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PROSPECT

Extracellular Matrix Dynamics in Heart Failure: A Prospect for Gene Therapy

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Abstract In chronic congestive heart failure, an illness affecting more than 4 million Americans, there is impairment of myocardial extracellular matrix (ECM) remodeling. Failing human ventricular myocardium contains activated matrix metalloproteinases (MMPs), which are involved in adverse ECM remodeling. Our studies support the concept that impaired ECM remodeling and MMP activation are, in part, responsible for the cardiac structural deformation and heart failure.

There is no known program that has declared its aim the investigation of the role of ECM gene therapy in heart failure. The development of transgenic technology, and emerging techniques for in vivo gene transfer, suggest a strategy for improving cardiac function by overexpressing or downregulation of the ECM components such as MMPs, tissue inhibitor of metalloproteinases (TIMPs), transforming growth factor-β1 (TGF-β), decorin, and collagen in cardiomyopathy and heart failure. J. Cell. Biochem. 68:403–410, 1998.

Key words: extracellular matrix; gene therapy; collagen; matrix metalloproteinase; tissue inhibitor of metalloproteinase; transforming growth factor; decorin; cardiomyopathy; hypertrophy; ischemia; fibrosis; functional genomics

EXTRACELLULAR MATRIX DYNAMICS AND GENETIC BASIS OF HEART FAILURE

The extracellular matrix (ECM) plays a significant role in maintaining the efficiency of cardiac function. Structural anatomic abnormalities lead to cardiac dysfunction and are, in part, contributory to congestive heart disease [22]. A number of studies have been focused on the cardiac dysrhythmias or conduction abnormalities. The ECM, including type I fibrillar collagen and its receptor integrins, which surround the cardiac cell, forms a 3-dimensional network and bridges the ECM and the cellular cytoskeletal myofibril [3,4]. This skeletal framework provides tensile strength to the tissue, governs tissue stiffness and preserves the alignment of cardiac myocytes [3,4]. Therefore, it indirectly governs the structural architecture and geometry of the myocardium and its ventricular chambers [7]. Morphologic evidence of fibrillar collagen disruption and myocyte slippage has been observed in heart failure [21]. Abnormalities in ECM composition and concentration during dilation, hypertrophy, and ischemic injury leads to heart failure (Fig. 1). In dilated cardiomyopathy (DCM) patients with cardiac amyloidosis, elevated myocardial collagenase activity was associated with fibrillar collagen degradation [24]. During the development of DCM in patients and animals, disruption and discontinuity in collagen fibers have been observed leading to adverse cardiac remodeling [4,44]. Remodeling of ECM implies an alteration in the ECM and in the spatial orientation of cells and intracellular components. Specialized proteinases that are capable of breaking down the ECM (i.e., matrix metalloproteinases, MMPs) and the inhibitors of proteinases (i.e., tissue inhibitor of metalloproteinases [TIMPs]), appear to be balanced in the normal myocardium thereby maintaining the integrity of the myocardium [27,28,31]. We have demonstrated that the equilibrium between proteinase and antiproteinase is altered following heart failure [34] (Fig. 2). We demonstrated that tissue plasminogen activator (tPA) con-

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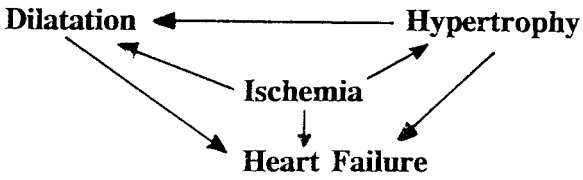

Fig. 1. Schematic representation of linkage between dilated, hypertrophic, and ischemic cardiomyopathies and heart failure.

