

The Aging Cardiomyocyte: A Mini-Review

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Key Words

Myocyte · Aging · Senescence

Abstract

Background: Aging per se is a risk factor for reduced cardiac function and heart diseases, even when adjusted for aging-associated cardiovascular risk factors. Accordingly, aging-related biochemical and cell-biological changes lead to pathophysiological conditions, especially reduced heart function and heart disease. **Objective:** In this review, we summarize the changes that occur as the heart ages from youth to old age on the basis of the cardiac myocyte. Aging phenotypes and underlying mechanisms shall be discussed that affect cardiomyocyte repair, signaling, structure, and function. **Methods:** Review of the literature. **Results:** The following factors play vital roles in the aging of cardiomyocytes: oxidative stress, inflammation, cellular protection and repair, telomere integrity, survival and death, metabolism, post-translational modifications, and altered gene expression. Importantly, non-cardiomyocyte-based aging processes (vascular, fibroblast, extracellular matrix, etc.) in the heart will interfere with cardiomyocyte aging and cardiac function. **Conclusion:** Based on our analyses, we postulate that the physiological aging process of the heart and of the cardiomyocyte is primarily driven by intrinsic aging factors. However, extrinsic aging factors, e.g. smoking, also make an important contribution to pathologically accelerated aging of the heart.

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Introduction

With aging, cardiac function declines. Cardiac reserve, i.e. the difference between the peak cardiac pumping level and the normal baseline resting level, is reduced. Cardiomyocyte loss, left-ventricular hypertrophy, changes in ventricle chamber diameter, and an accumulation of extracellular matrix lead to reduced cardiac output, decreased left-ventricular end-systolic pressure, fractional shortening, and decreased heart rate [1–3]. These facts clearly stress that the heart ages, indicating that the maintenance and repair potential of the heart is limited. Aging-associated phenomena have been described on the basis of signaling, structure, function, and repair not only at the macroscopic but also at the cellular and molecular levels.

Although the dogma from the 1920s that cardiomyocytes are a terminally differentiated cell type unable to undergo mitosis has changed in recent years due to the discovery of cardiomyocyte cell division in the adult heart [4], a net loss of total cardiomyocyte number during physiological aging can be observed [5, 6]. In addition, the majority of adult cardiomyocytes are hypertrophied and terminally differentiated. Advanced technologies allowed scientists to discover proliferation and cell division in a fraction of cardiomyocytes not only in the young but also in the adult and old heart. This cardiomyocyte proliferation, however, is limited to rather small cardiomyocytes [4, 7]. Nevertheless, these findings stress that – in

contrast to earlier beliefs – not all cardiomyocytes are as old as the organism and the organ, but show a high degree of divergence with respect to their age, as has been demonstrated by telomere length analyses. Accordingly, the story of the aging cardiomyocyte has changed.

Apart from small cardiomyocytes, the presence of cardiac stem cells (CSCs) in the heart or the recruitment of CSCs to the heart are still under discussion. Self-renewing, clonogenic, and multipotent c-kit (stem cell receptor)-positive cells have been described, which predominantly differentiate into cardiomyocytes [4, 5]. Although these findings have attracted a lot of scientific interest because these cells may serve as a cellular pool for cardiac repair after myocardial injury, the origin of CSCs is still a matter of debate.

The Aged Cardiomyocyte

Cardiac aging is a complex process which includes aging and deposition of extracellular matrix, aging of the coronary vasculature, aging of cardiac fibroblasts, and aging of the contractile apparatus of the heart [6, 8, 9]. Constituting the core of cardiac function and the contractile apparatus, cardiomyocytes display a number of physiological and morphological features, which are affected by the aging process, and these changes are thought to give rise to reduced cardiac function and heart disease. Some of these changes are described below.

