Highlights

- Adverse cardiac remodelling is an important feature of cardiac disease and leads to heart failure.
- Cysteine cathepsins contribute to key signalling pathways involved in adverse cardiac remodelling.
- They contribute to extracellular matrix remodeling, cardiomyocyte hypertrophy, calcium handling, cellular apoptosis, and autophagy.
- Cysteine cathepsins have potential to serve as biomarkers for cardiovascular disease.
- Cathepsins have translational potential as therapeutic targets in cardiac disease.

Signalling Pathways Linking Cysteine Cathepsins to Adverse Cardiac Remodelling

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Abbreviations

Extracellular matrix remodelling (ECM); excitation-contraction (E-C); myocardial infarction (MI); low density lipoprotein (LDL); abdominal aortic aneurysm (AAA)

Abstract

Adverse cardiac remodelling clinically manifests as deleterious changes to heart architecture (size, mass and geometry) and function. These changes, which include alterations to ventricular wall thickness, chamber dilation and poor contractility, are important because they progressively drive patients with cardiac disease towards heart failure and are associated with poor prognosis. Cysteine cathepsins contribute to key signalling pathways involved in adverse cardiac remodelling including synthesis and degradation of the cardiac extracellular matrix (ECM), cardiomyocyte hypertrophy, impaired cardiomyocyte contractility and apoptosis. In this review, we highlight the role of cathepsins in these signalling pathways as well as their translational potential as therapeutic targets in cardiac disease.

1 Introduction

An important pathophysiological consequence of several cardiovascular diseases is adverse cardiac remodelling, involving structural changes to the myocardium and extracellular matrix (ECM) [1,2]. These changes may present clinically as ventricular wall thinning, impaired contractility and chamber dilation. Driving these changes are events at the molecular and cellular level including impaired calcium handling, apoptosis, necrosis, inflammation, hypertrophy, autophagy and ECM remodelling including interstitial fibrosis [1–6](Figure 1). Clinically, these cellular alterations are associated with impaired cardiac contractility and arrhythmias that can lead to sudden death or development of heart failure (HF). Despite optimised treatment, mortality and morbidity rates for HF remain high and continue to increase [7,8]. There is a critical need to identify new molecular targets/pathways contributing to adverse cardiac remodelling and use these to develop novel therapeutic strategies that limit patients with cardiac disease developing HF [9–11].

One potential group of molecular targets that has been gaining increasing interest for their role in adverse cardiac remodelling are the lysosomal proteolytic enzymes; cathepsins. ‘Cathepsin,’ is a

term used to collectively describe cysteine, aspartic and serine proteases. Whilst aspartic (cathepsin D & E) and serine cathepsins (cathepsin A and G) have been shown to play a role in cardiac remodelling (e.g. cathepsin A is important for matrix remodelling and ventricular contractility) [12], the majority of cathepsins fall into the group of cysteine proteases [13]. The largest subfamily of cysteine proteases are the papain-like proteases. In humans, these papain-like cysteine proteases are made up of 11 members; cathepsin B, C, H, K, F, O, S, V, X, L and W [14]. Mice express 10 human orthologues with the exception of cathepsin V [15]. Four are exopeptidases (B, C, X and H), and five are endopeptidases (L, S, K, V, F) (summarized in Figure 1). Uniquely, cathepsin B can also act as an endopeptidase. The majority of these cathepsins are expressed by almost all human tissues, where they play important roles in the degradation of proteins within cells. However, some cathepsins such as cathepsin K, W, V, S and O are predominately associated with specific tissues. For instance, several studies have shown that cathepsin K is highly expressed by osteoclasts where it contributes to the degradation of articular collagen in inflammatory arthritis [16]. Linnevers et al., showed that cathepsin W tends to be expressed at high levels by lymphatic tissues and CD8+ T cells, suggesting that this cathepsin may have cytotoxic activity [17]. Indeed, natural killer cells use cathepsin W to mediate cytotoxicity [18]. Cathepsin V is mainly expressed by the testis and thymus [19], whereas, cathepsin S is predominantly expressed by antigen presenting cells (APCs) such as dendritic cells and B cells [20]. Finally, cathepsin O is primarily expressed by human breast carcinoma cells [21]. While it is currently unknown how certain subsets of cathepsins have different functions and cellular localizations, a possible explanation for this may be due to subtle structural differences between cathepsins. For example, cathepsin W contains a 21-amino acid insertion between histidine and asparagine residues in the active site and the C-terminal extension, which distinguishes it from other cathepsins such as cathepsins L and B [17]. However, further study is required to fully elucidate the relationship between the structure of cathepsins and cellular localization and function.

Papain-like cysteine proteases (herein referred to as cysteine cathepsins) were traditionally considered to be lysosome-restricted proteases, intrinsically involved in the proteolysis of unwanted proteins [22,23]. However, the past few decades have shed light on the observation that cysteine cathepsins can also be found in the extracellular environment, cytosol, nucleus, nuclear membrane and plasma membrane, where they are thought to have important roles in cell signaling and protein degradation, processing, and trafficking [22,23]. Murine knockout models have proved invaluable in elucidating the role, dysregulation and relative contribution of cysteine cathepsins to adverse cardiac remodelling and disease [24–26]. One of the most important ways in which cysteine cathepsins contribute to adverse cardiac remodeling is through pathological structural remodeling of

the ECM [27,28]. Additionally, several cysteine cathepsins have been shown to contribute to adverse cardiac remodeling by dysregulating hypertrophic, inflammatory, cellular calcium handling, apoptotic, and autophagy signaling in the heart, further strengthening their capacity as novel therapeutic targets [28,29,38–42,30–37]. Furthermore, serum and plasma cathepsins have shown biomarker potential in humans in relation to disease severity, prognosis and mortality in patients with cardiovascular disease. This review will focus on the role of key cysteine cathepsins in ECM remodeling, inflammation, cardiomyocyte hypertrophy, calcium handling, cellular apoptosis, and autophagy. Furthermore, we will examine the potential of cysteine cathepsins as both novel therapeutic targets and biomarkers in cardiovascular disease.

1091.1 Cysteine cathepsin structure and maturation

All cysteine cathepsins contain a catalytic domain, a signal peptide and a pro-peptide. The signal peptide is usually 10-20 amino acids long and signals the peptide's translocation into the endoplasmic reticulum during protein translation [14,43]. The pro-peptide prevents the premature activation of the catalytic domain, acts as a scaffold for the folding of the catalytic domain and also has an important role in the transport of the proenzyme to endosomal/lysosomal compartments [44]. Variable in length, the pro-peptide can be 251 amino acids long (cathepsin F) or only 36 amino acids long (cathepsin X) [44]. Conversely, the catalytic domain contains a highly conserved active site consisting of cysteine, histidine and asparagine residues [45]. Additionally variable in length, the catalytic domain of the human cathepsins can vary between 214 and 260 amino acids in length [45]. The folded structure of cathepsins is well conserved, specifically the active site of the peptide is located in both the L and R domains, with the L domain containing the cysteine residue and the R domain containing the histidine residue [46]. Translational modifications such as glycosylation, sulfurization and peptide folding take place in the endoplasmic reticulum. The cathepsins are then directed to the endosome. Lysosomal cysteine cathepsins are processed further in the Golgi apparatus where the mannose residues are modified to mannose-6-phosphate, thus facilitating the binding to the mannose-6-phosphate receptor to allow lysosomal targeting [43]. Following acidification in the early endosome, the N-terminal pro-peptide is cleaved and removed, and the enzyme is now proteolytically active [44,47]. Cysteine cathepsins can be further activated and are secreted into the extracellular space by calcium mediated endosome/lysosome fusion with the cell membrane [43] (summarized in Figure 2). Importantly for cathepsins B, K, X and L, the pro-peptide chain folds over the active site in the opposite direction to the substrate and subsequently blocks access to the active site [48–52]. For some cathepsins, autocatalytic activation by acidic conditions is the main mechanism of activation, and for other cathepsins, removal of the N-terminal pro-peptide

by other cathepsins such as aspartic proteinases, pepsin and elastase activates the enzyme [53–55]. The literature has long suggested that cysteine cathepsins exert optimal proteolytic activity at a very low pH in the endosomal/lysosomal compartments [56]. It is therefore, puzzling that cysteine cathepsins can exert such potent proteolytic activity at a less than optimal pH, such as when they are secreted into the extracellular environment to degrade the ECM. Many theories have been put forward to explain how cysteine cathepsins exert high proteolytic activity in the extracellular environment, and one such theory suggested by Punturieri et al., provides strong evidence that cathepsin-expressing cells can modulate the peri-cellular environment to optimize cathepsin activity [57]. Specifically they showed that monocyte-derived macrophages could express cysteine cathepsins K, L and S, while simultaneously increasing the expression of vacuolar-type H+ ATPase to acidify the peri-cellular space thus keeping cathepsin K active [57]. It is also likely that structural differences between cysteine cathepsins may significantly impact on their enzymatic potency at different pH [58,59].