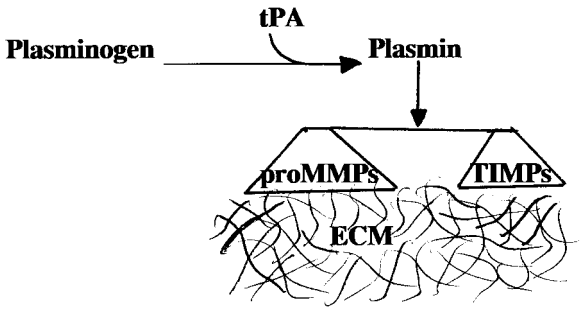

Fig. 2. Role of neutral serine proteinases tPA and plasmin in ECM remodeling and in activation of latent proMMPs and dissociation of TIMPs from proMMP/TIMP complex.

verts plasminogen to plasmin, which in turn activates MMPs and inactivates TIMP-1 post-translationally following ischemic cardiomyopathy [34] (Fig. 2). Furthermore, in ischemic heart failure, we observed the induction of gelatinase B (92 kDa), gelatinase A (66 kDa), and interstitial collagenase (MMP-1). In contrast to this in DCM heart (nonischemic), we found induction of only gelatinase A (66 kDa) and interstitial collagenase (MMP-1) and no expression of gelatinase B [35].

The genetic basis of ECM-disruption following DCM is not well addressed [14]. The inability of ECM to maintain cardiovascular integrity is the primary result of weakened connective tissue which may also be seen in rare single-gene disorders of structural architecture, such as Ehlers-Danlos IV, a defect in type III collagen gene [20]. Fibrillin is required for ECM assembly at the cell surface, and Marfan's syndrome is due to a defect in the fibrillin-1 gene [2]. Elastin deficiency causes Williams' syndrome and vasculopathy [6]. The mutations in the TIMP-3 gene [40] in patients with Sorsby's fundus dystrophy have been identified. Disruption of connective tissue turnover and the consequent effect on matrix stability has been implicated in a number of diseases [26,32,37], and the role of MMPs has been demonstrated in the ECM/MMP/TIMP family [26,32,33,37]. Collectively, these studies suggested that disruption of MMP-mediated matrix remodeling does affect the composition and function of tissues. It also suggests that more common genetic variation in these genes may contribute to the pathogenesis of common multifactorial disorders in cardiovasculature. It seems likely that mild mutations in some of the components of ECM may cause severe cardiomyopathic abnormalities. For the first time we have demonstrated, during DCM, an excessive upregulation of a MMP gene that regulates ECM remodeling [35]. TIMP-4 specifically and constitutively over expressed in the heart [8]. The differential role of ECM components during ischemic, dilated, and hypertrophic cardiomyopathies and heart failure is shown in Table I. Also, collagen and its receptor integrin β1 are induced in hypertrophic ischemic conditions [44].

GENE THERAPY IN THE MYOCARDIUM

Cell Infusion

The transduction of adult myocytes in vivo demonstrated the feasibility of expression of genes in rat and canine myocardium by direct injection of plasmid DNA. These studies, however, were limited by low efficiencies [17,39]. The adenoviruses, which can infect nondividing cells, are of great interest as vectors for gene transfer in adult myocardium. Studies have demonstrated higher levels of gene expression in rat myocardium after direct injection of adenovirus vectors compared with injection of plasmid DNA alone [13]. The adult myocardium in vivo has been activated by intracoronary administration of adenoviral vectors encoding reporter genes. In this model, gene expression was observed in both the coronary vasculature and the myocardium [9]. Levels of gene expression in the myocardium were 10- to 50-fold higher compared with direct DNA plasmid injection. The reporter gene expression peaked at 1 week, diminished at 2 weeks, and was present at low levels after 1 month in adult myocytes [9,13]. These studies demonstrated feasibility of gene transfer directly in the myocardium.

Promoter Elements Targeted

Gene transfer to the myocardium has proved a useful tool in understanding cardiac gene regulation in vivo. For example, transcription elements regulating basal and thyroid hormone-responsive cardiac alpha myosin heavy chain gene expression in adult rat hearts in vivo have

TABLE I. Differential Role(s) of Extracellular Matrix Components in Heart Failure

	Heart			
	Normal	Ischemic	Dilated	Hypertrophied
MMP-1; interstitial collagenase; 54 kDa		↑	↑	↓
MMP-2; gelatinase A				
66 kDa		↑	–	–
72 kDa	Constitutive	↑	↑	↑
Gelatinase B; 92 kDa		↑	↓	↓
MMP-3; stromelysin				
46-58 kDa		↑	–	–
MT-MMP; 66 kDa		↑	–	–
tPA; 66 kDa		↑	–	–
TIMP-1; 28 kDa		↓	↓	↑
TIMP-2; 22 kDa	Constitutive	–	–	–
TIMP-4; 27 kDa	Constitutive	↓	↓	↑
Collagen I		↑	↓	↑
Integrin β1		↑	↓	↑