First, the total number of cardiomyocytes in the heart decreases with age. The loss in cardiomyocyte cell number by apoptotic or necrotic cell death leads to replacement hypertrophy of the remaining cardiomyocytes and to the deposition of extracellular matrix (collagen) [6]. The maximal volume that can be reached by a cardiomyocyte is approximately $90,000 \mu\text{m}^3$, with a maximal cross-sectional area of $600\text{--}900 \mu\text{m}^2$ [5, 10]. Cardiomyocyte enlargement per se is a physiological process occurring in response to the increased need for performance (e.g. due to sports), and aging-related cardiomyocyte hypertrophy can be viewed in a similar fashion. However, in contrast to sports, aging-induced cellular hypertrophy is the result of an aging-dependent loss in the total number of cardiomyocytes, which increases the mechanical burden for the remaining cells. Importantly, cardiomyocyte hypertrophy occurs during the aging process, but the ability to enlarge is lost in the senescent cardiomyocyte [5, 10]. The ratio of senescent to nonsenescent cardiomyocytes, in combination with the total number of cardiomyocytes, may therefore define the potential of the

heart to maintain or lose its function. In support of this, studies have shown that cardiomyocyte loss and enlargement precede ventricular hypertrophy, and are therefore thought, at least in part, to be responsible for the phenomenon. Another factor that may contribute to cardiomyocyte hypertrophy is the aging-dependent change in myocyte mechanics and Ca^{2+} transients. Since myocyte activation, contraction, and relaxation are defective (see below), individual cells have to become hypertrophic in order to preserve their performance at the single cell level.

Young cardiomyocytes are able to undergo mitosis including cytokinesis. The finding that adult and senescent cardiomyocytes are often multinucleated suggests that DNA replication and karyogenesis are still functional in the adult cell and that cytokinesis is abrogated [11]. Although cell fusion of noncardiomyocytes with cardiomyocytes has been described [12], a fusion of differentiated adult cardiomyocytes has not been reported, making it unlikely that multinucleation is a result of cell fusion. At a certain stage, cardiomyocytes enter a terminally differentiated state characterized by an irreversible cell cycle arrest, which is mediated and indicated by an upregulation of p16/INK4A and p53, and defines the senescent cardiomyocyte. Senescent cardiomyocytes show a reduction in telomere length to approximately 15 kb instead of 30 kb, and uncapped telomeres [5, 10, 13]. Although telomere attrition initiates the identical senescence program in young and old cells, the number of cells differs in magnitude when the old and the young heart are compared.

With aging, cardiac function is impaired at the level of the organ as well as at the level of the cardiomyocyte. Alterations in myocyte activation, contraction, and relaxation in old cardiomyocytes based on changes in gene expression have been observed. Genomewide microarray analyses of total hearts as well as isolated cardiomyocytes from young and old hearts show that mainly genes with a relevance in heat shock/stress response, mitochondrial death signaling and function, cytoskeletal organization, survival/growth, and transcription are differentially expressed [14, 15]. In old cardiomyocytes, there is a general tendency towards (1) a reduced ability to cope with stress, e.g. via reduced expression of heat shock proteins (HSP70) and anti-oxidative enzymes (hemeoxygenase-1), (2) reduced and altered function of the mitochondrial respiratory chain (e.g. reduced expression of cytochrome c oxidase), which causes electron leakage and oxidative damage, (3) increased stiffness and reduced contractility/decelerated relaxation, related to downregulation of sarcoplasmic reticulum Ca^{2+} -ATPase (see below), in-

creased expression of cytoskeletal proteins, and a transcriptional switch (caused by de-differentiation) of contractile protein isoforms, e.g. from fast (type V₁) to slow (type V₃) myosin [16, 17], and (4) a shift from proliferation and survival signaling towards cell death signaling (reduced expression of survivin, modulation of the bcl2 rheostat towards a pro-apoptotic state) [14, 15].

