Cytopathological Basis of Heart Failure – Cardiomyocyte Apoptosis, Interstitial Fibrosis and Inflammatory Cell Response

(heart failure / cardiomyocyte apoptosis / extracellular matrix / inflammation)

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Abstract. A characteristic feature of heart failure is progressive deterioration of the left ventricular function. The mechanisms responsible for progression of heart failure are not known, but may be related to progressive loss of cardiomyocytes due to apoptosis or programmed cell death. Apoptosis of cardiomyocytes can cause scattered loss of cardiomyocytes and, when sufficiently widespread, this might cause heart failure. Beside cardiomyocyte apoptosis, progressive accumulation of interstitial collagen fibres in the heart occurs in the failing heart that may lead to ventricular diastolic or systolic dysfunction. Pathological processes in the failing heart (cardiomyocyte apoptosis, changes in interstitial tissue of the heart) are accompanied by an inflammatory cell response.

In this paper cardiomyocyte apoptosis, inflammatory cell response and changes in interstitial tissue of the heart are reviewed as potential factors responsible for progression of the left ventricular dysfunction in heart failure.

Progressive left ventricular dysfunction occurs in heart failure, which is the final clinical manifestation of a variety of cardiovascular diseases, such as coronary artery disease, arterial hypertension, valvular heart disease, myocarditis, dilated cardiomyopathy, or alcohol abuse. Progressive left ventricular dysfunction often occurs in the absence of clinically apparent intercurrent adverse events. The mechanisms that might lead to heart failure are cardiac cell death and increased extracellular matrix deposition (Weber et al., 1988; Anversa et al., 1996). There are two general mechanisms of cell death: necrosis and apoptosis (Arends and Wyllie, 1991). Despite several reports of the cardiomyocyte apoptosis in heart failure, some scepticism remains even today as to whether cardiomyocyte apoptosis plays a role in the progression of heart failure (Sabbah et al., 1995). The transition from compensated left ventricular hypertrophy to heart failure is associated with alterations in the myocardial interstitium, which is accompanied by an inflammatory cell response (Woodiwiss et al., 2001; Weber, 2004).

In this paper the cytopathological basis of heart failure – cardiomyocyte apoptosis, changes in interstitial tissue of the heart and inflammatory cell response are reviewed.

Cardiomyocyte apoptosis

Progressive LV dysfunction is a characteristic feature of the failing heart. Although the mechanisms that drive the progression of heart failure are not known, programmed cell death or apoptosis of cardiomyocytes is implied in the pathogenesis of heart failure (Arends and Wyllie, 1991; Anversa et al., 1996). Apoptosis, an evolutionarily conserved form of cell suicide, requires specialized machinery, and the central component of this machinery is a proteolytic system involving a family of proteases called caspases (Thornberry and Lazebnik, 1998). Terminally differentiated cells such as cardiomyocytes are not believed to undergo apoptosis under natural, but under pathological conditions (Cheng et al., 1995). Studies in end-stage explanted failed human hearts and in animal models of experimentally induced heart failure have provided evidence for the existence of cardiomyocyte apoptosis (Olivetti et al., 1995; Sabbah et al., 1995; Anversa et al., 1996; Olivetti et al., 1997; Saraste et al., 1999; Petrovic et al., 2000; Mani and Kitsis, 2003; Wencker et al., 2003). Apoptosis of cardiomyocytes can cause scattered loss of myocytes and, when sufficiently widespread, this might impair the ventricular function and cause heart failure (Anversa et al., 1996). Widespread loss of cardiomyocytes leads to compensatory mechanisms such as left ventricular hypertrophy, left ventricular dilation, and enhanced and sustained activity of the sympathetic nervous system and renin-angiotensin system.

However, to fully appreciate the significance of cardiomyocyte apoptosis in the pathogenesis of heart failure, the researcher must know the incidence of cardiomyocyte apoptosis in the failing heart. A big discrepancy regarding the incidence of cardiomyocyte apoptosis exists between early reports (more than 20%) and recent reports (0.023% to 0.25%) on heart failure (Olivetti et al., 1995; Narula et

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Abbreviations: ICAM – intracellular adhesion molecule, MI – myocardial infarction, TGF – transforming growth factor.