↑, increase; ↓, decrease; –, no change.

been studied by regulating promoter elements [15]. Direct injection of constructs into the myocardium is a model system for investigating DNA elements and regulatory pathways that control gene expression and growth in the heart. Lints et al. [18] created mice homozygous for ablation of the transcription factor responsible for the development of the heart. In these mice, Lints and colleagues observed early embryonic lethality secondary to arrested cardiac development, with a thin-walled, poor functioning (similar to DCM hearts), incompletely septated heart. These studies indicated a critical role of gene promoter binding proteins in proper adaptive function in maintaining cardiac specific gene structure and expression at different stages of cardiac development and function. The upregulation and/or downregulation of these genes may cause cardiac maladaptation and the progression of certain disease states. Ye et al. [42] described a common stromelysin promoter variant that was associated with the progression of atherosclerosis. For the first time, we demonstrated that the function of activator protein-1 (AP-1) in collagenase promoter is altered in DCM. Explanations for this abnormality include mutations in transcription factor or the overproduction of the transcription factor causing uncontrolled gene expression. However, these possibilities remain to be examined.

Vector-Directed

The most promising area is the direct injection of a gene by adenoviral vectors into skeletal muscle for production of secreted proteins [1]. Myoblasts, transduced by retroviral vectors expressing human growth hormone, injected into skeletal muscle, produced physiological levels of human growth hormone in the serum. Recent studies suggested that physiological levels of recombinant erythropoietin were secreted into the circulation after intramuscular injection of adenovirus into skeletal muscle of neonatal mice [25]. This suggested that similar approach of gene therapy can be applied to other secreted proteins, such as ECM components.

ISCHEMIC CARDIOMYOPATHY

Occlusion of a major coronary artery leads to a sequence of events that begins with myocardial infarction and ends with heart failure (Fig. 3). Our work and that of others have shown differential dynamic changes in the ECM and reduction-oxidation (redox) components, both at the site of infarction and in areas remote to infarction, leading to heart failure. We have demonstrated that at the site of infarction, early proteolytic and redox insults activate latent myocardial metalloproteinases which disrupt ECM. At the late stage, the levels of antiproteinases are reduced and the newly synthesized ECM continues to be degraded, leading to cardiac dilatation and systolic failure. The adverse ECM dynamic and redox changes lead to phenotypic shifts in the fibroblasts to myofibroblasts and induce activation of matrix metalloproteinases. At the site remote to infarction, ECM synthesis continues, leading to fibrosis and dia-