2.2 Regulation of cysteine cathepsin activity

Mature cysteine cathepsins are negatively regulated by endogenous reversible, competitive protein inhibitors known as cystatins, thyropins and serpins. Cystatins are regarded as the most important and widely studied endogenous cathepsin inhibitors [60,61]. Type 1 cystatins are known as stefins, and they are generally regarded as being intracellular proteins. These single-chain proteins contain approximately 100 amino acid residues, lack a signal peptide, carbohydrates and disulphide bonds [62]. Stefin A and B are generally considered the most commonly found stefins in mammals including humans [62]. Type 2 cystatins (commonly called cystatins) tend to be more widely distributed. They contain a single-chain with approximately 115 amino acid residues, contain 2 disulphide bridges (with the exception of cystatin F), and contain 7 known family members [63]. Finally type 3 cystatins or kininogens, are multidomain single-chain glycoproteins, found primarily in the blood plasma of mammals. Kininogens can be subdivided into three broad categories, low-molecular weight kininogen (LK), high molecular weight kininogen (HK) and T-kininogen [64]. The heavy chain of HK and LK are composed of 3 repeated type 2 cystatin-like domains with 8 disulphide bridges, and domain 2 and 3 directly inhibit cathepsins [65].

2 Cysteine cathepsin involvement in ECM remodelling and inflammation

The cardiac ECM, which is composed of proteins such as collagen and elastin, is crucial in supporting the structural integrity of cardiac tissues where it maintains tissue homeostasis. Dysregulated

synthesis, deposition and degradation of ECM components are involved in the pathogenesis of many cardiovascular diseases such as cardiomyopathies, atherosclerosis and aneurysm formation [66–69]. Inappropriate ECM deposition, mainly by fibroblasts, can lead to interstitial, epicardial and perivascular fibrosis which can impair ventricular compliance with consequent systolic and/or diastolic dysfunction [70]. Fibrosis is initially an adaptive response to maintain the structure and pump activity of the heart, but over time fibrosis significantly impedes ventricular compliance, contractility and excitation-contraction (E-C) coupling and directly contributes to heart failure [70]. The development and persistent activity of myofibroblasts (cells that have acquired a phenotype between a fibroblast and smooth muscle cell [71]) leads to further cardiomyocyte necrosis and hypertrophy due to the release of pro-hypertrophic and pro-inflammatory stimuli, thus exacerbating adverse cardiac remodelling [70,72].

Cardiac fibrosis is intimately and inextricably linked with inflammation in the heart where ECM components directly modulate inflammatory responses. For example, hyaluronic acid can mediate pro-inflammatory responses by directly interacting with Toll Like Receptors on dendritic cells, monocytes and lymphocytes [73]. Additionally, several immune cells such as eosinophils, neutrophils, leukocytes and macrophages can regulate ECM remodelling and fibrosis in the heart [74]. Macrophages for example can release cathepsins to remodel the ECM in cardiovascular diseases, can degrade collagen through mannose receptor interactions, and M2 macrophages can directly contribute to fibrosis by secreting pro-fibrotic mediators such as TGF-β, IL-10, IGF-1 and galectin-3 [75–79]. Resident myocardial cells such as cardiomyocytes and fibroblasts additionally secrete cytokines such as IL-6 in response to cardiac insult or hypoxia which can directly mediate myocardial fibrosis in addition to further attracting inflammatory cells into the myocardium, and thus contributing to ECM remodelling [80–83].

Importantly, the processes of synthesis, deposition and degradation of ECM proteins are tightly controlled by cysteine cathepsins, which are secreted by resident myocardial cells as well as invading inflammatory cells [84]. Owing to the importance of the ECM in normal physiological cardiac function, it therefore follows that enhanced activity of some cysteine cathepsins has been associated with pathological structural changes to the ECM and increased fibrosis in many cardiovascular diseases [27,28]. Additionally, cysteine cathepsins have been shown to direct pro-fibrotic inflammatory responses in cardiovascular disease by interacting with inflammatory cells [34–37]. Key roles for cysteine cathepsins K, B, L and S in ECM remodelling, fibrosis and inflammation have been found in several cardiovascular diseases and are summarised below (Figure 3).

1982.1 Cathepsin K in ECM remodeling

In myocardial infarction (MI), fibrosis can be both protective or damaging depending on the stage of cardiac dysfunction [85]. While fibrosis can impair ventricular compliance leading to systolic and diastolic dysfunction in late stages of myocardial remodelling, in the early remodelling phase, fibrosis and scar formation may limit cardiac damage and prevent cardiac rupture [85]. Studies have shown that whole body cathepsin K knockout mice have increased myocardial fibrosis and impaired cardiac function following MI, with increased collagen deposition but no change in collagen synthesis [86]. Cathepsin K knockout mice demonstrated decreased type-III collagenase activity and increased numbers of myofibroblasts in infarcted tissue which likely explains the enhanced myocardial fibrosis [86]. The enhanced fibrotic response observed in cathepsin K knockout mice highlights the prominant role that cathepsin K plays in ECM degradation. Interestingly, however, cathepsin K knockout did not increase inflammatory cell infiltration of the myocardium which suggests that cathepsin K directly degrades the ECM independent of orchestrating inflammatory cell infiltration of the myocardium [86]. In contrast, cathepsin K knockout in a murine model of diabetic cardiomyopathy significantly reduced perivascular fibrosis and the expression of collagen I in the heart, which was associated with improved cardiac function [28]. This discrepancy between studies suggests that cathepsin K plays different roles in ECM degradation dependent on cardiac disease aetiology. It is likely that this context specificity originates from the ability of cathepsin K to alter different ECM signaling pathways and key proteins (e.g. TGF-β) the importance of which differ between cardiac diseases. Further studies to delineate such interactions between cathepsin K and ECM signalling pathways is warranted to ensure the full therapeutic potential (and side effects) of cathepsin K can be realised.

2202.2 Cathepsin B in ECM remodeling

Fewer studies have established a link between cathepsin B and pathological ECM remodeling in the heart. However, whole body cathepsin B knockout can significantly decrease perivascular and interstitial fibrosis in a mouse model of pressure overload [35]. Interestingly, cathepsin B has been shown to be involved in the processing of TGF-β and subsequent differentiation of lung fibroblasts [87]. To the best of our knowledge the role that cathepsin B plays in TGF-β signaling in the heart remains unknown and warrants further investigation. Additionally the role that cathepsin B may play in ECM remodeling in other cardiac diseases remains unknown.

2.3 Cathepsin L in ECM remodeling

Several studies have shown that cathepsin L is intimately involved in ECM remodeling in cardiovascular disease. Importantly, whole body cathepsin L knockout mice have been shown to develop a dilated cardiomyopathy phenotype at 1 year of age and in addition to characteristic systolic dysfunction, these mice have extensive interstitial fibrosis [88,89]. In MI, cathepsin L has been shown to contribute to adaptive cardiac remodeling [36]. MI in cathepsin L knockout mice was associated with increased adverse cardiac remodeling, cardiac dysfunction and poorer scar healing due to decreased fibrosis and myofibroblast differentiation [36]. Furthermore, cathepsin L knockout in this in vivo model of MI led to reduced myocardial infiltration of monocytes, natural killer cells and c-kit positive cells, in addition to reduced expression of granulocyte-colony stimulating factor, stromal cell-derived factor-1 and stem cell factor, all of which are chemoattractant for inflammatory cells [36]. This suggests that cathepsin L is directly involved in attracting inflammatory cells to stabilize scar formation post-MI.

2.4 Cathepsin S in ECM remodeling

Cathepsin S has been shown to directly interact with inflammatory cells and inflammatory signaling pathways, which leads to myocardial fibrosis. Overall, studies suggest that cathepsin S may regulate scar formation in the heart and that an absence of cathepsin S is associated with uncontrolled fibrosis [90]. In studies utilizing murine models of angiotensin II-induced hypertension, cathepsin S expression was significantly increased in macrophages within the heart [37,90]. Angiotensin II increased cardiac fibrosis and the expression of TGF-β, collagen I, and myofibroblasts, all of which were significantly higher in whole body cathepsin S knockout mice [90]. Furthermore, cathepsin S deficiency was associated with increased macrophage infiltration and increased expression of pro-inflammatory and pro-fibrotic cytokines, TGF-β, IL-1β and TNF-α [90]. This suggests that cathepsin S may not only directly contribute to ECM degredation, but may also regulate inflammatory cell infiltration of the myocardium thereby reducing the production of pro-fibrotic cytokines in the heart. However, the exact mechanisms by which cathepsin S can orchestrate and modulate inflammatory responses in the heart remains unexplored and warrants further study.

In another study, the effect of pharmacological inhibition of cathepsin S with a nonselective cathepsin inhibitor, E64d, was explored in an in vivo murine model of MI [37]. Cathepsin S expression was significantly increased in infarcted tissue post-MI, with macrophage and CD4+ T cell infiltration of infarcted tissues significantly increasing following cathepsin S inhibition, as well as the expression of pro-inflammatory cytokines IL-1β, IL-6, IFN-γ, TNF-α and MCP-1 [37]. Importantly, cathepsin S inhibition prevented fibroblast differentiation and increased post-MI cardiac fibrosis,

and this was associated with systolic dysfunction [37]. Further study using isolated fibroblasts from cathepsin S knockout mice, showed that cathepsin S inhibition/knockout was associated with decreased cellular/myocardial Smad2 and Smad3 phosphorylation in response to TGF-β treatment, and an associated decrease in myofibroblast differentiation, which suggests that cathepsin S can interact directly with the TGF-β signaling pathway to regulate ECM remodeling in the heart [37]. Overall, research to date suggests that cathepsin S plays an important role in ECM remodeling and the regulation of cardiac fibrosis. Similar to cathepsin L, restoration of myocardial cathepsin S expression has the potential to decrease pathological ECM remodeling in cardiovascular disease. Importantly, therapeutic interventions targeting cysteine cathepsins should preferentially preserve the initial adaptive fibrotic response but disrupt chronic fibrosis which impairs cardiac function. Therefore, both the extent and timing of cathepsin inhibition will have important implications on patient outcome.

3 Cysteine cathepsin involvement in cardiac hypertrophy

Cardiac hypertrophy involves an increase in cardiomyocyte protein synthesis, altered sarcomeric organisation and an overall increase in cardiomyocyte size [91]. Cardiac hypertrophy is a feature of multiple cardiac diseases such as valvular dysfunction, hypertension, coronary arterial disease and myocardial failure [91]. Morphological changes accompanying cardiac hypertrophy such as increased ventricular dimensions can directly contribute to impaired cardiac function, with left ventricular hypertrophy being strongly associated with adverse patient outcome [92,93]. Dependent on the type of hemodynamic load on the heart, cardiac hypertrophy can be eccentric or concentric [94]. Eccentric hypertrophy, where sarcomeres are replicated in series, is characterised by cardiac chamber dilatation and elongation of cardiomyocytes [94]. In contrast, concentric hypertrophy, where sarcomeres are replicated in parallel, results in increased cardiomyocyte width, increased ventricular wall thickness and decreased ventricular luminal diameter in an effort to normalise systolic wall stress [94].

Importantly, several cysteine cathepsins have been shown to interact with molecular mediators of cardiomyocyte hypertrophy [35,38–42]. In the following section we will discuss the role of cysteine cathepsins K, B, L and S in hypertrophic signalling in cardiovascular disease (summarised in Figure 4).

3.1 Cathepsin K in cardiac hypertrophy

Several studies have shown that cathepsin K can exacerbate cardiac hypertrophy. In a model of obesity-associated cardiac hypertrophy, cathepsin K knockout mice were shown to have reduced cardiac and cardiomyocyte hypertrophy, with an associated improvement in cardiac contractile

function [95]. In this study, cathepsin K knockout was associated with reduced expression of pro-hypertrophic proteins NFATc, ANP and GATA binding protein 4 [95]. Furthermore, in an in vivo model of cardiac hypertrophy using abdominal aortic constriction, cathepsin K knockout was additionally shown to reduce cardiac and cardiomyocyte hypertrophy and improve systolic function [38]. In this study, the expression of the hypertrophic marker GATA4 was significantly reduced in cathepsin K knockout mice [38]. Similarly cathepsin K knockout mice had reduced phosphorylation of Akt, acetyl-CoA carboxylase (ACC) and AMP-activated protein kinase (AMPK) [38]. Akt can mediate hypertrophic responses in the heart and in cardiomyocytes by activating mTOR, and it was shown in this study that cathepsin K knockout decreased hypertrophic responses in cardiomyocytes by reducing mTOR signaling [38]. The mTOR signaling pathway has been widely accepted to directly contribute to pathological hypertrophy in the heart [96,97]. This finding was also supported by in vitro studies where plasmid-mediated transfection of the cathepsin K gene into cultured cardiomyocytes induced cardiomyocyte hypertrophy, which was significantly reduced in cardiomyocytes treated with rapamycin, an mTOR inhibitor [38]. Other studies have shown that cathepsin K knockout mice are afforded reduced age-associated cardiomyocyte hypertrophy and that cathepsin K can directly contribute to cardiac hypertrophy in murine models of diabetic cardiomyopathy by activating calcineurin which dephosphorylates NFATc3, thereby inducing the nuclear translocation of NFATc3 to induce the transcription of hypertrophic genes [28,98]. In summary, these studies suggest that cathepsin K directly interacts with several molecular pathways to augment cardiomyocyte hypertrophy. Therapies which decrease cathepsin K activity and expression may have the potential to reduce pathological hypertrophy in the heart thereby improving cardiac function. Future studies should aim to uncover the ways by which cathepsin K can contribute to cardiac hypertrophy in divergent cardiovascular diseases.

3203.2 Cathepsin B in cardiac hypertrophy

Importantly, cathepsin B has been shown to be upregulated in both an in vivo pressure-overload model of cardiac hypertrophy and in an in vitro angiotensin-II mediated model of cardiomyocyte hypertrophy [35]. In vivo studies revealed that cathepsin B knockout mice were protected from cardiac hypertrophy with both grossly decreased ventricular wall dimensions and cardiomyocyte cross-sectional areas [35]. In the same study, in vitro experiments revealed that cathepsin B downregulation in H9c2 cells using a shRNA-cathepsin B silencing lentiviral vector also afforded protection against cardiomyocyte hypertrophy, while lentiviral over-expression of cathepsin B was shown to significantly increase cardiomyocyte size [35]. Furthermore, it was revealed in both in vivo and in vitro studies that cathepsin B deficiency was associated with reduced cardiomyocyte

hypertrophy due to decreased activation of the TNF-α/ASK1/JNK signalling pathway, and that treating cardiomyocytes with SP600125 (a JNK inhibitor) reduced cardiomyocyte hypertrophy by inhibiting ASK1/JNK signalling [35]. In summary and in similarity to cathepsin K, cathepsin B has been shown to directly contribute to cardiac hypertrophy and mechanistically cathepsin B interacts with the TNF-α/ASK1/JNK signalling pathway to augment cardiomyocyte hypertrophy. Given the importance of the NFATc3 and mTOR signalling pathways in cardiac hypertrophy and the aforementioned interactions with cathepsin K, it would be of interest to explore possible interactions between cathepsin B and these signalling pathways within the heart. Overall, current research suggests that therapeutic targeting of cathepsin B has the potential to reduce cardiac hypertrophy thereby improving overall cardiac function.

3413.3 Cathepsin L in cardiac hypertrophy

Unlike other cysteine cathepsins, studies have shown that cathepsin L can suppress cardiomyocyte hypertrophy. Sun *et al*., showed that the expression and activity of cathepsin L was significantly increased in vitro following exposure of cardiomyocytes to phenylephrine, with cathepsin L deficient cardiomyocytes developing significantly increased hypertrophy [99]. Enhanced hypertrophic responses in cardiomyocytes are associated with impaired lysosomal function and autophagy, which reduce protein turnover and further exacerbate hypertrophic responses [99]. Cathepsin L has also been shown to suppress cardiac hypertrophy by interfering with the Akt/GSK3B signaling pathway [39]. In this study, transgenic mice overexpressing cathepsin L had reduced cardiac hypertrophy in response to aortic banding. This was attributed to reduced Akt/GSK3B signaling which was confirmed in in vitro studies where cardiomyocytes overexpressing cathepsin L via adenoviral transduction were treated with angiotensin II [39]. Additionally, both in vivo and in vitro studies revealed that the phosphorylation of mTOR was decreased in transgenic mice/cardiomyocytes overexpressing cathepsin L [39]. As mentioned, Akt can directly and indirectly mediate hypertrophic responses in the heart by activating mTOR kinase which in turn phosphorylates downstream targets leading to an increase in cellular hypertrophy [39]. Therefore, cathepsin L may inhibit cardiac hypertrophy by interfering with Akt and mTOR signaling pathways. Interestingly, unlike other cysteine cathepsins, the literature suggests that cathepsin L suppresses cardiomyocyte hypertrophy. Importantly, most studies have focused on whole body cathepsin L knockout and therefore it is important that future studies explore targeting of cathepsin L specifically within the heart. Additionally, it would be of interest to investigate potential interactions between cathepsin L and the NFATc3 signaling pathway, in addition to studying the role that cathepsin L may play in cardiac hypertrophy in other cardiovascular diseases such as coronary artery disease.

3.4 Cathepsin S in cardiac hypertrophy

The expression of cathepsin S can be markedly increased in the myocardium and in cardiomyocytes of both rats and humans with hypertension induced HF and associated cardiac hypertrophy [100]. In a separate study it was shown that the HMG-CoA reductase inhibitor simvastatin could decrease cardiac hypertrophy in hypercholesterolemic mice in part by reducing the expression of cathepsin S in the heart [101]. The molecular link between cathepsin S and cardiac hypertrophy is unknown and warrants further investigation. For example, it would be interesting and informative for future studies to explore potential interactions between cathepsin S and the aforementioned signaling pathways involved in cardiac hypertrophy such as NFATc3 and mTOR, as well as investigating the role that cathepsin S has in cardiomyocyte hypertrophy in other cardiovascular diseases.

4 Cysteine cathepsins and calcium handling dysfunction

Excitation-contraction (E-C) coupling is the process that links the electrical excitation of cardiomyocytes and their contractility [102]. The process of E-C coupling is well defined where depolarization of the sarcolemma opens L-type calcium channels and facilitates calcium entry into the cell. This increase in intracellular calcium interacts with and opens the ryanodine receptor located on the surface of the sarcoplasmic reticulum (SR) leading to calcium release from the SR and a transient rise in cytosolic calcium [102]. The binding of calcium to cardiac troponin facilitates cardiomyocyte contractility during systole. During diastole, calcium dissociates from cardiac troponin and is pumped back into the SR by the sarco-endoplasmic reticulum calcium ATPase (SERCA2a) or moves out of the cell predominantly via the sodium-calcium exchanger (NCX) [102,103]. Phospholamban (PLB) regulates SERCA2a activity whereby phosphorylation of PLB relieves its inhibitory effect on SERCA2a activity [104]. In HF, impaired cardiac contractility is often associated with a reduction in calcium transient amplitude, prolonged calcium transient decay and increased calcium transient duration [105–109] due to altered expression/activity of calcium handling proteins [105,110–112].

While several studies have associated dysregulated cysteine cathepsin activity with impaired cardiac contractility [28,38,113], only a few studies have demonstrated a direct effect of cysteine cathepsins on calcium handling protein expression and activity in cardiomyocytes. In the following section we will discuss the role of cysteine cathepsins K and L in calcium handling dysfunction in cardiovascular disease (summarised in Figure 5). To the best of our knowledge the role that cathepsins B and S play

in E-C coupling and calcium handling protein expression and activity in cardiovascular disease is unknown and warrants further investigation.

4.1 Cathepsin K and calcium handling dysfunction

Guo et al., showed that cathepsin K knockout mice were afforded better systolic function in a murine model of diabetic cardiomyopathy [28]. Importantly in this study cathepsin K was shown to directly contribute to murine diabetic-induced cardiomyopathy by activating calcineurin and inducing nuclear translocation of NFAT, resulting in the transcription of hypertrophic and pro-apoptotic genes [28]. Intriguingly, the same cathepsin K knockout model was associated with reduced left ventricular wall thinning, chamber dilatation, interstitial fibrosis and cardiomyocyte hypertrophy [28]. Isolated cardiomyocytes from mice with diabetic cardiomyopathy demonstrated contractile dysfunction, reduced intracellular calcium decay rates and depressed calcium transients in response to electrical stimulus, with such calcium handling abnormalities being normalized in cathepsin K knockout mice [28]. Importantly calcineurin is involved in regulating several ion channels including L-type calcium channels, ryanodine receptors and SERCA2a and therefore cathepsin K mediated alterations in calcineurin activity may potentially contribute to calcium handling dysfunction in cardiomyocytes [114].

Prolonged diastolic calcium transients and increases in end-diastolic calcium cytosolic concentrations are hallmarks of congestive heart failure and one of the most important reasons for this is due to reduced SR calcium uptake caused by SERCA2a dysfunction [110,111]. Importantly, mice with diabetic cardiomyopathy showed reduced protein expression of SERCA2a and decreased phosphorylation of phospholamban, both of which were normalized in cathepsin K knockout mice [28]. The combination of decreased SERCA2a protein expression and impaired SERCA2a pump activity (decreased phospholamban phosphorylation) is likely to have resulted in reduced SR calcium uptake during diastole thereby contributing to cytosolic calcium overload. Additionally, a later study by Guo et al., studied the effects of cathepsin K on cardiac function using a murine model of doxorubicin-induced cardiotoxicity [31]. Doxorubicin treated mice had impaired systolic function and evidence of adverse cardiac remodeling, both of which were normalized in cathepsin K knockout mice [31]. Isolated cardiomyocytes from doxorubicin treated, cathepsin K knockout mice had normalized contractility, intracellular calcium decay rates and intracellular calcium rise in response to electrical stimulation which further supports the idea that cathepsin K can contribute to dysfunctional calcium handling in cardiac disease [31]. Cathepsin K was further shown to contribute to calcium handling dysfunction in a murine model of obesity-associated cardiac disease [95]. In this

study, cardiomyocytes isolated from cathepsin K knockout mice had improved contractile function and normalised calcium handling [95]. In addition cathepsin K knockout animals had increased SERCA2a and phospholamban expression which supports aforementioned findings that cathepsin K can modulate calcium handling protein expression [95]. Overall the literature suggests that cathepsin K can directly impair calcium handling in the heart by decreasing calcium handling protein activity and expression. Therefore, therapeutic silencing of cathepsin K specifically within the heart has the potential to significantly improve cardiac contractility. In addition, assessing the role that cathepsin K may play in calcium handling dysfunction in other cardiovascular diseases may well prove informative.

3.2 Cathepsin L and calcium handling dysfunction

The role that other cathepsins play in calcium handling dysfunction remains largely unknown. However, cathepsin L derived from African trypanosomes and more recently mammalian cathepsin L have been shown to modulate calcium handling activity, resulting in cytosolic calcium overload and reduced cytosolic calcium removal. For instance, cathepsin L from Trypanosoma brucei has been shown to modulate SR function leading to increased calcium leakage from the SR, which contributed to increased calcium wave release frequency and cardiac arrhythmias [115,116]. Although little is known about the effects of mammalian cathepsin L on calcium handling in the heart, He et al., showed that the cathepsin L inhibitor CAA0225 could significantly improve left ventricular fractional shortening following murine in vivo ischaemia-reperfusion (IR) injury [32]. Furthermore, CAA0225 normalised NCX and SERCA2a activity in isolated rat cardiomyocytes with ex-vivo IR injury [32]. This suggests that cathepsin L can directly interfere with the activity of calcium handling proteins, contributing to cytosolic calcium overload and contractile dysfunction in cardiomyocytes. Therefore therapeutic inhibition of cathepsin L, similar to cathepsin K, has the potential to improve cardiac function via improved calcium handling in cardiomyocytes.

5 Cysteine cathepsins and cellular apoptosis

Apoptosis is a well-characterized form of programmed cell death [117–119]. This evolutionary conserved process can be mediated by two distinct pathways: the extrinsic, death receptor pathway initiated by external stimuli and the intrinsic, mitochondrial-mediated pathway which is initiated by internal stimuli [117–119]. Apoptosis has distinct phases and leads to morphological cellular alterations such as reduced cellular volume, chromosomal condensation, DNA fragmentation and apoptotic body formation [117–119]. Both intrinsic and extrinsic pathways ultimately result in the activation of caspases which degrade intracellular proteins [117–119]. Furthermore, apoptosis is

tightly controlled by BCL-2 family proteins which contain both pro- and anti-apoptotic proteins to balance cellular fate between survival and death [120]. In the heart, apoptosis of myocardial cells is a prominent feature of adverse cardiac remodeling [5,121].

Importantly, several cysteine cathepsins including cathepsin K and B have been shown to interact with various molecular mediators of apoptosis, leading to programmed death of cardiomyocytes [29–31]. One possible mechanism underlying cathepsin-mediated cellular apoptosis is lysosomal membrane permeabilization (LMP) which facilitates cytosolic translocation of cathepsins [122]. LMP also releases Bid which has been shown to be cleaved and activated into pro-apoptotic tBid, by cysteine cathepsins L, B, S and K [123,124]. tBid can activate Bax and induce cytochrome c release from the mitochondria resulting in caspase-3 and -9 activation [30]. In the following section, we will discuss the role of cysteine cathepsins K, B, L and S in apoptotic signalling in cardiovascular disease (summarised in Figure 6).

5.1 Cathepsin K and apoptosis

Cathepsin K has been shown to directly contribute to murine diabetic induced cardiomyopathy by activating calcineurin and inducing nuclear translocation of NFAT, resulting in the transcription of hypertrophic and pro-apoptotic genes [28]. In a separate study, augmented cathepsin K expression was observed in control animals following doxorubicin injection and correlated with a decrease in fractional shortening, decreased wall thickness and an overall decline in cardiac function [31]. Control animals given doxorubicin showed an increase in cardiomyocyte NFkB-p65 phosphorylation, which was normalised in cathepsin K knockout models [31]. This suggests that doxorubicin-induced cardiotoxicity may in part be mediated by cathepsin K which may enhance nuclear translocation of NF-kB leading to an increase in cardiomyocyte apoptosis, with enhanced transcription of tissue-damaging, pro-inflammatory cytokines such as IL-6 [31]. Furthermore, in a study by Hua et al., it was shown that aging mice (24 months of age) exhibited more significant cardiac remodelling, prolonged cardiomyocyte lengthening, decreased intracellular calcium release and diminished cardiac contractility in a model of age-induced cardiac dysfunction [98]. These changes were significantly ameliorated in global cathepsin K knockout mice and it was shown in separate complementary experiments using H9c2 cells (a rat cardiomyoblast cell line which is used as a model of various properties of cardiomyocytes) which were stimulated with doxorubicin to induce cellular apoptosis, that cathepsin K silencing by siRNA inhibited the nuclear translocation of Apoptosis Inducible Factor (AIF), reducing cardiomyocyte caspase-independent and dependent apoptosis [98]. Overall, cathepsin K can directly contribute to cardiomyocyte apoptosis and therefore targeting a reduction

of cathepsin K has the potential to reduce cardiomyocyte death and consequently improve overall cardiac function. However, further study is required to elucidate the role that cathepsin K may play in other cardiovascular diseases and to establish potential interactions between cathepsin K and specific apoptotic signalling pathways.

5045.2 Cathepsin B and apoptosis

At the cellular level, apoptosis of interstitial cells and cardiomyocytes is a significant feature of dilated cardiomyopathy in humans [29]. Ge et al., showed that cathepsin B is involved in cardiomyocyte apoptosis and the degradation of myofibrillar proteins in MI [27]. This study further showed that cathepsin B expression was increased in failing myocardium from patients with DCM compared with healthy controls, and that the levels of cathepsin B positively correlated with the magnitude of cardiomyocyte apoptosis [27]. The study suggested that cathepsins contribute to dilated cardiomyopathy and mechanistically may achieve this by initiating cellular apoptosis, in addition to remodelling the ECM leading to augmented interstitial fibrosis [125,126]. Additionally, cathepsin B knockout was shown to ameliorate hypertrophy, fibrosis and, apoptosis in the murine heart following aortic banding and pressure overload resulting in reduced activation of TNF-alpha, ASK1 and cytochrome c [127]. Similar findings (reduced hypertrophy and apoptosis) were observed in an *in vitro* cardiomyocyte model of pressure overload using angiotensin II [127]. Furthermore, in a murine model of viral myocarditis induced by coxsackievirus B3 infection, cathepsin B knockout mice were shown to incur significant reductions in inflammasome mediated pyroptosis [128]. This finding was further strengthened by the fact that cystatin c knockout mice had increased inflammasome-initiated pyroptosis with a concomitant increased severity of disease pathology [128]. Overall, and in similarity to cathepsin K, the literature suggest that cathepsin B can directly contribute to cardiomyocyte apoptosis in several different models of cardiovascular disease, suggesting that therapeutic targeting of cathepsin B may have the potential to improve cardiac function by decreasing cardiomyocyte apoptosis. Importantly, current reserach suggests that cathepsin B can interact with several molecular mediators of apoptosis such as ASK1 and cytochrome c. However, it will be important for future studies to uncover any potential discrepencies between the effects of global cathepsin B knockout and targeting of cathepsin B-dependent apoptosis in cardiomyocytes.

5295.3 Cathepsin L and apoptosis

The role that cathepsin L plays in apoptotic signalling in the heart remains unclear. While cathepsin L can activate tBid inducing apoptosis, studies have also suggested that cathepsin L may exert anti-apoptotic responses in the heart [123,124,129]. At the molecular level cathepsin L overexpression in

cardiomyocytes has been shown to suppress cardiac hypertrophy by interfering with the Akt/GSK3B signaling cascade [42]. In this study, using an aortic banding model of cardiac hypertrophy, transgenic mice overexpressing human cathepsin L were shown to have reduced cardiac hypertrophy, fibrosis and cardiomyocyte apoptosis which was mediated through blockade of Akt signaling [42]. One further observation was that cathepsin L reduced the gene and protein expression of pro-inflammatory cytokines by blunting NF-kB signaling, where NF-kB DNA binding activity, IkB-alpha phosphorylation and degradation were blocked in transgenic mice overexpressing human cathepsin L in comparison to control mice [42]. NF-kB activation has been shown to augment adverse cardiac remodelling and pro-apoptotic signalling in the heart and these results may suggest that cathepsin L mediates anti-apoptotic effects in the heart by decreasing NF-kB signalling [42,129]. However, further study is required to fully elucidate the role of cathepsin L in apoptotic signalling in the heart. In particular, establishing the effects that reducing cathepsin L expression/activity could have on cardiomcyoyte apoptosis, in addition to uncovering potential interactions between cathepsin L and specific apoptotic signalling pathways, would be useful and informative.

5485.4 Cathepsin S and apoptosis

Despite the fact that cathepsin S can activate pro-apoptotic tBid, the role that cathepsin S has in apoptotic signalling in cardiovascular disease is also unclear [123,124]. In a murine model of MI, cathepsin S inhibition was associated with increased adverse cardiac remodelling changes such as increased interstitial fibrosis, left ventricular chamber dilatation and impaired systolic dysfunction but interestingly apoptosis was unaffected [37]. Importantly, this study used a non-selective cathepsin S inhibitor, E64d, and therefore other cathepsins are likely to have been inhibited alongside cathepsin S which may have masked any specific effects that cathepsin S had on cardiac apoptosis [37]. Therefore, future studies should aim to explore the role that cathepsin S has on cardiomyocyte apoptosis via targeted knockdown approaches or using specific cathepsin S inhibitors.

5596 Cathepsins and autophagy

Autophagy is characterized as a catabolic process that facilitates cell survival in the face of pathological insult, be that infectious, toxic or due to metabolic stress [130–133]. Autophagy is a highly conserved and essential process which is integral to normal cellular homeostasis [134]. There are 3 types of autophagy; microautophagy, macroautophagy and chaperone-mediated autophagy [135]. Although these processes are mechanistically distinct, the end result of this process involves

the degradation of intracellular proteins in the lysosome [136,137]. The most common and major pathway in autophagy is macroautophagy, a process where cytosolic components including damaged organelles, dysfunctional and aggregated proteins and intracellular pathogens become incorporated into a double membrane vacuole termed the autophagosome [138,139]. This autophagosome can then merge with lysosomes to form the autophagolysosome which results in intra-vacuole protein degradation which can be recycled by the cell to maintain function [138,139]. Accumulations of abnormal proteins consequential to impaired lysosomal function are believed to contribute to the pathogenesis of cardiac disease [140].

Cysteine cathepsins are key lysosomal proteases and therefore have important roles in degrading proteins in lysosomes which inextricably links cathepsins with the process of autophagy. There is conflicting evidence regarding the role that autophagy may play in adverse cardiac remodeling. Some studies suggest that autophagy is protective against adverse cardiac remodeling and that preservation of autophagy is associated with reduced infarct size, decreased cardiac chamber dilatation and improved contractile function [141–143]. Conversely, however, increased activation of autophagy has been associated with increased cardiomyocyte death and augmented adverse cardiac remodeling consequences such as cardiac chamber dilatation, impaired systolic function and increased interstitial fibrosis [144]. Cathepsin L is the most extensively studied cysteine cathepsin linked with autophagy in cardiovascular disease and therefore this section will mainly focus on cathepsin L . Cathepsins B and S will be briefly discussed in this context but to the best of our knowledge the role that cathepsin K plays in autophagy in cardiovascular disease remains unknown.

5876.1 Role of cathepsin L in autophagy

Cathepsin L has been associated with autophagy in cardiovascular disease and many studies have suggested that cathepsin L can preserve cardiac function by facilitating autophagy [33]. For example, an *in vitro* study showed that phenylephrine induced cardiomyocyte hypertrophy stimulated the expression of cathepsin L [33]. Cardiomyocytes deficient in cathepsin L displayed enhanced hypertrophic responses and diminished cellular viability as a result of impaired lysosomal function with accumulations of autophagosomes and decreased protein degradation, thus suggesting that cathepsin L functions to preserve intracellular function in response to hypertrophic stimuli [33]. Importantly, restoring cathepsin L expression in cardiomyocytes through adeno-associated viral vector-mediated gene transfer restored normal protein degradation [33]. Interestingly, in cathepsin L deficient mice, there was a compensatory upregulation of the aspartic protease, cathepsin D which maintained some degree of lysosomal function in the absence of cathepsin L, which suggests that

cathepsins may work in concert to maintain optimal intracellular function [33]. In mice with a dilated cardiomyopathy phenotype, complete cathepsin L deficiency has been associated with accumulations of material in enlarged lysosomes [145]. An interesting study by Dennemarker et al., showed that a global cathepsin L knockout murine model was associated with impaired degradation of material in autophagolysosomes [146]. In this study, transgenic mice with a GFP tagged autophagy marker, LC3, were crossed with cathepsin L knockout mice and primary mouse embryonic fibroblasts (MEFs) were obtained [146]. Upon induction of autophagy, there was no significant alteration in the formation of autophagic vesicles, in the initiation of autophagy or in the fusion of autophagic vesicles and lysosomes in MEFs [146]. However, co-localisation studies of both Lamp1 and GFP-LC3 in cathepsin L knockout MEFs revealed the presence of abnormally enlarged autophagolysosomes and suggested that an absence of cathepsin L is associated with impaired autophagy [146]. However, while studies suggest that a deficiency of cathepsin L is associated with impaired autophagy related protein turnover, other studies have suggested that cathepsin L has a far more specific role in autophagy [147]. Indeed, in a study by Ueno et al., a cathepsin L specific inhibitor CAA0225 was shown to inhibit the degradation of autophagy related proteins, gamma-aminobutyric acid receptor-associated protein (GABARAP) and Microtubule-Associated Protein 1A/1B-Light Chain 3 II (LC3-II), which suggests that cathepsin L may have a specific role in degrading these autophagosomal membrane proteins [147]. Interestingly, in this study cathepsin L was preferentially associated with the lysosomal membrane rather than the lysosomal lumen [147]. While studies have suggested that cathepsin L is important in the process of autophagy in cardiac disease, other mechanisms by which cathepsin L may regulate autophagy have yet to be elucidated. While it is clear that cathepsin L plays an important role in normal autophagic signaling in the heart, further work is required to determine precisely how cathepsin L mediated autophagic signaling might contribute to adverse cardiac remodeling. It will also be important to assess the effect specific effects of cathepsin L on autophagic signaling in cardiomyocytes and thereby cardiac function.

6256.2 The role of cathepsins B and S in autophagy

Other cathepsin are known to be involved in autophagy and some cathepsins have been shown to have specific roles in this process. For example, Man et al., showed that cathepsin B could cleave the calcium channel Transient Receptor Potential Cation Channel Mucolipin 1 (TRPML1)/Mucolipin-1 (MCOLN1) in lysosomes and suppress transcription factor TFEB which is involved in lysosomal biogenesis, in addition to reducing autophagy related and lysosomal proteins [148]. This suggests that cathepsin B can impair autophagy, although further study is required to establish the role that cathepsin B plays in autophagic signaling in the heart. In an interesting study by Pan et al.,

angiotensin-II induced myocardial fibrosis and inflammation was associated with an increase in macrophage myocardial translocation, reduced mitophagy, and an increase in macrophage autophagosome accumulation in cathepsin S knockout mice [90]. A deficiency of cathepsin S was associated with reduced mitochondrial turnover leading to an increase in damaging oxygen free radicals and NF-kB in macrophages, which exacerbated the disease phenotype [90]. Therefore, in contrast to cathepsin B, this suggests that cathepsin S plays an important role in normal autophagic signalling in the heart. Further work is required to reconcile the contrasting roles that cathepsin B and S may play in autophagy in varying cardiovascular diseases, and in particular to determine how interactions between cathepsin B and S and autophagy may impact on adverse cardiac remodelling and overall cardiac function.

7 Cathepsins as therapeutic targets

Properties of the ideal cardiac biomarker include specificity for cardiac muscle (over skeletal muscle damage) and a high sensitivity to detect not only a small degree of myocardial injury but also the extent/reversibility of damage [149]. Cysteine cathepsins have shown promise as cardiac biomarkers in patients with cardiovascular disease where they are associated with disease severity, prognosis and mortality [150–154].

7.1 Cathepsins K, B, L and S as cardiac biomarkers

Cathepsins L, K and S have significant cardiac biomarker potential in patients with coronary artery disease. Serum cathepsin L levels positively correlated with the degree of coronary arterial stenosis and indeed, cathepsin L levels were shown to be highest in patients with coronary heart disease than those without, and higher in patients with unstable versus stable angina pectoris [155]. Similarly, patients with a history of MI displayed higher serum levels of cathepsin L compared to those with acute MI [155]. In comparison, patients with acute MI had elevated peripheral blood levels of cathepsins B and K with a decrease in their inhibitor cystatin C compared to healthy controls [75]. Another study showed that plasma cathepsin K levels were highest in patients with acute MI compared to those with unstable and stable angina pectoris and non-coronary heart disease controls [86]. Enhanced serum cathepsin S levels have also been documented in patients with atherosclerotic stenosis, patients with acute or previous MI and, unstable angina [156]. Other studies involving stable angina patients have shown that the plasma concentration and activity of cathepsin S is correlated with the levels of atherogenic low-density-lipoprotein (LDL), whereby lowering LDL levels by Atorvastatin lowers cathepsin S concentration [157]. Serum cathepsin S and C-reactive protein levels are both positively correlated with abdominal aortic aneurysm (AAA)

diameter, and combined levels are better in predicting the inflammatory activity of AAA lesions than either alone [158].

Serum concentrations of some cathepsins have been shown to correlate with the development of chronic cardiac disease. For example, studies have shown that serum cathepsin K levels correlate negatively with left ventricular ejection fraction and positively with left ventricular end-diastolic and end-systolic dimensions [150]. In peripheral blood mononuclear cells, the activities of cathepsin B and cathepsin L were negatively correlated with left ventricular ejection fraction in patients diagnosed with idiopathic dilated cardiomyopathy and in such, correlated with the severity of disease [151]. Similarly, young patients (median age 15) with clinically diagnosed hypertrophic cardiomyopathy had significantly higher serum concentrations of cathepsin S than disease-free patients or those who were genetically at high risk of developing hypertrophic cardiomyopathy [152]. Importantly, these serum concentrations were positively correlated with left ventricular mass index and mitral septal E/e' (a measure of left ventricular filling pressure, indicative of diastolic function) [152]. Of note, two clinical studies showed that enhanced serum cathepsin S levels were associated with increased cardiovascular-related mortality, suggesting that inhibition of cathepsins may prove clinically useful [153,154]. Thus, measurement of circulating cathepsins appears to be a promising biomarker to provide a non-invasive method of diagnosing and monitoring the extent of cardiac dysfunction in various cardiac diseases, however, cathepsins have yet to be shown to be superior to the existing gold standard cardiac biomarkers. For myocardial insult these include cardiac troponin I and T, B-type naturetic peptide (BNP) and N-terminal-proBNP (NT-proBNP) [159]. For myocardial remodelling these include C-reactive protein (inflammation) and, soluble ST2 and galectin-3 (hypertrophy/fibrosis) [159]. Indeed, with advances in personalised medicine, a multiplex biomarker approach may be more suitable to predict disease severity and whether cathepsins can contribute to this profile remains unexplored [160].

6927.2 Translational potential of cathepsin inhibitors

As previously mentioned, both secreted and membrane-bound proteases play important roles in ECM degradation and matrix metalloproteinases (MMPs) were long thought to be the main protease involved in exercising this function [161]. Although pharmacological inhibition of MMPs has shown some success in treating diseases where ECM degradation contributes to the disease pathology, such as rheumatoid arthritis and neoplasia [161,162], clinical trials using MMP inhibitors have failed [163]. In recent years it has become clear that MMPs do not contribute to the bulk of ECM degradation [164,165] but rather cysteine cathepsins are the key proteases involved in the

degradation and subsequent repair of the ECM [166]. It is therefore conceivable that cysteine cathepsins may represent better pharmacological targets than MMPs to ameliorate pathological ECM remodeling.

E64d, a broad spectrum and irreversible cathepsin inhibitor, has shown potential in ameliorating cardiac dysfunction through cathepsin S, B and K inhibition [167]. E64d reduces cathepsin induced elastolytic activity in the left ventricle of rats with hypertensive cardiac failure, and the severity of left ventricular fibrosis, hypertrophy and coronary remodelling were all ameliorated [25]. As previously highlighted and in contrast, inhibition of cathepsin S by E64d in mice with MI lead to increased scar formation in the ischaemic myocardium and suppressed fibroblast differentiation, ultimately leading to defective ECM formation [168]. The cathepsin inhibitor, K11777, has shown effectiveness in the treatment of parasitic infectious diseases such as schistosomiasis and toxoplasmosis, through targeting cathepsin B and L [116,169,170]. K11777 protected T.cruzi-infected dogs from cardiac dysfunction, as demonstrated by a reduction in serum cardiac troponin I levels and histopathological lesion scores [171].

A selective, reversible and potent cathepsin K inhibitor (IC50 6 nM), cathepsin K inhibitor II, was effective at reducing doxorubicin-induced cardiotoxicity in isolated adult murine cardiomyocytes [31]. The specific cathepsin B inhibitor, CA-074Me, has been shown to reduce cardiac dysfunction, cardiomyocyte hypertrophy and fibrosis following MI in Sprague-Dawley rats [172]. Interestingly, CA-074Me not only reduced cathepsin B activity but also reduced NLRP3 inflammasome activation [172]. Generally, cathepsin L inhibitors have poor selectivity and potency, however, compound SID 26681509 reversibly bound to cathepsin L with high potency (IC50 1nM) [173]. Indeed, this compound had a 7-50-fold selectivity for cathepsin L (over cathepsins B and S), suggesting that this compound may be useful in treating cardiovascular disease [173]. Another cathepsin L inhibitor, CAA0225, is a selective irreversible and cell permeable compound that has demonstrated significant inhibition of cathepsin L activity in rat liver (IC50 1.9nM) while leaving cathepsin B activity intact [174]. CAA0225 is effective at reducing cathepsin L activity during ischaemia-reperfusion in ex vivo adult rat hearts and in so doing improves both systolic and diastolic cardiac function [175].

Clinical trials of different cathepsin inhibitors have had variable levels of success to date. A number of phase I clinical trials of cathepsin S inhibitors have been halted, although one inhibitor, RWJ-445380, has been assessed in phase IIa trials for rheumatoid arthritis [176]. Although a cathepsin B inhibitor, VBY-376 (Virobay), went through phase I trials after showing preclinical effectiveness in

liver injury, there is no public data to demonstrate the efficacy of the drug [176]. On the other hand, cathepsin K inhibitors have shown significant promise in phase I-III clinical trials for treating osteoporosis and osteoarthritis. The cathepsin K inhibitor, L-873724 (Merck), is a non-lysosomotropic, potent and selective (>800-fold over other cathepsins) inhibitor, that was shown to suppress bone resorption in rabbit and rhesus monkey [177]. Due to the short half-life and clearance (Cl = 7.5 mL/min/kg) of L-873724 in monkeys, a modification of the L-873724 compound was designed (odanacatib or MK-0822) to make it metabolicaly stable thereby increasing the selectivity for cathepsin K [178]. Indeed, odanacatib has been assessed in several phase II and III trials of osteoporosis in both males and females with positive outcomes such as increasing bone density and reducing bone turnover, thus reducing the risk of fractures [179] [176,180]. However, the development of odanacatib was discontinued by Merck due to patients displaying an increased risk of stroke and atrial fibrillation [181]. Similarly, the cathepsin K inhibitor Balicatib (AAE581; Novartis), that although displayed >100-fold selectivity for cathepsin K over other cathepsins, and reduced bone resorption in an osteoporosis trial [179], was found to accumulate in lysosomes and cause morphea-like (localised scleroderma) skin reactions; therefore trials were discontinued [182]. A more promising cathepsin K inhibitor is ONO-5334 (Ono Pharma). In the phase II trial (the OCEAN study), ONO-5334 significantly increased areal bone mineral density in the hip and spine without altering bone size in patients with postmenopausal osteoporosis and, this effect was persistent over 2 years [183]. Nevertheless, to date there have been no clinical trials using cathepsin inhibitors in cardiac or cardiovascular diseases despite their importance in adverse cardiac remodelling.

Conclusions

As discussed, the cysteine cathepsins K, B, L and S play critical roles in the process of adverse cardiac remodelling and contractile dysfunction following myocardial insult. They do this via manipulating ECM synthesis and degradation, by modulating the inflammatory response, altering cardiac hypertrophy, calcium handling signalling as well as the processes of apoptosis and autophagy. Given that cathepsin activity/expression is altered during cardiac disease and serum concentrations of some cathepsins correlate with the development/severity of cardiac disease, it is clear that more work is required to realise the therapeutic potential of cysteine cathepsins to prevent adverse cardiac remodelling.

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References

[1] K.T. Weber, Extracellular matrix remodeling in heart failure: A role for de novo angiotensin II generation, Circulation. 96 (1997) 4065–4082. https://doi.org/10.1161/01.CIR.96.11.4065.

[2] J.S. Burchfield, M. Xie, J.A. Hill, Pathological ventricular remodeling: Mechanisms: Part 1 of 2, Circulation. 128 (2013) 388–400. https://doi.org/10.1161/CIRCULATIONAHA.113.001878.

[3] E. Mezzaroma, S. Toldo, D. Farkas, I.M. Seropian, B.W. Van Tassell, F.N. Salloum, H.R. Kannan, A.C. Menna, N.F. Voelkel, A. Abbate, The inflammasome promotes adverse cardiac remodeling following acute myocardial infarction in the mouse, Proc. Natl. Acad. Sci. U. S. A. 108 (2011) 19725–19730. https://doi.org/10.1073/pnas.1108586108.

[4] L. Schirone, M. Forte, S. Palmerio, D. Yee, C. Nocella, F. Angelini, F. Pagano, S. Schiavon, A. Bordin, A. Carrizzo, C. Vecchione, V. Valenti, I. Chimenti, E. De Falco, S. Sciarretta, G. Frati, A Review of the Molecular Mechanisms Underlying the Development and Progression of Cardiac Remodeling, Oxid. Med. Cell. Longev. 2017 (2017) 3920195. https://doi.org/10.1155/2017/3920195.

[5] P.S. Azevedo, B.F. Polegato, M.F. Minicucci, S.A.R. Paiva, L.A.M. Zornoff, Cardiac Remodeling: Concepts, Clinical Impact, Pathophysiological Mechanisms and Pharmacologic Treatment, Arq. Bras. Cardiol. 106 (2016) 62–69. https://doi.org/10.5935/abc.20160005.

[6] C.S. McCarroll, W. He, K. Foote, A. Bradley, K. McGlynn, F. Vidler, C. Nixon, K. Nather, C. Fattah, A. Riddell, P. Bowman, E.B. Elliott, M. Bell, C. Hawksby, S.M. MacKenzie, L.J. Morrison, A. Terry, K. Blyth, G.L. Smith, M.W. McBride, T. Kubin, T. Braun, S.A. Nicklin, E.R. Cameron, C.M. Loughrey, Runx1 deficiency protects against adverse cardiac remodeling after myocardial infarction, Circulation. 137 (2018) 57–70. https://doi.org/10.1161/CIRCULATIONAHA.117.028911.

[7] M. Arrigo, M. Jessup, W. Mullens, N. Reza, A.M. Shah, K. Sliwa, A. Mebazaa, Acute heart failure, Nat. Rev. Dis. Prim. 6 (2020) 16. https://doi.org/10.1038/s41572-020-0151-7.

[8] S.S. Virani, A. Alonso, E.J. Benjamin, M.S. Bittencourt, C.W. Callaway, A.P. Carson, A.M. Chamberlain, A.R. Chang, S. Cheng, F.N. Delling, L. Djousse, M.S.V. Elkind, J.F. Ferguson, M. Fornage, S.S. Khan, B.M. Kissela, K.L. Knutson, T.W. Kwan, D.T. Lackland, T.T. Lewis, J.H. Lichtman, C.T. Longenecker, M.S. Loop, P.L. Lutsey, S.S. Martin, K. Matsushita, A.E. Moran,

M.E. Mussolino, A.M. Perak, W.D. Rosamond, G.A. Roth, U.K.A. Sampson, G.M. Satou, E.B. Schroeder, S.H. Shah, C.M. Shay, N.L. Spartano, A. Stokes, D.L. Tirschwell, L.B. VanWagner, C.W. Tsao, S.S. Wong, D.G. Heard, Heart disease and stroke statistics—2020 update: A report from the American Heart Association, Circulation. 141 (2020) E139–E596. https://doi.org/10.1161/CIR.0000000000000757.

[9] G. Savarese, L.H. Lund, Global Public Health Burden of Heart Failure, Card. Fail. Rev. 03 (2017) 7. https://doi.org/10.15420/cfr.2016:25:2.

[10] D.M. Henkel, M.M. Redfield, S.A. Weston, Y. Gerber, V.L. Roger, Death in heart failure: a community perspective., Circ. Heart Fail. 1 (2008) 91–97. https://doi.org/10.1161/CIRCHEARTFAILURE.107.743146.

[11] A. Riddell, M. McBride, T. Braun, S.A. Nicklin, E. Cameron, C.M. Loughrey, T.P. Martin, RUNX1: an emerging therapeutic target for cardiovascular disease, Cardiovasc. Res. 116 (2020) 1410–1423. https://doi.org/10.1093/cvr/cvaa034.

[12] M. Hohl, M. Mayr, L. Lang, A. Nickel, J. Barallobre Barreiro, X. Yin, T. Speer, S.R. Selejan, C. Goettsch, K. Erb, C. Fecher-Trost, J.-C. Reil, B. Linz, S. Ruf, T. Hübschle, C. Maack, M. Böhm, T. Sadowski, D. Linz, Cathepsin A contributes to left ventricular remodeling by degrading extracellular superoxide dismutase in mice, J. Biol. Chem. 31 (2020) jbc.RA120.013488. https://doi.org/10.1074/jbc.RA120.013488.

[13] N.D. Rawlings, A.J. Barrett, P.D. Thomas, X. Huang, A. Bateman, R.D. Finn, The MEROPS database of proteolytic enzymes, their substrates and inhibitors in 2017 and a comparison with peptidases in the PANTHER database, Nucleic Acids Res. 46 (2018) D624–D632. https://doi.org/10.1093/nar/gkx1134.

[14] V. Turk, V. Stoka, O. Vasiljeva, M. Renko, T. Sun, B. Turk, D. Turk, Cysteine cathepsins: From structure, function and regulation to new frontiers, Biochim. Biophys. Acta - Proteins Proteomics. 1824 (2012) 68–88. https://doi.org/10.1016/j.bbapap.2011.10.002.

[15] R.W. Mason, Emerging Functions of Placental Cathepsins, Placenta. 29 (2008) 385–390. https://doi.org/10.1016/j.placenta.2008.02.006.

[16] H.J. Salminen-Mankonen, J. Morko, E. Vuorio, Role of cathepsin K in normal joints and in the development of arthritis., Curr. Drug Targets. 8 (2007) 315–23.

[17] C. Linnevers, S.P. Smeekens, D. Brömme, Human cathepsin W, a putative cysteine protease predominantly expressed in CD8+ T-lymphocytes., FEBS Lett. 405 (1997) 253–9.

[18] T. Wex, H. Wex, R. Hartig, S. Wilhelmsen, P. Malfertheiner, Functional involvement of cathepsin W in the cytotoxic activity of NK-92 cells., FEBS Lett. 552 (2003) 115–9.

[19] D. Brömme, Z. Li, M. Barnes, E. Mehler, Human Cathepsin V Functional Expression, Tissue

Distribution, Electrostatic Surface Potential, Enzymatic Characterization, and Chromosomal Localization ‡, Biochemistry. 38 (1999) 2377–2385. https://doi.org/10.1021/bi982175f.

[20] L.C. Hsing, A.Y. Rudensky, The lysosomal cysteine proteases in MHC class II antigen presentation, Immunol. Rev. 207 (2005) 229–241. https://doi.org/10.1111/j.0105-2896.2005.00310.x.

[21] G. Velasco, A.A. Ferrando, X.S. Puente, L.M. Sánchez, C. López-Otín, Human cathepsin O. Molecular cloning from a breast carcinoma, production of the active enzyme in Escherichia coli, and expression analysis in human tissues., J. Biol. Chem. 269 (1994) 27136–42.

[22] B. Turk, V. Turk, D. Turk, Structural and functional aspects of papain-like cysteine proteinases and their protein inhibitors., Biol. Chem. 378 (n.d.) 141–50.

[23] B. Turk, D. Turk, G.S. Salvesen, Regulating cysteine protease activity: essential role of protease inhibitors as guardians and regulators., Curr. Pharm. Des. 8 (2002) 1623–37.

[24] X.W. Cheng, K. Obata, M. Kuzuya, H. Izawa, K. Nakamura, E. Asai, T. Nagasaka, M. Saka, T. Kimata, A. Noda, K. Nagata, H. Jin, G.-P. Shi, A. Iguchi, T. Murohara, M. Yokota, Elastolytic Cathepsin Induction/Activation System Exists in Myocardium and Is Upregulated in Hypertensive Heart Failure, Hypertension. 48 (2006) 979–987. https://doi.org/10.1161/01.HYP.0000242331.99369.2f.

[25] X.W. Cheng, T. Murohara, M. Kuzuya, H. Izawa, T. Sasaki, K. Obata, K. Nagata, T. Nishizawa, M. Kobayashi, T. Yamada, W. Kim, K. Sato, G.-P. Shi, K. Okumura, M. Yokota, Superoxide-dependent cathepsin activation is associated with hypertensive myocardial remodeling and represents a target for angiotensin II type 1 receptor blocker treatment., Am. J. Pathol. 173 (2008) 358–69. https://doi.org/10.2353/ajpath.2008.071126.

[26] E. Rabkin, M. Aikawa, J.R. Stone, Y. Fukumoto, P. Libby, F.J. Schoen, Activated interstitial myofibroblasts express catabolic enzymes and mediate matrix remodeling in myxomatous heart valves., Circulation. 104 (2001) 2525–32.

[27] J. Ge, G. Zhao, R. Chen, S. Li, S. Wang, X. Zhang, Y. Zhuang, J. Du, X. Yu, G. Li, Y. Yang, Enhanced myocardial cathepsin B expression in patients with dilated cardiomyopathy., Eur. J. Heart Fail. 8 (2006) 284–9. https://doi.org/10.1016/j.ejheart.2005.09.004.

[28] R. Guo, Y. Hua, O. Rogers, T.E. Brown, J. Ren, S. Nair, Cathepsin K knockout protects against cardiac dysfunction in diabetic mice, Sci. Rep. 7 (2017) 8703. https://doi.org/10.1038/s41598-017-09037-z.

[29] B.K. Hong, H.M. Kwon, K.H. Byun, D. Kim, E.Y. Choi, T.S. Kang, S.M. Kang, K.J. Chun, Y. Jang, H.S. Kim, M. Kim, Apoptosis in dilated cardiomyopathy, Korean J. Intern. Med. 15 (2000) 56–64. https://doi.org/10.3904/kjim.2000.15.1.56.

[30] V. Stoka, B. Turk, S.L. Schendel, T.H. Kim, T. Cirman, S.J. Snipas, L.M. Ellerby, D. Bredesen, H. Freeze, M. Abrahamson, D. Brömme, S. