; LVEDD, LV end-diastolic diamter; LVESD, LV end-systolic diamter; ΔP mean, mean ventricular-aortic pressure gradient.

Table 2
List of antibodies.

Antibody	Company	Host	WB/IHC
Collagen I	Rockland	Rabbit	IHC
Collagen III	Rockland	Rabbit	IHC
Collagen IV	Rockland	Rabbit	IHC/WB
Collagen V	Rockland	Rabbit	IHC/WB
Collagen VI	Rockland	Rabbit	IHC/WB
BMP1	Oncogene	Rabbit	WB
RECK	BD Biosciences	Mouse	WB
Osteonectin	Santa Cruz	Rabbit	WB
Osteopontin	Acris	Rabbit	WB
Tenascin	Sigma	Mouse	IHC
Tenascin C	R&D	Rat	IHC
TSP1	Calbiochem	Mouse	IHC/WB
TSP2	Santa Cruz	Rabbit	WB
TSP4	Santa Cruz	Mouse	WB
CTGF	Santa Cruz	Rabbit	IHC
CTGF	Abcam	Rabbit	WB
TGFβ1	Santa Cruz	Rabbit	WB
TGFβ2	Abcam	Rabbit	WB
TGFβ3	Abcam	Rabbit	WB
TAK1	Cell Signaling	Rabbit	WB
Smad1	Cell Signaling	Rabbit	WB
Smad2	Cell Signaling	Mouse	WB
Smad3	Cell Signaling	Rabbit	WB
Smad 7	Santa Cruz	Rabbit	WB
TAK1	Cell Signaling	Rabbit	WB
TNFα	Sigma	Mouse	WB
TNFα-R1	Biovision	Mouse	WB
TNFα-R2	Abcam	Rabbit	WB
Actin (HHF35)	Sigma	Mouse	WB

WB, Western blot, IHC, immunohistochemistry.

1. Introduction

Aortic valve stenosis (AS) is often associated with heart failure and high mortality [1–3]. Myocardial remodeling is a determinant stage of heart failure (HF), which is characterized by changes of ventricular size, shape and function [4]. The process of left ventricular (LV) remodeling is accompanied by cardiomyocyte hypertrophy and loss, extracellular matrix (ECM) reorganization and the increased ECM elements [5–8]. Despite numerous studies, the exact cellular and molecular mechanisms of cardiac remodeling in AS remain mostly unknown and are limited mainly to experimental animal data [9–14]. We and others have previously shown that the extent of ECM remodeling and myocardial fibrosis may not be reversible after delayed AS surgery [3,5,15–17]. Therefore, detection of the structural and molecular components involved in myocardial ECM remodeling could help to find promising therapeutic targets and prospective biomarkers to optimize the time of aortic valve replacement.

The collagens are the most abundant proteins of the ECM and well-accepted tissue markers of cardiac remodeling [18]. The adult myocardium consists of fibrillar collagen type I and collagen type III [19]. Collagen type V is another fibrillar collagen that in humans is encoded by the COL5A1 gene [20]. The distribution and expression of collagen V in the normal or pathological heart are almost unknown.

Although collagen type I and III are the most prevalent in myocardial ECM and their role in myocardial remodeling is increasingly studied, the role of non-fibrillar collagens such as collagen type IV and VI is less studied. Recently it was demonstrated that deletion of collagen VI in mice has a protective effect after myocardial infarction by reducing fibrosis and pathological remodeling [21]. It is reasonable therefore to hypothesize that collagen VI is also involved in ECM remodeling in the hypertrophied heart.

The fibrillar collagens are secreted into the ECM as N-terminal propeptides PINP and PIIINP, carboxyterminal (ICTP) and aminoterminal telopeptides. PINP and PIIINP represent the newly synthesized collagen I and collagen III, respectively. ICTP detects degraded collagen I, whereas IIINTP is a marker of cross-linked mature collagen III [15, 22–25].

Collagen turnover is essential in ECM remodeling. Removal of c-terminal propeptides of fibrillar procollagens is a crucial event in collagen fibril-formation and is accomplished by procollagen c-proteinases which are identical to bone morphogenetic protein-1 (BMP1), a member of the tolloid family of Zn-dependent astacin-like metalloproteinases [26]. Collagen turnover is regulated basically by matrix metalloproteinases and their tissue inhibitors. A newly discovered alternative inhibitor of matrix metalloproteinases is RECK (reversion-inducing cysteine-rich protein with Kazal morifs) [27]. However, neither BMP1 nor RECK has been studied in relation to compensated or decompensated myocardial hypertrophy.

Matricellular proteins have been classified as a family of non-structural family of extracellular proteins that do not play primarily a structural role in the ECM but influence collagen fibril assembly [28]. The role of this protein family in myocardial remodeling in the hypertrophied and/or failing myocardium still remains obscure. As an example, tenascin, thrombospondin-1 and 2, osteonectin and osteopontin are highly expressed during embryogenesis and are very low or absent in adult normal hearts. In contrast, during HF progression these proteins appear to be up-regulated [29]. In addition, the role of connective tissue growth factor in the heart which also belongs to matricellular proteins is limited to myocardial infarction [30].

Myocardial ECM remodeling is essentially regulated by profibrotic and anti-fibrotic factors. Among them, an important role has TGF-β, consisting of three highly conserved isoforms, designated TGFβ-1, TGFβ-2, and TGFβ-3. From all these 3 isoforms, only TGFβ-1 has intensively been studied in the pathological heart. Tumor necrosis factor-alpha (TNFα) is another factor which is believed to be involved in the regulation of fibrosis [31]. However, more studies are needed to substantiate its role in fibrosis, especially in the transition from compensated to decompensated cardiac hypertrophy.

Fig. 1. Representative confocal micrographs of collagen type I (green) and type III (red) in controls and in patients with AS demonstrating a gradual increase accumulation of these fibrillar collagens from controls to AS-1, AS-2 and AS-3. Merged images (right panels) show the prevalence of collagen III over that of collagen I in control and partially in AS-1 patients, whereas in the AS-2 and AS-3 groups, the quantity of both collagens is apparently equal. Nuclei are shown in white after staining with DAPI and F-actin is shown in blue after labeling with Alexa633-phalloidin. Lower diagrams represent quantitative data of collagen I, collagen III, and the collagen III/collagen I ratio in control and in AS groups. Notice that the AS-2 and AS-3 groups exhibit significantly lower collagen III/collagen I ratio in comparison with the AS-1 group and controls.