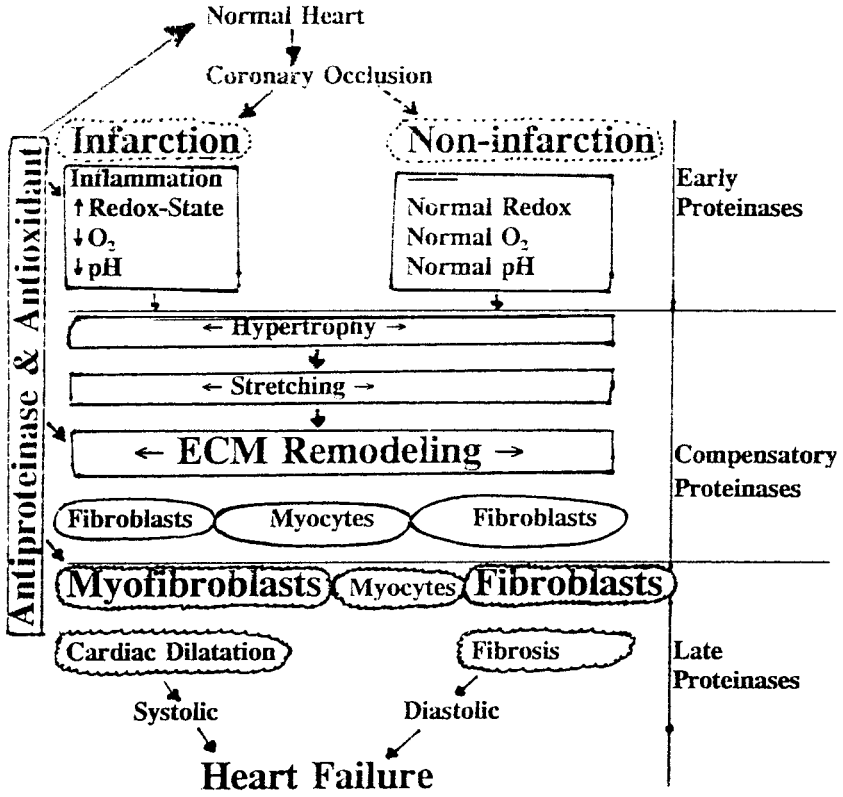

Fig. 3. Schematic presentation of events followed by ischemic insult, leading to heart failure. Initially redox-insult and proteolytic cascades are activated that induce adverse ECM dynamic process, leading to the transformation of fibroblasts to maladaptive myofibroblast cells at the site of infarction. The ventricle is dilated. However, at the site remote to infarction (i.e., noninfarction) ECM is accumulated and wall becomes fibrotic. This leads to systolic and diastolic heart failure. The treatment of antioxidant and antiproteinase may reduce the severity of heart failure. The squiggly ellipse represents ECM around the cardiac cells. The significance of one squiggly ellipse around myocyte suggests susceptibility to apoptosis in ischemic condition.

stolic failure (Fig. 3). In the myocardial infarction (MI) model of heart failure, induction of overall structural remodeling results in generalized hypertrophy and stretching, leading to fibrosis in the noninfarcted heart and to dilation in the infarcted heart [34,37]. We have shown that at the site of infarction, MMP-1 and MMP-2 (gelatinase A and B) are activated and the levels of TIMP are significantly reduced. Therefore, the ratio of MMP/TIMP increased to 6–7, as compared to 1.1 in the normal heart [31]. Genetic manipulations of these components may prevent adverse ECM remodeling and heart failure (Table I).

Transgenic mice that overexpress TIMP-1 have been developed [19]. It is of great interest to determine whether these animals also overexpress TIMP in the heart. We have demonstrated that TIMPs in general are repressed at the site of infarction and heart failure [34]. We suggest that adverse ECM dynamic alteration during myocardial infarction and heart failure will be reduced in these animals which overproduce TIMP-1. The temporal alterations in the ECM components following coronary artery occlusion in these transgenic mice will demonstrate that the induction of TIMP gene improves myocardial damage in response to ischemic-insult. In this model we expect that the ratio between MMP/TIMP will not increase as much as it increases in the hearts of animals after coronary ligation [36]. These studies will further support the idea that TIMP-1 therapy following MI may be a useful approach to the treatment of ischemic heart disease. Other gene therapies targeted toward the downregulation of MMP-1 and MMP-9 in ischemic heart failure would also be of great importance. Upregulation of collagen by inducing promoter activity in the ischemic heart would halt or reverse heart failure.

IDIOPATHIC CARDIOMYOPATHY

In the DCM the ventricular wall become thin, myocytes are hypertrophied and ECM is disrupted, leading to systolic heart failure. In ham-

