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Caveolin and caveolae in age associated cardiovascular disease

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Abstract

It is estimated that the elderly (> 65 years of age) will increase from 13%–14% to 25% by 2035. If this trend continues, > 50% of the United States population and more than two billion people worldwide will be "aged" in the next 50 years. Aged individuals face formidable challenges to their health, as aging is associated with a myriad of diseases. Cardiovascular disease is the leading cause of morbidity and mortality in the United States with > 50% of mortality attributed to coronary artery disease and > 80% of these deaths occurring in those age 65 and older. Therefore, age is an important predictor of cardiovascular disease. The efficiency of youth is built upon cellular signaling scaffolds that provide tight and coordinated signaling. Lipid rafts are one such scaffold of which caveolae are a subset. In this review, we consider the importance of caveolae in common cardiovascular diseases of the aged and as potential therapeutic targets. We specifically address the role of caveolin in heart failure, myocardial ischemia, and pulmonary hypertension.

1 Introduction

Cardiovascular disease is the leading cause of mortality in the United States and more than 80% of these deaths occur in those 65 years or older.[1,2] Advanced age is the most important predictor of poor outcome in patients with acute coronary syndromes[3] and with the projected increase in the elderly population worldwide, it is important to understand the risk factors associated with aging in order to identify appropriate treatments. The aged heart has an increased sensitivity and decreased tolerance to ischemia/reperfusion (I/R) injury.[4,5] Aging also results in a loss of the heart's ability to respond to cardioprotective stimuli such as pharmacological and ischemic preconditioning.[6,7] It is believed that the aging cardiomyocyte develops a reduced tolerance to stress because of decreased mitochondrial function, increased oxidative stress, changes in gene expression, and aberrant cell signaling.[8]

Lipid rafts are subcellular microdomains of the plasma membrane that consist of lipid clusters enriched in cholesterol and sphingolipids, in which particular proteins are concentrated.[9] Caveolae, a subset of lipid rafts, have a unique flask-like structure that is generated by caveolin and cavin proteins.[10-14] Caveolae and caveolins act to coordinate cellular signaling events in many cells, including those of the cardiovascular system.[15] Age-associated alterations in the composition of lipid rafts and caveolae could affect a variety of cellular functions and have been linked to diseases, such as Alzheimer's, atherosclerosis, and diabetes, which are more prevalent in the elderly population.[16] The focus of this review will be on the role of lipids rafts and caveolae in the progression of age related cardiovascular diseases, such as heart failure, myocardial infarction, and pulmonary hypertension.

2 Caveolae and caveolins

Caveolae, or "little caves", are cholesterol and sphingolipid-enriched invaginations of the plasma membrane.[10] Caveolins, structural proteins of caveolae, are present in three isoforms.[11] Caveolin-1 (Cav-1) is required for caveolae formation in many non-muscle cell types and for the membrane localization of Cav-2,[17] while Cav-3 is required for caveolae formation in striated (skeletal and cardiac) and smooth muscle cells.[18] Caveolins function as chaperones and scaffolds for signaling molecules in caveolae by providing temporal and spatial regulation of signal transduction.[11] Through their caveolin scaffolding domain (CSD),

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caveolins not only anchor other proteins in caveolae, but also inhibit or enhance that protein's signaling capacity. Caveolins have a variety of other functions including vesicular transport, cholesterol and calcium homeostasis, and t-tubule formation.[19,20]

The generation of mice lacking the caveolin genes has made it possible to better understand the significance of each caveolin isoform and its contribution to whole animal physiology and human disease. While all caveolin null mice [Cav-1, Cav-2, Cav-3 single knockouts and Cav-1/3 double knockout (KO)] are viable and fertile, each displays a unique phenotype. Cav-1 KO mice have a complete loss of caveolae in non-muscle cells and a 90% loss of Cav-2 expression due to protein degradation.[21,22] Thus, Cav-1/3 double KO mice lack expression of all three caveolin proteins and do not form visible caveolae in any cell type.[23] Caveolin protein expression and association with caveolae is decreased with age and studies of the caveolin KO mice support the hypothesis that the loss of caveolin protein causes an aged phenotype.[24,25] Caveolins are, therefore, potential therapeutic targets in the treatment of age related disorders, such as cardiovascular disease.