al., 1996; Olivetti et al., 1997; Saraste et al., 1999; Petrovic et al., 2000; Mani and Kitsis, 2003; Wencker et al., 2003). The first impression is that the incidence of cardiomyocyte apoptosis of less than 0.1% is not important. However, if we assume that the human left ventricle contains up to 6 x 10^9 cardiomyocytes (Olivetti et al., 1995), and that the period for cardiomyocyte death to occur due to apoptosis is 24 h, we may speculate an almost 4% loss of left ventricular contractile mass from apoptosis per year, and an even greater loss of left ventricular contractile mass from apoptosis per year at a higher rate of apoptotic process. Moreover, using transgenic mice that express a conditionally active caspase exclusively in the myocardium, it has been recently demonstrated that very low levels of myocyte apoptosis (0.023%) are sufficient to cause a lethal, dilated cardiomyopathy (Wencker et al., 2003). A large discrepancy in the incidence of cardiomyocyte apoptosis in heart failure is primarily due to methodological reasons, but an accurate determination of the rate of cardiomyocyte apoptosis in heart failure would undoubtedly help to solve current uncertainties regarding the incidence of cardiomyocyte apoptosis in a failing heart and in heart failure.

Recently it has been demonstrated that cardiomyocyte apoptosis is strongly associated with post-infarction heart failure and may be a major determinant of unfavourable left ventricular remodelling and early symptomatic post-infarction heart failure (Abbate et al., 2003). Although the precise cellular and molecular bases of the remodelling events are not known, cardiomyocyte apoptosis in the non-infarcted remote myocardium appears to be involved (Mani and Kitsis, 2003). It is interesting to note that the frequency of cardiomyocyte apoptosis in the remote myocardium following myocardial infarction is very similar to that observed in end-stage dilated cardiomyopathy (Olivetti et al., 1997; Petrovic et al., 2000; Mani and Kitsis, 2003).

Additionally, cardiomyocyte apoptosis was also implicated as a prognostic factor and discriminator between progressive and non-progressive heart failure due to dilated cardiomyopathy (Zorc et al., 2003). Further studies in this regard are mandatory before further appreciation of cardiomyocyte apoptosis as a prognostic factor in heart failure.

While these findings have generated considerable enthusiasm, some scepticism remains even today as to whether cardiomyocyte apoptosis plays a role in the progression of heart failure (Sabbah et al., 1998). Recently it has been reported that inhibition of cardiac myocyte death in the murine model largely prevents the development of cardiac dilation and contractile dysfunction, the hallmarks of heart failure (Hayakawa et al., 2003; Wencker et al., 2003). Favourable results of pharmacologic interventions with inhibitors of apoptosis in animal models of failing heart are clearly another piece of evidence for the importance of cardiomyocyte apoptosis in the pathogenesis of heart failure (Hayakawa et al., 2003; Wencker et al., 2003). Moreover, reduction of cardiomyocyte apoptosis by caspase inhibition indicates that caspase inhibition may provide a novel therapeutic target for heart failure (Hayakawa et al., 2003; Wencker et al., 2003).

Connective tissue skeleton of normal human heart

The stroma of the heart (interstitial tissue or extracellular matrix) composed predominantly of the connective tissue represents the fibrous skeleton of the human heart. The cardiac extracellular matrix maintains the structure of the myocardium and determines the tissue tensile strength and stiffness (Weber et al., 1993). Moreover, it contributes to ventricular function through the transmission of myocyte-generated force to the atrial and ventricular chambers, and to the re-lengthening of myocytes in diastole (Robinson et al., 1986). However, the cell-extracellular matrix interaction provides not only structural and mechanical support, but also important biological signalling during tissue remodelling (migration, differentiation, proliferation, and the expression of different genes). Importantly, the heart remodels myocardial tissue in the physiological and pathological response. Several molecules are involved in the process of the cell-extracellular matrix interaction, and integrins play an important role in the communication between cells and the matrix (Bosman and Stamenkovic, 2003; Imanaka-Yoshida et al., 2004). Moreover, integrins regulate the expression of matrix-degrading enzymes, namely matrix metalloproteinases (Ivaska and Heins, 2000). Important target cells in the heart are fibroblasts, which are under the control of extracellular matrix molecules (Eckes et al., 1999). Extracellular matrix molecules directly modulate fibroblast metabolism and biosynthetic activity via integrin receptors (Eckes et al., 1999).

The epimysium, the perimysium and the endomysium represent the cardiac extracellular matrix. The epimysium envelops the entire cardiac chamber, the perimysium enwraps groups of myocytes, and the endomysium envelops individual myocytes. Moreover, endomysial weave is connected to adjacent myocytes by lateral sprouts (Rossi et al., 1998). Rossi and co-workers were the first to demonstrate the three-dimensional architecture of collagen fibrils in human myocardium after digestion of the cellular elements (Rossi et al., 1998).