Other investigations on the nucleic acid and protein levels have shown that not only cytoskeletal, but also nuclear structure (crucial for the regulation of gene expression and genomic stability) is affected by aging. The intermediate filaments lamin A and C, vital for the maintenance of the nuclear shape, are downregulated in aged cardiomyocytes [18]. Electrical coupling and cell-cell communication deteriorates during aging. Connexin 43 expression, crucial for cardiac gap junctions and cell-cell coupling in the myocardium is downregulated in aged cells. In the guinea pig sinoatrial node, connexin 43 protein levels decrease with age [19], and aging-dependent changes in connexin 43 distribution from the longitudinal end to the lateral cardiomyocyte cell borders, which was accompanied by a slowing of conduction in the ventricle, have been described [20]. In line with the results from the microarray analyses, survivin, an apoptosis inhibitor, was downregulated, especially in aged, spontaneously hypertensive rats [21]. Further enzymes and pathways involved in survival and growth like PKC, Akt, AP1 are repressed and cell cycle inhibitors like p21 and p16 are upregulated. Although some inhibitors of the cell cycle may confer resistance to cell death, and although non-cycling cells are generally more resistant to many death stimuli, the overall change in the balance between pro- and anti-death signals clearly shifts towards death in the senescent cardiomyocyte. This is underlined by analyses of metallothioneins, which inhibit oxidant activity and cell death, and antagonize senescence-associated phenotypes. A number of metallothioneins are downregulated in old cardiomyocytes [22–25].

Numerous proteins involved in cardiomyocyte function and signaling are affected by the aging process. Aging was shown to significantly reduce the sensitivity of myofilaments to Ca²⁺. Changes in sarcoplasmic reticulum, by e.g. function impairment or downregulation of sarcoplasmic reticulum Ca²⁺-ATPase and Na⁺/Ca²⁺ exchanger, may explain the prolonged duration of cardiomyocyte relaxation [14]. Reduced fluidity and elasticity of aged cardiomyocyte plasma membranes are a further cause of the reported biomechanical properties of the cells [26]. Altered signaling, affecting Ca²⁺ transients, also contributes to reduced cardiomyocyte function, as

does the deposition of cellular ‘garbage’-like lipofuscin granules due to imperfect autophagy. Lipofuscin particles are a typical sign of aged cardiomyocytes and a marker of senescence [27]. Senescent cardiomyocytes show also changes in the α -adrenergic and angiotensin-signaling pathways [28]. The list of differences between old and young cardiomyocytes is much longer. However, nearly all observations indicate that the aging cardiomyocyte gradually loses its structural and functional properties.

Finally, at the end of life, the senescent cardiomyocyte has 2 options: either to undergo cell death by apoptosis, which displays a rather ‘silent way’ of dying, since the remains of the cardiomyocyte are phagocytosed by neighboring cells or macrophages, or to undergo cell death by necrosis (see below).

Mechanisms in Cardiomyocyte Aging

Although they are not always easy to separate, one can basically name 2 independent causes of aging of a system like a cell: intrinsic and extrinsic factors. Extrinsic or environmental factors can influence intrinsic factors like genes, and interact (positively or negatively) to finally determine the pace of aging of a system.

Oxidative Stress, Oxidant Defense, and Repair Mechanisms

A typical aging factor that may stem from both intrinsic and extrinsic sources is oxidative stress. Oxidative stress can be caused by environmental factors (ionizing irradiation or chemicals taken up via nutrition and drinking water, inhalation, or skin) or may stem from intrinsic sources like the respiratory chain. A prototypic reaction involved in the generation of oxidative stress is the so-called Fenton reaction catalyzed by metals like copper and iron, which produces hydroxyl radicals thereby increasing oxidative stress. The core principle in oxidative stress-mediated aging is the deterioration of cellular function by the generation of reactive oxygen or nitrogen species and/or radicals that interact and thereby change the properties of biological molecules. Alteration of nucleic acids, lipids, sugars, and proteins plays a central role in the aging process, and oxidants are known to interfere with these classes of molecules [29]. In the case of proteins, damage may either occur directly by protein oxidation or indirectly via DNA mutations within protein-coding regions of the nuclear and mitochondrial genomes. Irrespective of the oxidant source (intrinsic or