3   Heart failure

Despite advances in diagnosis and treatment, heart failure remains one of the most common, costly, and deadly diseases.[26] The prevalence of the disease is significantly increased in people over 65 years of age and arises as a consequence of abnormal cardiac structure, function, rhythm, or conduction.[26] Ventricular dysfunction resulting from myocardial infarction and/or hypertension is frequently the cause of heart failure and each will be discussed in more detail in the sections below.

Although Cav-3 is the predominant caveolin in cardiac myocytes, Cav-1 KO mice develop a severe cardiomyopathy, which appears to contribute to their significantly shortened life span. At 24 months, Cav-1 KO mice show a 50% reduction in viability, with a major decline between 27 and 65 weeks of age.[27] The hearts of Cav-1 KO mice are structurally and functionally abnormal at 2–4 months of age. Imaging and functional studies revealed that Cav-1 KO hearts have significantly enlarged ventricular chambers, abnormal ventricular wall thickness, hypertrophy, and decreased contractility.[28,29] These defects are worse by 12 months of age, which shows that loss of Cav-1 causes a progressive cardiomyopathy.[27] Increased fibrosis in the heart has also been reported in Cav-1 KO mice.[22,27] This could affect stiffness of the heart and contribute to impaired myocardial function.

Since Cav-1 is expressed in both cardiac myocytes and fibroblasts,[30] it is not clear if this decline with aging is due to altered function of a specific cardiac cell (i.e., myocyte death and replacement by fibroblasts vs. increased activation of fibroblasts). As signaling scaffolds, caveolins have been shown to interact with receptor tyrosine kinases, Src family tyrosine kinases, endothelial nitric oxide synthase (eNOS), and members of the p42/44 MAP kinase cascade (MEK1/2 and ERK1/2).[31] At the molecular level, loss of Cav-1 is thought to cause cardiac hypertrophy through disruption of signaling pathways. Cav-1 is thought to negatively regulate the p42/44 MAP kinase cascade in cardiac fibroblasts[32] and activation of the p42/44 MAPK pathway in cardiac myocytes can drive cardiac hypertrophy.[33] In Cav-1 KO mice the p42/44 MAP kinase cascade is hyperactivated, which contributes to the cardiac defects seen in these mice.[28] This finding suggests that the major effect of Cav-1 loss may come from its function in the supporting cells of the heart (fibroblasts and endothelium). However, Cav-1 is also found in the cardiac myocyte and its role in these cells cannot be dismissed. In heart failure, β-adrenergic signaling is reduced and this may lead to further deterioration of heart function since, with decreased contraction, the heart is unable to meet its needs.[34] In Cav-1 KO hearts, the levels of cyclic AMP (cAMP), an important second messenger of β-adrenergic signaling, and ATP are decreased.[35]

Cardiac myocytes from Cav-3 KO mice completely lack caveolae.[36] Cav-3 KO mice develop a progressive cardiomyopathy marked by significant hypertrophy, dilation, and reduced fractional shortening.[37] Similar to Cav-1 KO mice, the p42/44 MAP kinase cascade is hyperactivated in the hearts of Cav-3 KO mice and may partly account for these cardiac defects.[37] Similarly, a mutation in Cav-3 has been found in familial hypertrophic cardiomyopathy.[38] In cardiac myocytes, t-tubules ensure rapid, uniform cell activation. In heart failure there is an extensive remodeling of the t-tubule network and this change may contribute to abnormal calcium handling.[39] The disorganization of t-tubules in Cav-3 KO skeletal muscle suggests that cardiac myocytes may display a similar phenotype that contributes to the progression of heart failure.

The activity of dozens of ion channel proteins and membrane transporters generate the flux of ions across the sarcolemmal membrane, which is responsible for activation and contraction. Many of these ion channels reside in caveolae and abnormalities in the function or regulation of these channels result in arrhythmias that can lead to heart failure.[40] Therefore, the loss of Cav-3 and caveolae in the cardiac myocyte could contribute to arrhythmias as another potential source of heart failure. In fact, mutations in Cav-3

have been found in the inherited arrhythmogenic syndrome, long-QT congenital syndrome.[41] Mice with a constitutive overexpression of α1-adenosine receptor demonstrate cardiac dilation and decreased left ventricular function.[42] In this model of heart failure, Cav-3 expression is decreased (Cav-1 and Cav-2 are unaffected) and there is a direct relationship between Cav-3 expression and ventricular dysfunction.[42] Cav-3 levels are also decreased in the failing human heart.[42]

Consistent with the findings that loss of Cav-3 contributes to heart failure, overexpression of Cav-3 has been shown to attenuate heart failure. Natriuretic peptides are endogenous hormones released by the heart to modulate cardiac hypertrophy.[43] Caveolae contain natriuretic peptide receptors such as atrial natriuretic peptide (ANP), which is closely associated with Cav-3.[44] Overexpression of Cav-3 attenuates cardiac hypertrophy, at least partially, by increasing natriuretic peptide expression and signaling.[45] Overexpression of Cav-3 may also prevent hypertrophy in cardiomyocytes by suppression of ERK1/2 signaling.[46] These results suggest Cav-3 may not only be a marker for heart failure, but also a therapeutic target.

Cav-1/3 double KO mice lack all caveolin protein expression and caveolae formation. This combined loss of caveolin protein does not produce any new phenotypes and only cardiac defects were exacerbated compared to single knockout animals.[23] Cav-1/3 double KO mice display a severe cardiomyopathy by two months of age in which left ventricular wall thickness, hypertrophy, ventricular dilation, and decreased fractional shortening that is more pronounced. The cardiac tissue also shows signs of interstitial inflammation, fibrosis, and myocyte necrosis. These results clearly establish a role for both Cav-1 and Cav-3 in maintaining normal cardiac function and further suggest that a loss of caveolin proteins with age could be a major contributing factor to the development of cardiovascular disease.

4 Myocardial infarction

Ischemic heart disease and myocardial infarction result in loss of blood flow and oxygen to part of the heart, which causes damage and reduced cardiac function. The incidence of myocardial infarction is increased in the elderly population.[1] Evidence suggests caveolae and caveolins are involved in the pathogenesis of ischemic injuries. Changes in caveolin protein expression have been identified in renal, cerebral, hindlimb, and myocardial ischemia.[47–49] Cerebral artery occlusion causes a marked increase in endothelial Cav-1 and Cav-1 KO mice display a significant increase in the volume of cerebral infarcts, as compared with wild-type

mice.[50] In this model, Cav-1 KO mice displayed decreased proliferation of endothelial cells and increased apoptosis. In a model of myocardial infarction, Cav-1 KO mice subjected to left anterior descending coronary artery ligation display reduced survival and despite similar infarct sizes, Cav-1 KO mice showed reduced cardiac function compared to wild-type mice.[51] Mechanistically, it appears that caveolins provide protection from ischemic injury through scaffolding signaling molecules and promoting cell survival.[49,51] Specifically, reduced Cav-1 expression alters β-adrenergic signaling through decreased cAMP production and protein kinase A phosphorylation, which alters cardiac contractility.[51] Recent evidence suggests that caveolins may also induce cardioprotection through epigenetic regulation.[52]

Ischemic preconditioning is an innate protective mechanism by which brief episodes of ischemia protect the heart from the damaging effects of prolonged I/R injury.[53] In addition to sublethal ischemia, several pharmaceutical agents, such as opioids and volatile anesthetics, produce a similar preconditioning protective effect.[54] Preconditioning activates a complex signaling cascade known as the reperfusion injury salvage kinase pathway and many of these signaling molecules associate with caveolins in caveolae.[55–57] Caveolae play a pivotal role in the generation of survival signals in cardioprotection and ischemic preconditioning increases the number of caveolae present in the sarcolemmal membrane.[58] Methyl-β-cyclodextrin disrupts lipid rafts and caveolae by removing cholesterol from the membrane. Preconditioning the heart in the presence of methyl-β-cyclodextrin abolishes the cardioprotective effects leading to decreased ventricular performance, increased myocardial infract size and cardiomyocyte apoptosis.[48,59] Additionally, aging is associated with a decrease in the protective effects of preconditioning in animal models and human patients.[6–8,60,61] The loss of caveolae and caveolins with age may, therefore, contribute to the loss of signal regulation and protection.

Ischemic preconditioning-induced protection is lost in caveolin KO mice.[52,58,62] Caveolins also function in preconditioning with isoflurane, a volatile anesthetic, and opioids. Cardiac myocytes are protected from simulated I/R injury when incubated with δ-opioid receptor agonists. This effect is lost in the presence of methyl-β-cyclodextrin, indicating a critical role for caveolae.[48] Cav-3 KO mice cannot be preconditioned with δ-opioid receptor stimulation.[63] Isoflurane-induced cardioprotection is dependent on the presence of caveolae, and is lost in both Cav-1 and Cav-3 KO mice.[64–66] Perhaps the most interesting evidence for the role of caveolins in cardioprotection comes from analysis of a transgenic mouse with myocyte specific overexpression of

Cav-3 (Cav-3 OE).[58] Cav-3 overexpression causes enhanced formation of caveolae on the cardiac myocyte sarcolemmal membrane. Cav-3 OE mice subjected to I/R injury have significantly improved functional recovery, reduced infarct size and apoptosis relative to wild-type mice. This innate cardioprotection in Cav-3 OE mice is similar to that seen in wild-type mice undergoing ischemic preconditioning.[58] The precise molecular mechanism by which caveolins protect the heart from I/R injury remains to be determined. However, a generalized pathway has emerged in which preconditioning stimuli enhance release of agonists of one or more G-protein coupled receptor families (opioid, adenosine, bradykinin) to enhance activity of pro-survival kinase pathways (including PI3-K/Akt and MAPKs) and inhibit activity of pro-death pathways (GSK3β).[67] The ability of caveolin to preserve such signaling as the heart ages may be a key feature of caveolin mediated stress adaptation.

5   Pulmonary hypertension

Severe pulmonary hypertension (PH) is characterized by a progressive increase in pulmonary vascular resistance and vascular remodeling leading to right heart failure and early death. The ability of the right ventricle to respond to the increased vascular resistance is influenced by several factors, including age. Cav-1 is highly expressed in the lung and is found in pulmonary endothelial cells. Disruption of the function of Cav-1 in the vascular system can have profound effects on cardiac function and may serve as a marker for pulmonary hypertension, which would allow earlier detection and improved treatment strategies.

Cav-1 KO mice show lung abnormalities, with reduced alveolar spaces, increased wall thickening, fibrosis, and hypercellularity.[22,68] Direct measurement of pulmonary artery pressure and histological analysis revealed that Cav-1 KO mice exhibit pulmonary hypertension, which may contribute to the right ventricle hypertrophy seen in these mice.[29] Additionally, lungs from Cav-1 KO mice exhibit increased pulmonary vascular resistance associated with pulmonary vascular remodeling including increased medial thickness and muscularization of distal pulmonary vessels, which is an underlying feature of pulmonary vascular remodeling in PH.[69] Reduced expression of Cav-1 in the lungs after myocardial infarction has been suggested to initiate the development of PH and lung structural remodeling.[70] Patients with severe pulmonary hypertension show reduced levels of Cav-1 in total lung tissue and pulmonary vascular endothelial cells.[65] Cav-1 polymorphisms have also been identified in pulmonary hypertension patients.[71] These findings suggest an antihypertensive function of

Cav-1 with respect to the pulmonary vascular architecture.

