Review

This Review is in a thematic series on **Aging and the Cardiovascular System,** which includes the following articles:

Mechanical Regulation of Cardiac Aging in Model Systems
Aging and Autophagy in the Heart
Macromolecular Degradation Systems and Cardiovascular Aging
Mitochondrial Metabolism in Aging Heart
Dietary Interventions, Cardiovascular Aging, and Disease: Animal Models and Human Studies
Pharmacological Strategies to Retard Cardiovascular Aging

Guest Editors: Guido Kroemer and Lorenzo Galluzzi

Mechanical Regulation of Cardiac Aging in Model Systems

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Abstract: **Unlike diet and exercise, which individuals can modulate according to their lifestyle, aging is unavoidable. With normal or healthy aging, the heart undergoes extensive vascular, cellular, and interstitial molecular changes that result in stiffer less compliant hearts that experience a general decline in organ function. Although these molecular changes deemed cardiac remodeling were once thought to be concomitant with advanced cardiovascular disease, they can be found in patients without manifestation of clinical disease. It is now mostly acknowledged that these age-related mechanical changes confer vulnerability of the heart to cardiovascular stresses associated with disease, such as hypertension and atherosclerosis. However, recent studies have aimed at differentiating the initial compensatory changes that occur within the heart with age to maintain contractile function from the maladaptive responses associated with disease. This work has identified new targets to improve cardiac function during aging. Spanning invertebrate to vertebrate models, we use this review to delineate some hallmarks of physiological versus pathological remodeling that occur in the cardiomyocyte and its microenvironment, focusing especially on the mechanical changes that occur within the sarcomere, intercalated disc, costamere, and extracellular matrix.** **(*Circ Res*. 2016;118:1553-1562. DOI: 10.1161/CIRCRESAHA.116.307472.)**

Key Words: cardiomyocyte hypertrophy ■ cardiomyopathy ■ cardiovascular disease ■ *Drosophila* ■ pathophysiology ■ physiology

The majority of people afflicted by cardiovascular disease (CVD) and heart failure are of advanced age. Although aging specifically is not thought to be the cause, it is an independent risk factor for CVD because both the incidence and the prevalence of CVD increases dramatically after the age of 50 years for men and women.[1] The heart undergoes extensive structural changes with aging independent of disease; this is marked by an asymmetrical increase in septum thickness and overall decrease in ventricular tissue compliance.[2] However, when changes are superimposed with cardiac stresses associated with disease, the occurrence and severity of disease increases. Regardless of pathology, however, these global age-related, organ-level structural changes are all rooted in intra- and extracellular remodeling, leading to hypertrophied cardiomyocytes regardless of sex or disease state. This remodeling is thought at least, in part, to reinforce organ structure under increased systolic pressure and afterload experienced with age.[3,4] Cardiomyocytes must maintain function with increasing loads via compensatory molecular mechanisms that span the sarcomere, intercalated disc (ID), and the sarcolemma. Proteins within these units remodel with increased stress and strain associated with aging to maintain function, but the accumulation of remodeling events may lead to a pathological response resulting to heart failure. Recent studies have focused on differentiating which specific molecular changes that accumulate with age help maintain cardiomyocyte function, and can be deemed hallmarks of physiological aging, versus those that aid in

Nonstandard Abbreviations and Acronyms	
α-CAT	α-catenin
β-CAT	β-catenin
BM	basement membrane
CVD	cardiovascular disease
cTnT	cardiac tropomyosin–binding troponin
DCM	dilated cardiomyopathy
ECM	extracellular matrix
HF	heart failure
ID	intercalated disc
LV	left ventricle
MHC	myosin heavy chain
MLC	myosin light chain
MMP	matrix metalloproteinase
N-CAD	N-cadherin

disease progression during advanced age, and can be deemed pathological aging.

The past few decades have seen the emergence of many likely and unlikely animal models used for their unique ability to study certain aspects of physiological and pathological cardiac aging, disease, or both. Recent aging studies have used large mammalian models such as simians, pigs, and dogs because they may more closely model functional decline and respond similarly to treatments. However, these models are costly and slow to age. Smaller, cheaper, and easier to use models are ideal to elucidate cellular and molecular mechanisms of aging. For that reason, the bulk of cardiac aging and disease research has focused on rat and mouse models, rather than larger mammalian counterparts. Yet, mice and rats have significantly increased heart rates over humans, and still require 2 years to age from young to old. The need for a rapidly aging, genetically tractable, and genetically homologous model has led to the recent popularity of the fruit fly, *Drosophila melanogaster*, as a model for age-related CVD. The fruit fly is uniquely suited to study cardiovascular aging and disease because of its relatively short lifespan ≈5 to 7 weeks, conservation of cardiac disease genes,[5] the breadth of transgenic systems,[6–8] and ease of in situ measurement of cardiac parameters.[9–12] With all of these models and tools, the scientific community can use multivariate approaches to explore how the age-related mechanical changes that occur in cardiomyocytes progress from maintenance of physiological function to those that induce pathological disease.