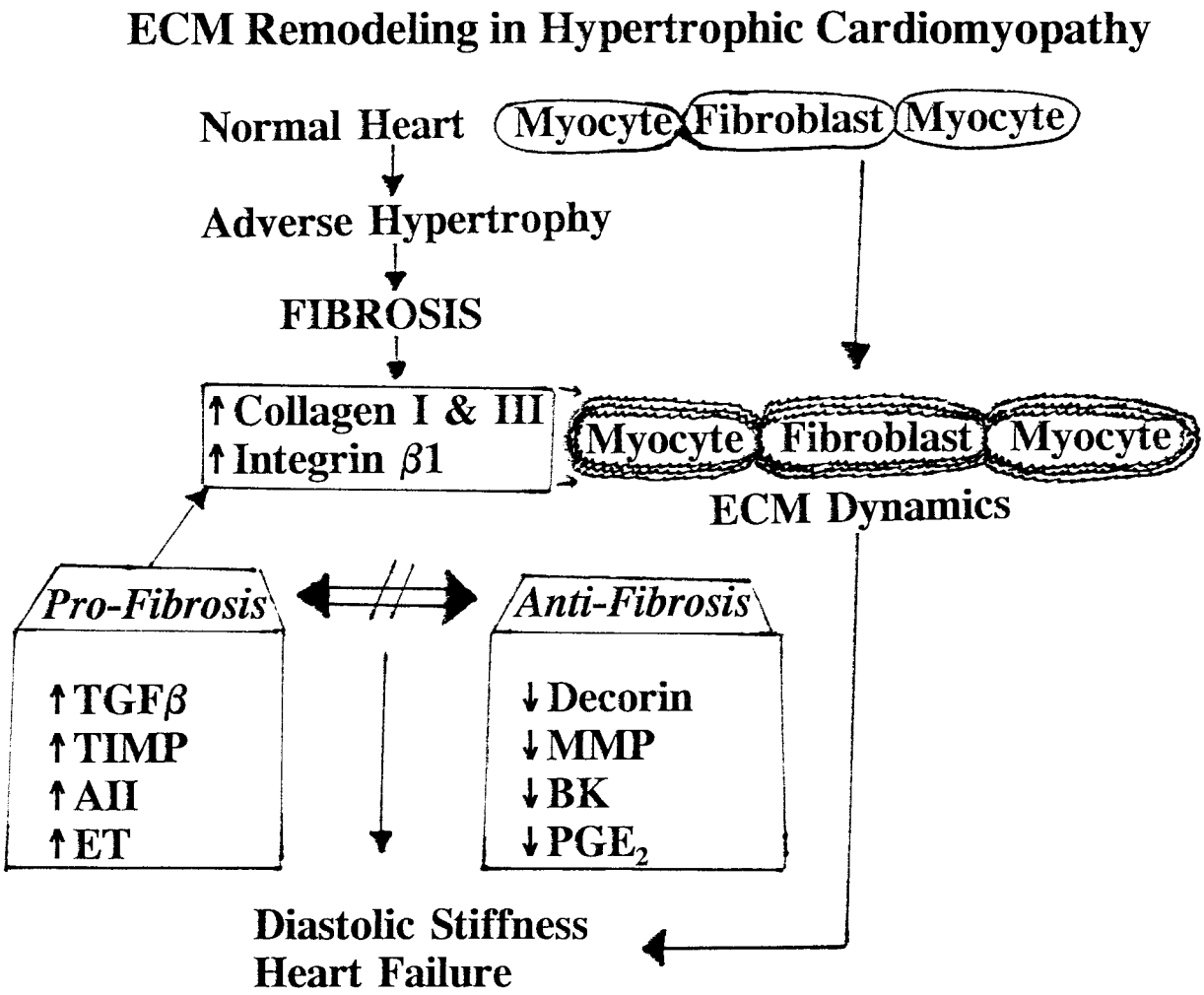

Fig. 4. Plausible relationship between fibrosis and hypertrophic cardiomyopathy and diastolic heart failure. In a pathophysiological state, adverse hypertrophic response leads to induction of ECM components, especially collagen I and III. The heart become fibrotic and stiffer. The balance between profibrotic and antifibrotic components is disturbed. TGF-β, transforming growth factor-β; TIMP, AII, angiotensin II; ET, endothelin are profibrosis and decorin, MMP, BK, bradykinin; prostagladilin E_2 are antifibrosis. The significant of three squiggly ellipses around the myocyte, fibroblast and myocyte suggests excess fibrous tissue around these cell, leading to reduction in cardiac contractility.

ster model of DCM, we have demonstrated temporal expression of MMP activity and ECM disruption [33,34]. MMP activity was increased at day 180 and remained elevated for up to 310 days when the heart failed [29]. This MMP activity was associated with a reduction in TIMP expression [29]. Also, we have shown that MMP-1 was specifically and differentially induced, and the level of TIMP was repressed following dilated cardiomyopathy and heart failure in human (Table I). There are two possible consequences of this specific MMP-1 gene induction in DCM. One is associated with the mutation at the propeptide site of MMP-1, which will produce constitutively active MMP-1. This possibility will avoid the post-translational requirement of MMP activation. The isolation of a full-length cDNA clone of MMP-1 from DCM tissue and fibroblast cells is in progress. However, the temporal expression of this gene may partly explain the presence of active MMP-1 in the DCM tissue. Further animal studies are needed to support this conclusion. The other possible consequence is that the level of TIMP is dramatically repressed (Table I). The reduced level of TIMP in the DCM heart may be due to the mutation in TIMP gene. Mutations in the TIMP-3 gene have been identified in noncardiac tissue [40]. However, this remains to be elucidated in the heart.