Cav-1 KO mice display hyperactivation of eNOS and altered vasoconstriction and vasorelaxation responses of isolated aortic rings.[22,29,68,72] Disruption of eNOS activity in Cav-1 KO mice is likely involved in the development of the observed cardiopulmonary pathologies. eNOS is a critical mediator of cardiovascular homeostasis regulating multiple physiologic and pathophysiologic processes including vascular tone, vascular remodeling, platelet aggregation, and angiogenesis.[73] Through interactions with Cav-1, eNOS is targeted to caveolae in endothelial cells.[74] Several studies have demonstrated a role for Cav-1 as a negative regulator of eNOS. Direct interaction of eNOS with Cav-1 significantly inhibits its enzyme activity *in vitro*[75] and infusion of a membrane permeable Cav-1 CSD peptide performs similarly *in vivo*.[76] The hyperactivation of eNOS in Cav-1 KO mice results in increased NO production, which is believed to cause the majority of the cardiovascular defects. Double KO of Cav-1 and eNOS (NOS3) completely blocks the increase in NO production in lung tissue.[69] These double KO mice which do not develop pulmonary hypertension, have normal pulmonary vasculature and lung morphology, and no right ventricle hypertrophy. The adverse effects of Cav-1 deficiency on lung architecture and pulmonary hypertension can also be reversed by inhibition of eNOS by L-NAME.[77] Interestingly, endothelial specific expression of Cav-1 in Cav-1 KO mice rescues the development of pulmonary and vascular defects.[78] These mice show no pulmonary hypertension or cardiac hypertrophy phenotype. These data indicate that loss of Cav-1 in the vasculature can have profound effects on cardiac function and the progression of pulmonary hypertension. The effects of Cav-1 may function primarily through eNOS, but regulation of other pathways, including p42/44 and angiotensin-converting enzyme, can also influence hypercellularity and endothelial dysfunction contributing to pulmonary hypertension.[79]

Cav-1 expression is ubiquitous and evidence suggests that its role in pulmonary hypertension may be cell specific. Pulmonary hypertension may be characterized by a reduction of Cav-1 in lung tissue, but Cav-1 levels in vascular smooth muscle are elevated.[80,81] Human patients with pulmonary hypertension exhibit increased Cav-1 levels and also display altered Ca^{2+} regulation.[82] Enhanced influx of Ca^{2+} in vascular smooth muscle causes hyper-proliferation of vascular smooth muscle, which leads to hypertrophy of the pulmonary vascular wall and increased pulmonary vascular resistance.[80,83] Additionally, Cav-1 functions within caveolae to inhibit cell proliferation, but during increased mechanical stress, Cav-1 translocates out of caveolae to other sites within the plasma membrane of smooth muscle

cells.[84] Endothelial damage sustained during early stages of pulmonary hypertension may result in exposure of smooth muscle cells to high cyclic pressure, thus resulting in enhanced expression of Cav-1 and cell proliferation. This data suggests that Cav-1 may have a cell specific dual role in pulmonary hypertension, similar to how caveolin can both promote and inhibit tumor progression in cancer.[85,86] Two stages of Cav-1 expression change promote pulmonary hypertension; an initial loss of Cav-1 in endothelial cells which is followed by subsequent increased expression of Cav-1 in smooth muscle cells. Conversely, there is evidence suggesting an increase in Cav-1 in endothelial cells can have detrimental effects. Increased Cav-1 levels are associated with pathologic angiogenesis, breakdown of the blood-brain barrier, cardiac hypertrophy, and diabetic angiopathy.[87–90] This must be considered when selecting therapeutic strategies.