extrinsic), oxidative DNA damage (e.g. formation of 8-hydroxy-dGTP) may affect proteins of the mitochondrial respiratory chain. Altered respiratory-chain function may in turn result in increased (intrinsic) oxidative stress causing further damage. As one of the most relevant intrinsic sources of oxidative stress, the respiratory chain physiologically produces oxidants and radicals that cause cardiomyocyte aging [30, 31]. Since cardiomyocytes are physiologically highly active cells, they comprise a large number of mitochondria, which make up to one third of total cellular mass. Accordingly, radicals and oxidants produced by respiratory chain function are thought to be very important factors in cardiomyocyte aging [32]. Apart from mitochondria, a number of other cellular structures and proteins, like NADPH oxidase, contribute to the intrinsic generation of oxidative stress, e.g. NADPH oxidase subunits gp91^{phox} and p47^{phox} are increased in senescent cardiomyocytes [33]. Importantly, environmental factors like cigarette smoking can cause oxidative stress via several mechanisms in parallel. In the case of smoking, not only oxidants and radicals contained in cigarette smoke but also oxidation reaction catalyzing metals are delivered to the human body. Furthermore, smoking has been shown to reduce the bioavailability of antioxidants like selenium and zinc, and to downregulate genes important for oxidant defense [34–36]. In line with the latter, some aging factors increase oxidative stress by reducing cellular defense and repair mechanisms. Chemicals may decrease oxidant defense and cause a shift from reduced glutathione (GSH) towards oxidized glutathione (GSSG). Genes involved in DNA repair may be affected by oxidant damage, reducing the ability of a cell to repair DNA damage, a vital physiological antiaging mechanism as highlighted by progeria patients who often show defects in DNA repair genes. Not only mutation, but also a downregulation of repair genes will reduce the capability of a cell to repair its damaged nucleic acids. Reduced expression of genes like Ku86/Ku70, PARP, and TRF2 has been described to coincide with aging [14, 15, 37]. In summary, oxidative stress and consequent damage leads to defective cardiomyocyte structures (e.g. contractile apparatus) and defective enzymes and receptors, which are typical for aged cells. The accumulation of these defects makes up a major part of the aging process.

Inflammation

A phenomenon that is closely linked to oxidative stress is inflammation. Generally, inflammation is a tool of the immune system used to defend itself against pathogens, but also plays an important role in processes like tissue

remodeling and carcinogenesis. Physiologically there is a balance between inflammatory and anti-inflammatory factors which shifts towards the former during aging. The precise reasons for this shift are not yet known but the increased death of cells (necrosis) promotes repair and inflammation and the increased presence of autoantigens (e.g. oxidatively modified proteins) may also be involved in the phenomenon. Although inflammation is a vital process, it also causes oxidative stress and damage, and oxidative damage causes inflammation. Through this interplay, a vicious circle is initiated that contributes to the aging of cardiomyocytes.

Post-Translational Modifications and Metabolism

Post-translational modification of proteins plays a central role in aging and heart disease. Protein oxidation, like carbonyl formation and other protein modifications, has been described and linked to cardiac aging. The presence of advanced glycation endproducts (AGE), for example, is increased 2.5-fold in aged hearts. Prolonged cardiomyocyte relaxation duration has been indicated in the aging myocardium, and oxidative stress, protein oxidation and AGE formation contribute to prolonged duration of cardiomyocyte relengthening [33]. Aging-associated pathophysiological changes in metabolism like diabetes crucially contribute to the generation of oxidized proteins and AGE [38].

Systemic and Cellular ‘Garbage’ Management

Especially in long-living postmitotic cells, cellular ‘garbage’ accumulates mainly due to a lack of dilution by mitosis [27]. A typical sign of garbage accumulation in senescent cardiomyocytes are lipofuscin particles consisting of aggregated oxidized proteins and lipid residues. Lipofuscin particles represent intralysosomal garbage rich in iron. According to the ‘lysosomal-mitochondrial-axis’ hypothesis [39], these high intralysosomal iron levels are responsible for the particular redox sensitivity of lysosomes. Since iron catalyses the generation of superoxide in the Fenton reaction, high levels of mainly free low-molecular-weight iron causes excessive oxidative stress, which damages lysosomal membranes and leads to the release of oxidants and radicals, as well as to the release of lysosomal enzymes like cathepsins. Through this mechanism, the accumulation of garbage contributes to oxidative stress and cell death. In addition, aged cells contain high levels of extralysosomal garbage like defective enlarged ‘giant’ mitochondria, due to problems with macroautophagy. These giant mitochondria, which accumulate during aging – possibly due to a selectivity in

macroautophagy – often contain mutated DNA sequences and defective respiratory chain components that cause electron leakage and thereby contribute to oxidative stress and damage [27, 39].