The fibrillar type I collagen is the major structural component of the cardiac extracellular matrix – 85% of the total collagen consists of fibrillar type I collagen. Type I collagen is synthesized by cardiac fibroblasts and myofibroblasts (Swynghedauw, 1999). In addition to type I collagen, the myocardium also contains types III and V collagens (fibril-forming collagens), and types IV and VI collagens located in the basement membrane. A balance of extracellular matrix synthesis and degradation determines the maintenance of cardiac extracellular matrix (Heeneman et al., 2003). The normal rate

of extracellular matrix synthesis in the heart is very low, but it is increased very much in pathological conditions (Cleutjens et al., 1995).

Alterations in the myocardial interstitium in the failing heart

The transition from compensated left ventricular hypertrophy to heart failure is associated with alterations in the myocardial interstitium (Woodiwiss et al., 2001). The progressive accumulation of interstitial collagen fibres in heart failure may be expected to decrease myocardial compliance and ventricular systolic and diastolic dysfunctions (Anversa et al., 1996; Rossi et al., 1998; Sabbah et al., 1998). Interstitial fibrosis, which occurs in heart failure, is undoubtedly an important determinant of pathologic hypertrophy in heart failure (Sabbah et al., 1998; Brilla, 2000).

The exact mechanism that promotes the accumulation of collagen in the interstitial compartment is still controversial. Two distinct phases have been distinguished in the development of myocardial fibrosis in experimental hypertensive heart disease: reactive accumulation of connective tissue in the perivascular and interstitial space by *de novo* synthesis of collagen (in the absence of myocyte loss), and reparative (replacement) fibrosis or scarring, which is an adaptation to the loss of cardiomyocytes (Brilla et al., 1995; Heeneman et al., 2003). During pathological conditions, such as myocardial infarction (MI), collagen synthesis and deposition is increased not only in the infarcted, but also in the non-infarcted myocardium (Cleutjens et al., 1995). Collagen synthesis is regulated by several growth factors [transforming growth factor β (TGFβ), platelet-derived growth factor (PDGF), fibroblast-growth factor (FGF)], cytokines [interleukin-1 (IL-1, IL-4, tumour-necrosis factor α (TNFα)] and hormones/factors (aldosterone, angiotensin II, endothelin) (Bishop et al., 1995; Brilla et al., 1995; Lijnen et al., 2000). Several matrix metalloproteinases are involved in the degradation of collagens (Li et al., 2000). In cardiovascular pathologies (dilated cardiomyopathy, myocardial infarction), collagen turnover (collagen synthesis and degradation) is an integral part of the disease.

Interstitial fibrosis is of great importance in heart failure because it results in reduced capillary density and increased oxygen diffusion distance that lead to hypoxia and dysfunction of the collagen-encircled myocyte. In subjects with chronic heart failure, cardiomyocytes of left ventricular regions that manifest severe interstitial fibrosis may be subjected to chronic hypoxia, a condition that can adversely affect the function and viability of collagen-encircled cardiomyocytes (Sabbah et al., 1995). Moreover, chronic hypoxia due to reduced capillary density and increased oxygen diffusion distance in the hypertrophic heart with severe interstitial fibrosis may also lead to cardiomyocyte necrosis (Sabbah et al., 1995).

Several cells are involved in increased extracellular matrix synthesis and turnover: inflammatory cells, fibroblasts, myofibroblasts, and mast cells.

Inflammatory cells, especially monocytes/macrophages and lymphocytes, play a crucial role in the fibrous response to cardiomyocyte necrosis and in failing heart (Weber, 2004). The reaction of cardiac tissue to acute injury involves interacting cascades of cellular and molecular responses that encompass inflammation, hormonal signalling, extracellular matrix remodelling, and compensatory adaptation of cardiomyocytes (Leferovich et al., 2001). The inflammatory cell response that appears at the site of cardiomyocyte necrosis is followed by fibrous tissue. The infarct scar is composed of fibroblasts and phenotypically transformed fibroblast-like cells, termed myofibroblasts. Myofibroblasts express alpha-smooth muscle actin, and these microfilaments confer contractile behaviour in response to various peptides and amines. These cells are nourished by neovasculature and are persistent at the MI site, where they express several peptides (angiotensin I-converting enzyme, receptors for angiotensin II, TGFβ). Moreover, TGFβ induces a phenotypic change from fibroblasts to myofibroblasts. Fibroblasts and myofibroblasts continue to elaborate fibrillar type I collagen, a stiff structural protein. Their generation of these peptides contributes to the ongoing scar tissue collagen turnover and to fibrous tissue formation of non-infarcted myocardium. Myofibroblasts do not only produce collagen, but also contract the fibrotic area, prevent dilatation by cell-cell and cell-matrix interactions, and play an important role in the archictectural control of scar tissue formation (Cleutjens et al., 1999). The infarct scar is a dynamic tissue: cellular, vascularized, metabolically active and contractile. Pharmacologic interventions with angiotensin-converting enzyme inhibitor or angiotensin 1 receptor antagonist have proved effective in attenuating scar tissue metabolic activity, and minimizing adverse accumulation of fibrous tissue in the non-infarcted myocardium (Sun and Weber, 2000).