6   Therapeutics

Given the evidence presented here, it can be hypothesized that the aged population is vulnerable to cardiovascular disease as a consequence of lost caveolin expression. Therefore, treatments focused on restoring caveolin expression may reverse these effects. Up regulation or overexpression of caveolins promote signaling *via* enhanced receptor-effector coupling or enhanced receptor affinity.[91] This improved signaling could prevent aging and the development of disease, or rescue impaired cardiac function. Treatments already in use today may have success because they positively affect caveolin expression. For example, in the failing heart, a left ventricular assist device initiates structural and functional changes through mechanical unloading. This device improves cardiac adrenergic responsiveness and lipid metabolism, processes regulated by caveolae. Implantation of a left ventricular assist device in human patients increases expression of Cav-1 and causes redistribution of Cav-3.[92] These data suggest that enhanced caveolin expression in the failing heart in response to mechanical unloading leads to reverse remodeling. It has also been shown that exercise confers cardioprotection from I/R injury.[93] Mild exercise causes an increase in Cav-3 expression, which may partially account for these results.[94] Similarly, exercise training of spontaneously hypertensive rats, which undergo pathologic hypertrophy as a consequence of hypertension, produces a reversal of this phenotype with increased expression of Cav-3.[95]

Treatments that more directly target caveolin protein levels may also have therapeutic potential. Infusion of a caveolin scaffolding domain (CSD) peptide has cardioprotective effects against I/R injury.[96] This peptide significantly attenuates cardiac contractile dysfunction, similar to preconditioning. Administration of the Cav-1 CSD has also been shown to effectively prevent the development of pulmonary hypertension and right ventricular hypertrophy.[97] Success has also been found in treating the downstream effects of lost caveolin. Hyperactivation of eNOS caused by loss of Cav-1 leads to an imbalance with vascular tetrahydrobiopterin (BH4), which acts as an essential eNOS cofactor. The resultant oxidative stress contributes to the development of cardiac and pulmonary defects. Donation of BH4 to Cav-1 KO mice causes improvement of both systolic and diastolic heart function and marked improvement of the impaired lung phenotype.[98] Additional therapies targeted at downstream signaling molecules may have similar results.

Statins were originally developed as lipid lowering drugs, but have additionally been shown to have vasoprotective properties through NO dependent pathways.[99,100] Studies also suggest that statins may be useful in the treatment of pulmonary hypertension.[101] Fluvastatin causes dissociation of eNOS and Cav-1, which has been shown to increase eNOS activity and improve endothelial function.[102] This suggests that decreasing Cav-1 expression in some cases could potentially be therapeutic as well. In contrast, the importance of caveolin/caveolae in cardiac systems suggest that though there may be beneficial effects of statins on multiple cellular systems that impact proper cardiovascular function, muscle specific myopathies associated with statin use may be of concern possibly through disruption of cardiac myocyte caveolae. Further studies in aging systems looking specifically at statins and muscle pathology are necessary.

7   Conclusions

From the evidence presented above, caveolins are central players in a number of cardiovascular diseases, and caveolin based therapeutics may be a powerful approach for addressing age-associated cardiovascular pathologies. Certain limitations need to be addressed as future therapy is considered: as caveolins are ubiquitously expressed, can they be targeted to specific cell types; current therapies are limited to delivery of gene, is it possible to develop pharmacologic agents that enhance the expression of caveolin; what is the role of caveolin as a protein *vs.* its property as a structural protein for caveolae in modulating disease phenotypes; is the regulation of caveolin expression transcriptional or translational with age, and can secondary processes, such as microRNA or transcription factors, be potential targets? Consideration of these questions will be vital to advancing the therapeutic potential of caveolin.

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