Collagen expression in DCM heart is quite low. It will be important to induce collagen gene expression in the DCM hearts by inducing collagen promoter elements in the DCM heart. Also,

gene therapy designed to overproduce TIMP and reduce MMP-1 in the DCM heart will significantly reduce the risk of heart failure.

HYPERTROPHIC CARDIOMYOPATHY

In hypertrophic cardiomyopathy, the cardiac wall is thick, myocytes become hypertrophied, and ECM accumulates (fibrosis), leading to diastolic heart failure. There is no effective therapy for the progressive fibrotic diseases. Molecular studies have demonstrated that the development of cardiac hypertrophy is associated with changes in ECM components and concentrations [3,4,7,21,22,41,44]. In spontaneously hypertensive rats (SHR) at heart failure, the collagenase activity (i.e., MMP-2, gelatinase A) was increased, suggesting active ECM remodeling in the hypertrophied heart (Table I). However, in hypertrophic cardiomyopathy the TIMP level was ~100-fold higher than the normal control, suggesting a role of TIMP in hypertrophic cardiomyopathy [30]. Our data pointed to generalized induction of MMP and indicated differential gene expression of proteinase antiproteinase under hypertrophic cardiomyopathy (Table I). A critical balance between profibrotic and antifibrotic components is necessary for normal tissue constitution (Fig. 4). It is known that transforming growth factor-β (TGF-β) induces collagen and TIMP expression and reduces MMPs [35]. However, during hypertrophic remodeling pro-fibrotic components are elevated, leading to the development of cardiovascular fibrosis and diastolic dysfunction.

TGF-β is profibrosis and induces ECM components, particularly collagen. Decorin, a component of proteoglycans, is antifibrosis and inhibits the effect of TGF-β [10]. The gene transfer of decorin cDNA into rat skeletal muscle increases the amount of decorin mRNA and protein present in skeletal muscle, as well as the amounts of decorin protein present in other tissues. In nonskeletal muscle, decorin expression has a marked antifibrotic therapeutic effect. Furthermore, in the model of decorin gene transfer, the generalized expression of ECM in all tissues was significantly reduced. This study suggested that therapy with decorin cDNA may reduce the cause of fibrotic heart disease.

Furthermore, direct inhibition of promoter elements in collagen and TIMP genes may also produce beneficial results. Collagen synthesis, for example, was significantly reduced by the use of an oligoprobe complementary to the promoter sequence [16]. We have suggested the induction of TIMP by inducing nuclear transcription factors [38]. It is of great interest to inhibit TIMP expression by binding to promoter sequence elements and, at the same time, to induce the expression of MMP-2, gelatinase A, in hypertrophic cardiomyopathy.

CONCLUSION

We have studied human myocardium and provided data that are directly relevant to clinical heart failure [34,35]. By taking advantage of transgenic mice to alter gene expression and to study the cause-and-effect relationships, it may be possible to develop gene therapy for heart failure. Also, transgenic mice can be used to study the hypertension model of volume and pressure overload. As the technology for gene therapy develops in vitro and in vivo in animal models, the three principles of gene therapy are (1) recombinant retroviruses, (2) recombinant adenovirus, and (3) direct DNA delivery. High-level transient expression after in vivo recombinant adenoviral therapy and moderate-level transient expression after in vivo administration of a synthetic DNA have been achieved [8]. Transgenic models of cardiac disease are being generated, and new insights are being made toward the goal of gene therapy in heart failure. As the repertoire of techniques for transferring DNA into heart cells expands and becomes available, the prospect for efficient long-term expression of exogenous DNA in heart cells will improve. Replacement gene therapy as well as use of promoter-specific complementary sequences to act on genetic regulatory elements will enhance the future treatment of cardiovascular disease and heart failure.

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