On the organ level, ‘waste management’ is primarily based on the death and consequent renewal of damaged cells. Since proliferation and stem cell-based renewal of cardiomyocytes are not able to maintain cardiomyocyte number during aging, the stem cell-based systemic and/or local renewal and repair system is only able to slow down the aging and deterioration of myocardial function with age but not to inhibit it. In addition, the aging of cardiac stem cells was also reported under certain age-related diseases like diabetes [11].

Telomeres

Telomeres are special chromatin structures at the end of eukaryotic chromosomes preventing the recognition of chromosome ends as DNA brakes, thereby inhibiting end-to-end fusion and cellular DNA damage response. Telomeric length is reduced in the course of cell division of adult cells which express no or only very low levels of telomerase. The shortening of telomeres and telomere attrition has been shown to contribute crucially to a number of factors associated with cardiac aging like oxidative stress, and the finding of different telomeric lengths in old and young cardiomyocytes suggests that cell division and consequent telomeric shortening may play a role [13, 40]. As a matter of fact, old cardiomyocytes show a reduction in telomere length from 30 to 15 kb. In addition, in a telomerase knockout mouse line, the absence of enzyme activity and consequent shortening of telomeres in early and late generations were observed causing a cardiac phenotype in late mouse generations [13]. However, since telomerase knockout causes problems not only in the adult organism but already during development, and since cardiomyocytes are not highly proliferative cells, the role of telomeric shortening and attrition in cardiomyocyte aging needs further investigation. Nevertheless, telomere uncapping and activation of repair with consequent problems, e.g. in cardiomyocyte karyogenesis during multinucleation, cannot be excluded.

Cellular Hypertrophy, Survival and Death

Changes in the cellular balance between life and death as well as in the type of cell demise – from apoptosis to necrosis – occur during the aging process. Discernible by the loss in the total cell number of cardiomyocytes during aging, the balance between cell renewal and growth shifts towards death. Pro-survival/growth signaling via akt-1,

PKC, STAT 3, anti-apoptotic factors like survivin, and various cytokines is repressed and the expression of cell cycle inhibitors like p21, p16, and death stimuli is increased [22–25] (also see above). Some of these changes in gene expression not only result in cell demise but also contribute to cardiomyocyte hypertrophy. In vivo and in vitro studies in animals have demonstrated that cytokines of the IL-6 family are involved in cardiac hypertrophy as well as, however, in the protection of cardiomyocytes against apoptosis [41]. Another example for the involvement of cytokines in adaptive myocardial growth stems from experimental and clinical analyses of kinins, which inhibit the growth of cardiomyocytes [42].

In contrast to apoptotic cell death, necrosis leads to the rupture of the plasma membrane, and leakage of cytosolic constituents into the surrounding tissue area. These constituents provoke an inflammatory immune response that in turn increases oxidative stress. The oxidative burden on the surrounding area may even cause the death of neighboring cells. In general, it is the magnitude of oxidative stress that defines whether a cell survives (low), undergoes apoptosis (medium), or dies by necrosis (high). The mechanisms underlying oxidative stress, inflammation, and cell demise in the aging heart are described above.

Discussion

The core hypothesis of this review is that the central principle that initiates the physiological aging of cardiomyocytes is intrinsic oxidative stress and damage. The main sources for oxidants are factors like the mitochondrial respiratory chain, lysosomes, and enzymes like NADPH oxidase. The expression of a defined set of genes is changed directly by causing mutations and indirectly by modifying proteins (posttranslational), lipids, and sugars, with consequent functional alteration. Modified (pro-aging) gene expression constitutes a central element in the aging process of cardiomyocytes, and makes up the driving force behind aging progression (fig. 1). Altered gene expression and mutations memorize and amplify the aged character of the cardiomyocyte, e.g. reduced DNA repair due to mutations in crucial DNA repair genes will allow further persisting mutations. The hypothesis of ‘oxidant stress-caused altered – pro-aging – gene expression’ is also underlined by studies on genes which exert myocardial anti-aging activity. As in many other organs and cells, proteins involved in metabolism and IGF signaling also play an important role in cardiomyo-

Fig. 1. Factors and interactions involved in the mechanisms underlying cardiomyocyte aging. It is thought that cardiomyocyte aging is initiated by oxidative stress, and that mutations and changes in gene expression are the central element in aging 'memorization' and progression. Although cell death is another central element in the aging process, it is rather a consequence of processes other than an initial event. The numbers indicate the number of direct interactions with other aging mechanism. 'Accumulation of post-translational modifications' is due to and refers to the accumulation of altered biomolecules, like lipoxidation products and advanced glycation endproducts, and an accumulation of 'garbage', e.g. lipofuscin particles. An important element causing these accumulations are changes in the protein degradation machinery and altered autophagy.

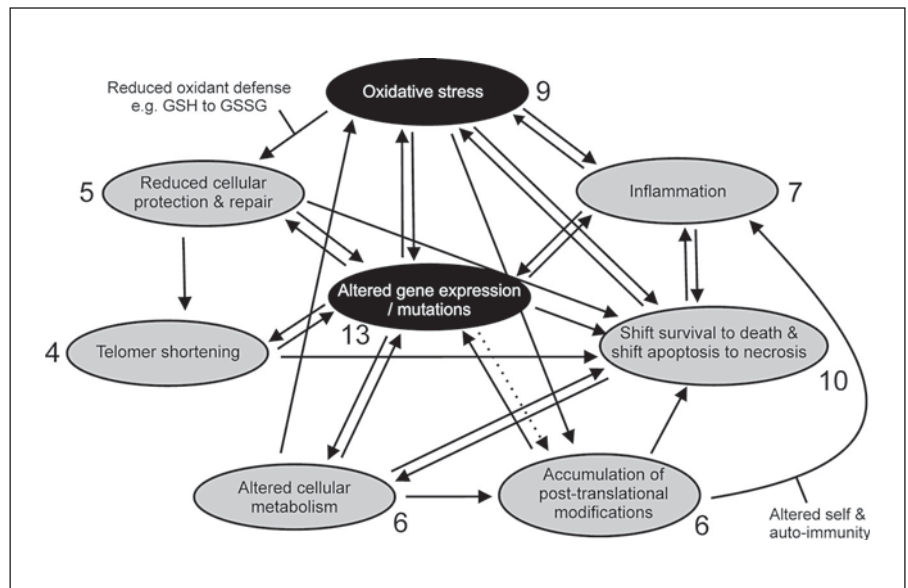
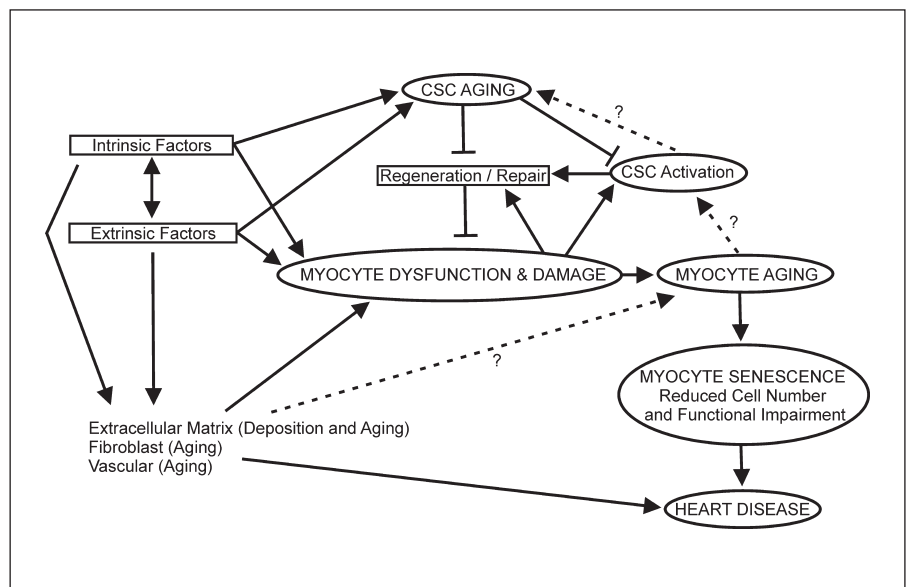


Fig. 2. Mechanisms and processes in cardiac aging, senescence, and disease. Apart from noncardiomyocyte-based processes, which are also affected by intrinsic and extrinsic factors, cardiomyocyte and cardiac stem cell aging constitute the central elements on the way from a young to a senescent heart.



cyte aging and aging of CSCs, e.g. transgenic mice expressing IGF-1 attenuated the expression of growth arrest and cell cycle arrest proteins p27/KIP, p53, p21/INK4a, p19/ARF, and attenuated the reduction in aging-caused telomerase activity compared to wild-type mice, which was paralleled by Akt phosphorylation [37]. A deletion of proteins that protect cells against oxidative stress, like HSP 70 or catalase, is associated with an acceleration of aging-induced cardiomyocyte relaxation dysfunction, reduced cardiac function in general, and a reduced life span, whereas a transgenic overexpression of these pro-

teins prolongs life span and reduces myocardial dysfunction and damage [43, 44].

Similar to cellular repair mechanisms and cell renewal via stem cell pools, multinucleation of cardiomyocytes may also constitute a system that decelerates cardiomyocyte aging. Due to the high basal level of intracellular oxidative stress, a lot of genomic mutations occur in cardiomyocytes. Multinucleation may reflect a mechanism to increase the number of alleles per cell, to reduce the likelihood of a cardiomyocyte ending up with only defective alleles of vital genes. Multinucleation is a result of the

inability of adult cardiomyocytes to undergo cytokinesis. This loss of the ability to divide may have 2 reasons, and both may play a role *in vivo*. First, due to the physiological activity of cardiomyocytes at a high level, many mutations may occur which are critical for carcinogenesis. Second, cardiomyocytes represent a highly specialized cell type, and the specialization may determine the inability to divide. Despite the proliferation of young cardiomyocyte and stem cells, myocardium is a tissue with low proliferation rates and cell replacement. Due to this, we suggest that mechanisms other than telomere attrition are the central elements in cardiomyocyte aging. Nevertheless, some telomere-based phenomena may well contribute to cardiomyocyte aging, and a prolonged activity of telomerase reverse transcriptase activity promotes cardiac muscle cell proliferation, hypertrophy, and survival [45].

In summary, the following factors play roles in the aging of cardiomyocytes: oxidative stress, inflammation, cellular protection and repair, telomere integrity, survival and death, metabolism, post-translational modifications, and altered gene expression. Importantly, also non-cardiomyocyte-based aging processes (vascular, fibroblast, extracellular matrix, etc.) in the heart interfere with cardiomyocyte aging and cardiac function (fig. 2). Although we are, at present, far from understanding the

processes that determine the aging of cardiomyocytes, and although the relevance of the mentioned factors differs between individuals, it is clear that a large number of different processes work together in the pacing of the aging process. Due to the fact that the average life span of an organism like man can be increased to a certain extent by providing ideal environmental conditions (low levels of extrinsic aging accelerators), but also that the maximum achievable life span is limited, intrinsic factors of aging seem to be the final determinants of the length of the life span. Since the heart is a slowly proliferating organ (low cellular renewal rate) with high physiological activity (high generation of intrinsic aging accelerators), we postulate that the physiological aging process of the heart and of the cardiomyocyte is primarily driven by intrinsic aging factors. However, extrinsic factor-based aging, e.g. from smoking, importantly contributes to pathologically accelerated aging of the heart.

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