Using the model systems introduced above, this review highlights a range of protein complexes implicated in regulating the mechanical sensing and transmitting of forces necessary for physiological cellular remodeling under increased load and stress,[13–15] which with age may become increasingly dysregulated. We will first focus on the intracellular cytoskeletal and sarcomeric remodeling that leads to age-related cardiac hypertrophy. In this context, we will pay close attention to separate physiological and pathological cardiac aging to highlight differences, which to date have been less appreciated.

Intracellular Remodeling as a Result of Stress, Age, or Disease

Cardiomyocytes undergo extensive morphological changes during development, stress, and aging due in large part to outside-in transduction of mechanical signals to the sarcomeres, cytoskeleton, and junctions, such as IDs and costameres. Internal remodeling gives cardiomyocytes plasticity to adjust to increased mechanical loads, especially when cells must compensate to preserve function. Here, we detail each structure and outline its pathological remodeling with disease and its potential physiological remodeling with age. For reference, Figure 1 contains many of the proteins referenced in this section and is color-coded based on whether expression changes have been shown to change with physiological aging before disease progression or still only associated with pathological cardiac aging; proteins in black are mentioned in the text without indicating conclusively if they contribute to either, whereas those in gray are shown to indicate overall structure.

Sarcomeric Remodeling and Maintenance

The sarcomere is a complex assembly of thick (myosin) and thin (actin) filaments responsible for force generation within the cardiomyocyte. Sarcomeres generate the contractile force within myocytes together with regulatory proteins, such as troponins and tropomyosin, and cytoskeletal components, such as titin and myosin-binding protein C. Sarcomere function is spatially and temporally regulated by post-translational modifications and alternative splice isoforms. Therefore, aberrant expression of certain isoforms with age may result in reduced contraction, reduced mechanical response, and disease.

In mammalian hearts, 2 myosin heavy chain (MHC) isoforms, α and β, exist. α-MHC is associated with higher ATPase activity, greater contractile velocity,[16] and it is the dominant form of MHC in rodent left ventricle (LV), that is, >90%.[17] In adult humans, β-MHC comprises ≈95% of MHC found in ventricles[18] and produces more force per power stroke.[16] Failing human myocardium experiences a further decrease in α-MHC expression (Figure 1, blue).[19,20] Rat hearts experience a similar decrease in α-MHC with aortic banding,[21] suggesting that cardiomyocyte regulation of MHC isoforms during pressure overload is not retarded by age and is more closely associated with pathology. Because MHC isoforms have unique affects on contractility, decreasing α-MHC could negatively affect contractile velocity and impair adaptation to increased cardiac demand. Unfortunately, age-related MHC expression data outside of disease are lacking, which could further clarify if this is a response to pressure changes or downstream activator of pathological remodeling. Similarly, essential myosin light chain (MLC) undergoes isoform shifts during disease, with an increase in the ratio of atrial to ventricular isoforms expressed with hypertrophic cardiomyopathy.[22] In addition to isoform switches, phosphorylation of ventricular MLC-2 regulates cross-bridge cycling; when ventricular MLC-2 is phosphorylated, hearts exhibit greater force production per myosin power stroke by slowing down cycling kinetics and prolonging the duty cycle, allowing more myosin heads to come into contact with actin.[23,24] Conversely, reduced phosphorylation in mice exhibit dilated cardiomyopathy (DCM)–like
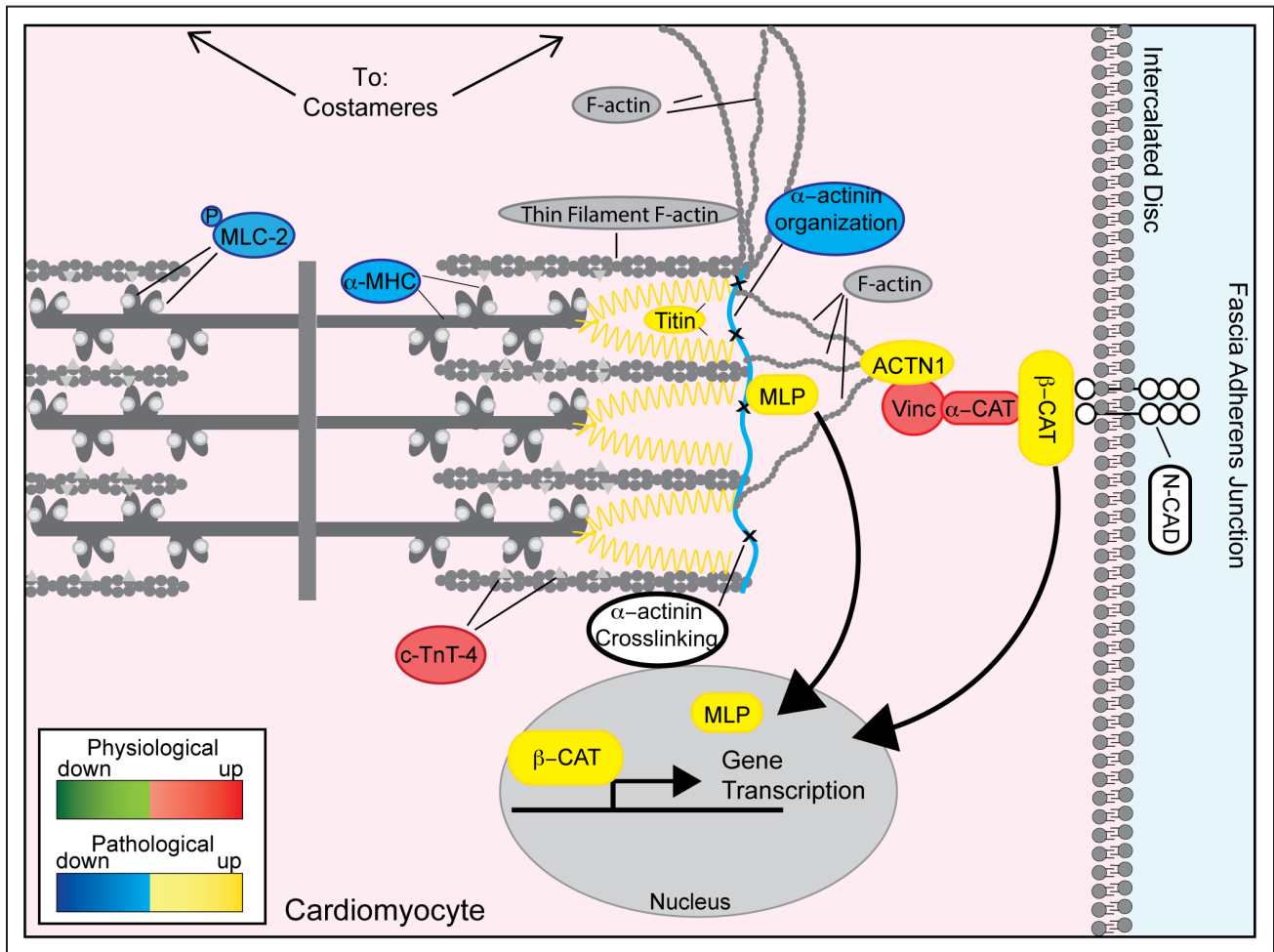
Figure 1. Physiological vs pathological age–related changes of the sarcomere and intercalated disc (ID). Schematic representation of sarcomere and ID components in the cardiomyocyte implicated in physiological aging (red, upregulated; green, downregulated), pathological aging (yellow, upregulated; blue, downregulated), or yet undetermined (black). Arrows to nucleus indicate translocation of protein into the nucleus with age. α-CAT indicates α-catenin; ACTN1, α-actinin-1; α-MHC, α-myosin heavy chain; β-CAT, β-catenin; c-TnT4, cardiac troponin T-4; F-actin, filamentous actin; MLP, muscle-binding limb protein; N-CAD, N-cadherin; P-MLC-2, phosphorylated myosin light chain-2; and Vinc, vinculin.

remodeling.[24,25] Humans also shift to more dephosphorylated MLC-2 in diseased and failing myocardium,[26–28] suggesting that cross-bridge cycling becomes increasingly dysregulated with disease (Figure 1, blue). Yet much of how both MHC and MLC isoforms change with physiological aging have yet to be discovered, owing largely to a focus on how mutations cause familial hypertrophic cardiomyopathy. Because of the strong correlation of heart failure and the decline of α-MHC and phosphorylated MLC-2 across species, these data suggest that a threshold of α-MHC expression and MLC-2 phosphorylation is needed for proper cardiomyocyte contraction; thus declining levels of either protein may be a marker for pathology.

In addition to thick filaments, some thin filament proteins change with aging and disease. For example, cardiac tropomyosin–binding protein troponin T (cTnT) undergoes alternative splicing to confer increased myofilament Ca^{2+} sensitivity in failing human hearts.[29,30] However, a cTnT3 to cTnT4 isoform switch does not enhance disease progression (Figure 1, red),[31] suggesting that the switch to more Ca^{2+}-sensitive isoform might compensate for increased hemodynamic stress. In

Drosophila, mutations adjacent to a highly conserved TnT1 tropomyosin-binding domain result in excessive cross-bridge formation at rest even under low Ca^{2+}, prolonged muscle activation, and restrictive cardiomyopathy.[32] Because these cTnT changes result in diastolic dysfunction for fly hearts, this model could explain why aging hearts experience increased resting tension and stiffness and ultimately impaired function. However a cTnT isoform expression profile with age is needed to confirm that the isoform switch is part of physiological compensation under increased stress because prolonged stress could have adverse consequences.

Maintaining myofilament structure and function with age and increased load is a combinatory process across the sarcomere. Major mechanical regulators of sarcomere structure, such as titin[14]–a giant sarcomeric protein that spans from Z-disc to M-band,[33] seem to contribute to this balance between physiological compensation and pathological remodeling. Titin acts as a sarcomeric ruler,[33,34] generates resting stiffness, and transduces signals in times of mechanical overload.[35] The role of titin in mechanically regulating the switch from physiological

aging to pathological remodeling, and heart failure has been extensively reviewed (Figure 1, yellow).[36] Recent work studying titin-associated proteins demonstrated that only patients with heart failure with preserved ejection fraction exhibited a significant increase in titin-dependent stiffness,[37] reinforcing the importance of this protein and proteins in complex with it in regulating the switch from normal to pathological function. As another example, the Z-disc protein α-actinin anchors actin filaments at the Z-disc.[14,38,39] Mutations in α-actinin are associated with familial hypertrophic cardiomyopathy and arrhythmias, suggesting that it is integral for proper sarcomeric growth and force propagation.[40] Patients who recover enough LV function to warrant explantation of their left ventricular assist device often experience a significant increase in α-actinin,[41] implying that α-actinin could scale with sarcomeric function. In addition to concentration, α-actinin lattice organization has been implicated in disease severity; canine heart failure models show partial rescue of α-actinin lattice structure post cardiac resynchronization therapy (Figure 1, blue).[42] However, it remains undetermined if these changes precede pathology because aging or stress-induced studies involving α-actinin are lacking (Figure 1, black). However, nonsarcomeric α-actinin, which aids costameric and ID adhesion complexes, does experience an increase in aged diseased hearts (Figure 1 and 2, yellow),[43] but how these levels change with physiological aging have not yet been studied. Beyond these most commonly discussed proteins, a variety of other models and proteins have been studied to a lesser extent, such as *Drosophila* and muscle limb protein expression and localization changes (Figure 1, yellow). Common muscle limb protein polymorphisms result in DCM,[44] creating cardiomyocytes unable to sense load.[45] Muscle limb protein is also shuttled to the nucleus on hypertrophic response,[46,47] suggesting that mislocalization results in impaired mechanosensing (Figure 1, arrow).[48] These data clearly imply that muscle limb protein and α-actinin are tied to mechanical sensing, but future work needs to explore their changes with age outside of disease.

ID Reinforcement and Gap Junction Remodeling With Age

The ID forms a specialized junction between adjacent cardiomyocytes to propagate longitudinal forces from sarcomeres to adjacent cells. Coupling is achieved through actin cytoskeleton bridges that occur specifically within the fascia adherens and reinforced by the desmosome; electric coupling is achieved through gap junctions.[13] In recent years, the ID has emerged as a key mechanical component, permitting the cell to sense and respond to stress, and load via ultrastructural changes as in volume-overloaded rabbit[49] and mouse hearts[50] as well as human DCM patients.[50] These correlations imply that IDs may have a more regulatory role in cardiomyocyte hypertrophy, which is evident when examining key components of ID and gap junction substructures. Both are regulated with age across animal models,[51–53] suggesting that cardiomyocytes transition from adaptive remodeling to pathological loss of sarcomere regulation.

Within these junctions, N-cadherins (N-CAD), vinculin, and the catenins form Ca^{2+}-dependent cell–cell adhesions that

are linked to the cortical actin cytoskeleton.[54] By forming a mechanical link to adjacent cardiomyocytes,[36] they may help to induce pathological remodeling in the heart. For example, N-CAD knockout mice lose ID and gap junction structures but exhibit growth in the LV long axis,[55] suggesting that N-CAD could regulate eccentric or concentric sarcomeric growth. Yet, mouse aging studies show that although other junction components change expression with age, such as connexin 43 and β-catenin (β-CAT), N-CAD expression remains unchanged.[52] These data imply that although N-CAD may mechanically regulate differential cell growth (Figure 1, black), other junctional proteins may regulate disease. The catenins, for example, link N-CAD's cytoplasmic tail to actin via vinculin, and β-CAT in particular changes its localization to the nucleus with age (Figure 1, arrow),[56] mediating fetal gene reactivation through novel transcriptional activity (Figure 1, yellow).[57] Recent work showed that although β-CAT overexpression has no measurable effects, its knockdown induces significant dysfunction.[58] Although β-CAT could have a role in pathological disease, we have recently demonstrated that its counterpart, α-catenin (α-CAT), was upregulated in aged, nondiseased simian, and fly hearts using proteomic and genetic analyses. Data suggest that α-CAT seems upregulated with physiological aging[51] and may precede pathological remodeling (Figure 1, red). Indeed mechanical stimulation of α-CAT via N-CAD triggers vinculin recruitment to junctions, demonstrating that α-CAT is necessary for force-mediated remodeling.[59,60] Although consensus is building for force-dependent regulation of the junctions,[61,62] further work is needed to explore how α-CAT combines with other signals to affect function with age.

Finally vinculin, which is another critical linker protein within the ID, binds to α-CAT to translate sarcomeric contraction into cell shortening. In aging patients with heart failure, vinculin undergoes remodeling,[53,63] which can be reversed with left ventricular assist devices.[64,65] In a recent comprehensive study across simian, rat, and fly, we demonstrated that upregulation of vinculin with age is conserved from invertebrates to mammals and that its role is compensatory rather than deleterious in aging (Figure 1, red).[51] Vinculin overexpression resulted in increased cortical stiffness most significantly at the ID where it increased longitudinal myofibril anchoring between adjacent cells and increased force transmission. Increased cardiac vinculin correlated with better force production and fractional shortening in fly hearts,[51] further supporting the idea that vinculin upregulation and localization to the ID with age aids the cardiomyocyte in preserving contraction, even under mechanical load. Metavinculin, a muscle-specific alternative splicing, is more associated to actin bundling[66] and involved in DCM versus aging.[66,67] Metavinculin expression levels have not be examined as a function of age, but these molecular interactions could play a large role in the mechanical regulation of vinculin-mediated cytoskeletal reinforcement mentioned above, ultimately adding another potential switch from normal to maladaptive remodeling.

Whether considering cardiomyocyte hypertrophy or subcellular remodeling, it is important to keep in mind that such mechanisms are often interrelated and complex, especially because multiple proteins within a single complex, such as

the ID, can act in opposing directions with age and disease (Figure 1). Thus, we think that although it is difficult to dissect and identify, it is critical to recognize the existence of intracellular mechanisms that counterbalance aging without necessarily contributing to disease.

Costamere and Extracellular Matrix Remodeling in Response to Age, Stress, and Disease

In addition to internal remodeling, cardiomyocytes experience changes in their surrounding extracellular environment with age. Under physiological conditions, cardiac extracellular matrix (ECM) provides structural support for cardiomyocytes and facilitates force transduction. However, increased ECM deposition and crosslinking along with decreased ECM degradation have all been implicated in aged patients.[68–70] These changes affect cardiomyocyte function[71] not only by changing the mechanical properties of the tissue but also by altering transmembrane receptors.[72–74] Cardiac ECM has been recently reviewed,[75–77] so we will focus on compositional changes that have been implicated in switching from adaptive to maladaptive remodeling (Figure 2, top). On the opposing side of the sarcolemma (Figure 2, bottom), costameres aid in cell–ECM adhesion by adhering Z-discs to ECM.[78,79] Given their changes with age, we will begin our discussion with costamere complexes, specifically the vinculin/talin/integrin structure given its known changes with age,[80] and transition to ECM using Figure 2 to illustrate our conclusions on physiological and pathological changes.

Costamere Complex Reinforcement

Costameres are periodic vinculin-containing structures on the lateral surface of cardiomyocytes[81] that link Z-discs to integrins via talin. Vinculin is integral in responding to mechanical stimulus, and patients with DCM and hypertrophic cardiomyopathy often have vinculin mutations.[66] In addition to disease, we have shown that as in humans,[53,63] age-related increases in cardiac vinculin are observed in simians and flies. Vinculin overexpression in *Drosophila* increased cortical stiffness at both the ID and the costameres. Improved assembly maintained myofilament lattice spacing but significantly improved lattice order (Figure 2, red).[51] These findings confirmed data in mice, which showed that cardiac vinculin knockout resulted in significantly more compliant cardiomyocytes and greater myofilament spacing before dysfunction.[82] Improved myofilament spacing may enhance cross-bridge cycling, which increases force production. Future work examining aged, nondiseased human myocardium will be necessary to corroborate vinculin upregulation with age in simian and *Drosophila* models, but these data establish a link between vinculin and compensatory nonmaladaptive response within aged tissue.

Together with vinculin, talin links the actin cytoskeleton to integrins and regulates their binding affinity across species.[83–85] Talin has 2 genes in vertebrates, with talin-2 largely expressed in adult myocardium.[86] Until recently, their role in the heart was unknown, but pharmachalogical and mechanical stress of mouse hearts revealed an upregulation of talin-1, which may be a compensatory response to stress.[87] However

in pressure-overloaded hearts, talin-1 ablation reduced hypertrophy and improved function,[87] suggesting that pressure-induced upregulation of talin-1 may be deleterious and thus a pathological marker (Figure 2, yellow). Conversely talin-2 is elevated in aged mice (Figure 2, red),[88] specifically between middle aged (10–16 months) and aged (20–24 months) mice.[88] When hearts are unloaded, for example, via left ventricular assist device, talin-2 is downregulated.[41] Although these data suggest that talin is involved in remodeling, aged nondiseased patient samples are needed to better explore if regulation is age-associated or merely a response to external load. Invertebrate studies may prove useful for future work because they only have 1 talin gene and age rapidly.

A final major component in this complex is integrin, which occurs as a heterodimer of α and β subunits[89,90] and directly transduces signals to and from ECM and costameres. The range of integrin dimer pairs confer ECM ligand specificity,[91] so their modulation with age could affect costamere function. α7β1 heterodimer is the most prevalent pair in adult cardiomyocytes,[92] but additional α and β subunits have been detected after pressure overload, post myocardial infarction, and with age.[93–95] In *Drosophila*, β1-integrin increases with age, but moderate attenuation correlates with improved cardiac function and significantly increased organismal lifespan.[73] Ventricular expression of β1-integrin is also increased in aged monkeys;[51] however, cardiac ablation of β1-integrin results in DCM during pressure overload,[74] suggesting that integrins are an integral regulator of stretch and load sensing. Knockdown of integrin-linked kinase, a β1-integrin binding protein, also correlated with improved cardiac function and lifespan.[73] These results confirmed the correlation of integrin-linked kinase inhibition and extended lifespan in *Caenorhabditis elegans*.[96,97] Although increased expression of β1-integrin and integrin-linked kinase with age correlate with impaired function, a moderate reduction in their expression can be cardioprotective, which suggests that tightly controlling β1-integrin (Figure 2, red and yellow stripes) and integrin-linked kinase (Figure 2, yellow) expression could have beneficial effects on function of aged hearts.

ECM Protein Composition and Remodeling Changes With Age

Cardiac ECM consists of a complex arrangement of interacting structural proteins, which include a basement membrane (BM) lattice of laminin and collagen IV linked together with fibronectin-mediating attachment to collagen I and III.[98] Accumulation of collagen I and collagen III with age has been documented across mammals[52,53,99,100] and including aged healthy human myocardium, suggesting it occurs with physiological aging.[101] Although these changes are thought to increase LV tissue stiffness[37] and impair ventricular relaxation, the propensity of data in patients with no known CVD suggests that an increase per se is not indicative of pathology. In addition, accumulation with age can occur in the absence of excessive mRNA[102] unlike in CVD,[103] suggesting that genetic regulation correlates with pathological conditions, but not necessary in physiological aging (Figure 2, yellow). However, an increase of collagen III relative to collagen I has been implicated in disease, but it is not clear how collagen III changes
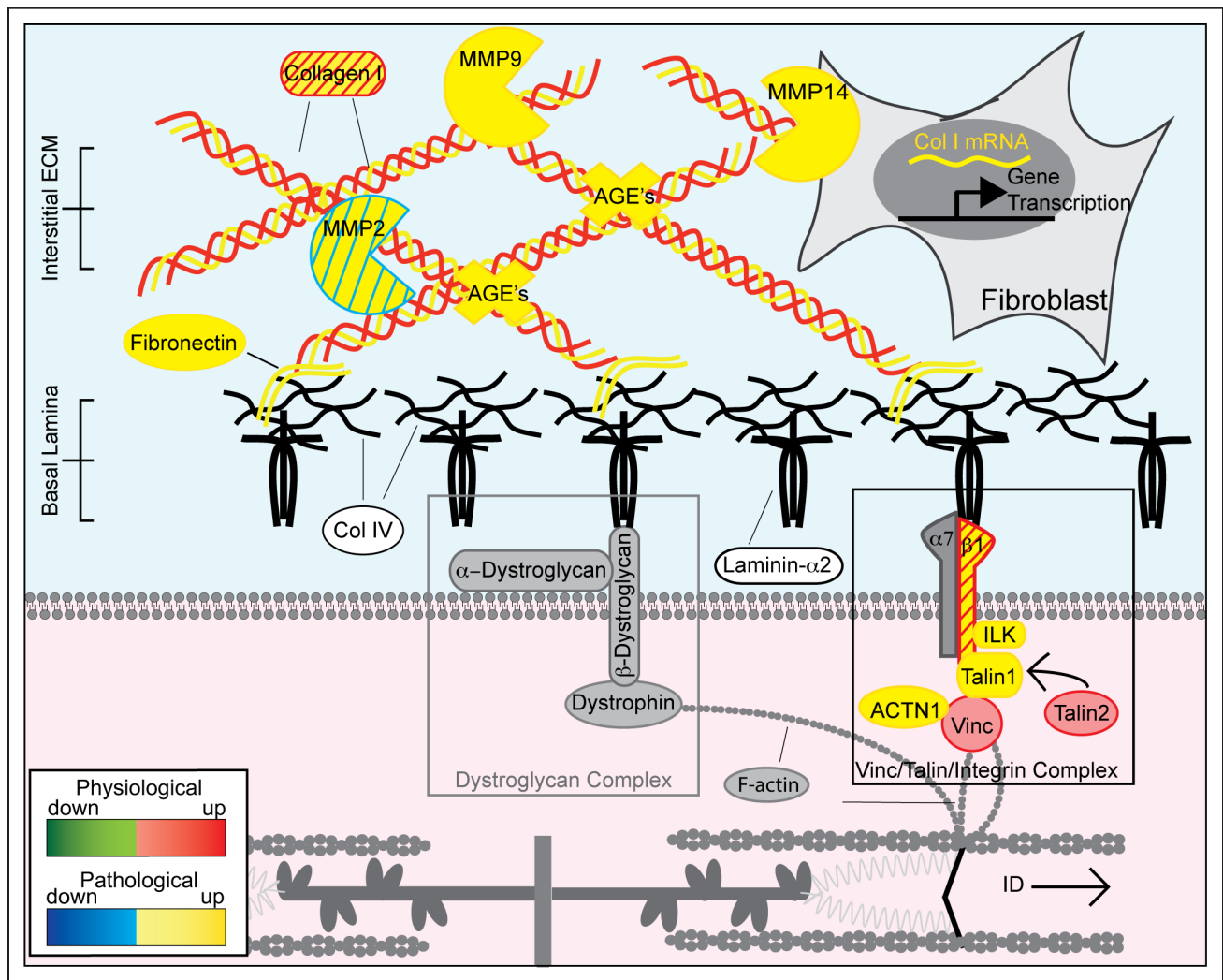
Figure 2. Physiological vs pathological age–related changes of costamere and extracellular matrix (ECM). Schematic representation of Costamere and ECM of the cardiomyocyte implicated in physiological aging (red, upregulated; green, downregulated), pathological aging (yellow, upregulated; blue, downregulated), or yet undetermined (black). Stripes of 2 colors indicate lack of consensus on expression levels across species or involvement in physiological vs pathological aging. α7 indicates α7-integrin; β1, β1-integrin; ACTN1, α-actinin-1; AGE's, advanced glycosylation endproducts; Col I, collagen type 1; Col IV, collagen type IV; F-actin, filamentous actin; ID, intercalated disc; ILK, integrin-linked kinase; MMP2, matrix mettaloproteinase-2; MMP9, matrix metalloproteinase-9; MMP14, matrix metalloproteinase-14; and Vinc, vinculin.

with age.[104,105] If anything, an increase in collagen III has been linked to increased tissue plasticity and not rigidity as with collagen I.[106] Classically though, interstitial ECM accumulation is implicated in less compliant myocardium, and excessive collagen I can induce diastolic dysfunction making its role seem more context specific (Figure 2, red and yellow stripes). In addition to accumulation with age, the degree of crosslinking can have a significant effect on tissue stiffness and function.[107] Collagen crosslinking, measured by changes in hydroxylated lysine and proline levels, increases significantly with age in sedentary rats.[108] Collagen crosslinking via advanced glycosylation endproducts also increases with age in canine and human myocardium (Figure 2, yellow),[109,110] which when inhibited, improved tissue compliance.[110] Such pathological crosslinking is at least partly reversible with exercise as well.[107,108]

Fibronectin, an adhesion molecule that links collagen fibers to BM, changes its expression with collagen, increasing in mice from 12 to 20 months (Figure 2, yellow).[95] When fibronectin is knocked out, mice experience less cardiac remodeling and hypertrophy with transaortic constriction,[111] which together suggest that fibronectin is associated with pathological remodeling with age. In addition to fibronectin, cardiac BM has an important role in transducing mechanical signals between cells. For example, laminin α2 decreases as a function of age in both human and rat myocardium, but only in rats it is associated with pathological remodeling because of a decrease in myocyte number (Figure 2, black).[112] Conversely, BM collagen IV increases with age in mice, suggesting an overall age-related BM thickening,[113] although it is not clear if age-related changes contribute to pathological remodeling because their response to stress is not clear (Figure 2, black). However, collagen IV is downregulated after left ventricular assist device unloading, providing evidence that collagen IV expression is responding to pressure overload.[64] Regardless, extensive

studies are necessary to explore how reinforcement of the BM with age affects signal transduction across the sarcolemma.

Finally, all of these age-associated ECM changes—especially collagen remodeling—occur in part because matrix metalloproteinases (MMPs), a large family of endopeptidases, degrade ECM.[114] MMP expression changes with age, for example, upregulation of MMPs, such as MMP-2, MMP-9, and MMP-14 (Figure 2, yellow), modulate collagen persistence, turnover, and fibrotic remodeling, but there is not yet consensus on whether the remodeling is maladaptive. Aged rats have significant interstitial fibrosis and depressed MMP-2 levels, which suggested that decreased MMP-2–mediated proteolysis led to ECM accumulation.[115] However in pressure overload, MMP-2 loss improved diastolic dysfunction and suppressed fibrosis.[116] These studies highlight the different actions by which MMP-2 mediates ECM remodeling with age: MMP-2 decrease eventually leads to ECM accumulation, but acute upregulation and chronic pressure overload initiates pathological activation of matrix degradation (Figure 2, blue and yellow stripes). Indeed, mice with impaired MMP expression experience reduced age-related fibrosis but total loss results in DCM.[100] More specifically, MMP-14 is an important regulator of ECM degradation with age via its sensitivity to mechanical load and its ability to activate MMP-2. Increased myocardial MMP-14 has been noted in patients with LV pressure overload,[117] and in vitro studies have shown MMP-14 upregulation with increased wall tension.[118] Induction of pressure overload in mice also leads to increased MMP-14 activation, which correlates with increased ECM accumulation.[119] Thus, it would seem that MMP-14, and subsequent MMP-2 activation, in part regulate adverse cardiac remodeling under stress conditions that mimic aging (Figure 2, yellow). MMP-9 also increases with age in conjunction with diastolic dysfunction (Figure 2, yellow),[120] whereas its deletion prevents age-related myocardial stiffening.[88] Yet in the larger discussion of whether MMPs contribute to pathological or physiological ECM remodeling, it is critical to note that MMPs degrade matrix, preventing accumulation, which is physiological for collagen I but pathological for collagen III. Thus, the context of all of these changes matter significantly to their effect(s) on aging.

Conclusions

Cardiac aging is a complex process that involves many molecular changes both inside and outside of the cardiomyocyte. In this review, we have highlighted some of the age-related structural changes that occur within the sarcomere and cytoskeletal complexes that longitudinally transmit forces between adjacent cardiomyocytes. For example, we have indicated that age-related vinculin reinforcement of the ID and costamere is beneficial to cardiomyocyte function, whereas decreasing proportion of α-MHC and phosphorylated MLC-2 correlates strongly with disease progression. However, because of the overlap in function of multiple complexes and proteins within the sarcomere and fascia adherens junction, conclusive age-related data for other cytoskeletal components has been lacking. Although matrix changes often seem associated with pathological changes, we also noted the underappreciated role it has during normal aging, for example, the increase in interstitial collagens that

can precede heart disease. To that end, we have provided evidence that although collagen accumulation can lead to pathological fibrosis, genetic upregulation of collagen mRNA is clearly implicated in disease progression. Despite the evidence we presented, question remains about what constitutes causal evidence of physiological responses such as: how much fibrillar collagen is too much, or is it the extent of crosslinking that determines pathology? How do BM components affect lateral signal transduction from the sarcomere to the ECM? As aging studies increase, phenotypes that were once thought to be solely maladaptive have become increasingly complex because of some instances in which they are actually mechanisms of physiological adaptation of cardiomyocytes with age. We will need more multi-model approaches to stratify some of these molecular changes that have been previously noted with aging in the context of advance disease into hallmarks of physiological reinforcement versus pathological progression to provide causal evidence for our hypotheses.

Disclosures

None.

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