The inflammatory cell response is of great importance in fibrous tissue formation in arterial hypertension (pressure overload model). In hypertension TGFβ plays a causal role in myocardial fibrosis through fibroblast activation, and reactive myocardial fibrosis expands from the perivascular to intermuscular interstitium (Kuwahara et al., 2002). In the intramyocardial arterioles of the pressure overload model, intracellular adhesion molecule (ICAM-1) expression has been demonstrated that triggers perivascular macrophage accumulation. Moreover, a crucial role in ICAM-1-mediated macrophage accumulation was suggested in the development of myocardial fibrosis, through TGFβ induction and fibroblast activation (Kuwahara et al., 2003). The importance of this inflammatory cell response leading to fibrous tissue formation is underscored by an anti-monocyte chemoattractant protein 1 monoclonal neutralizing antibody, which inhibits not only the accumulation of macrophages, but

also fibroblast proliferation, the induction of TGFβ, and the appearance of fibrosis with accompanying diastolic dysfunction (Kuwahara et al., 2004).

Mast cells, which are also found in human heart in heart failure, have been demonstrated to play an important role in interstitial fibrosis, and mast cell-fibroblast interactions are known to be important in the structure and function of connective tissue (Li et al., 1992; Galli, 1993; Petrovic et al., 1999; Gerling et al., 2003; Shiota et al., 2003). Moreover, cardiac fibroblasts are known to have high-affinity corticoid receptors for aldosterone and account for the accumulation of collagen within the myocardial interstitium in hypertension (Gerling et al., 2003). By synthesizing and secreting prohypertrophic cytokines and profibrotic growth factors, cardiac mast cells participate in the induction of cardiac hypertrophy and cardiac fibrosis, which are the key steps in the transition to heart failure (Shiota et al., 2003).

We should acknowledge that not only quantitative (interstitial fibrosis), but also qualitative changes occur in the myocardial interstitium in the failing heart (Woodiwiss et al., 2001). Importantly, a decreased ratio of myocardial insoluble to soluble collagen has been demonstrated in the animal model of a failing heart, which might lead to reduced myocardial collagen cross-linking and left ventricular dilatation in the failing heart (Woodiwiss et al., 2001). Several molecules are involved in the process of the cell-extracellular matrix interaction and ventricular remodelling (Benevolensky et al., 2000; Imanaka-Yoshida et al., 2004). Annexins were suggested to contribute to ventricular remodelling in the failing heart (Benevolensky et al., 2000). Overexpression of annexins II and V, as well as translocation of annexin V from cardiomyocytes to interstitial tissue was demonstrated in the failing heart (Benevolensky et al., 2000). Tenascin-C, an extracellular matrix glycoprotein, also plays an important role in tissue remodelling (Imanaka-Yoshida et al., 2001). Tenascin-C is not normally expressed in adults but reappears under pathologic conditions. It modulates adhesion of cardiomyocytes to the extracellular matrix during tissue remodelling after myocardial infarction (Imanaka-Yoshida et al., 2001). During the acute phase after myocardial infarction, interstitial cells in the border zone synthesize tenascin-C, which may loosen the strong adhesion of surviving cardiomyocytes to connective tissue and thereby facilitate tissue reorganization (Imanaka-Yoshida et al., 2001).

Conclusions

In heart failure several pathological processes can be encountered, i.e. cardiomyocyte apoptosis, changes in interstitial tissue of the heart, and inflammatory cell response. Apoptosis of cardiomyocytes in heart failure is implicated as a key factor in the pathogenesis of the heart failure. Moreover, in failing heart alterations in the myocardial interstitium (fibrosis) occur that may be associated with the inflammatory cell response.

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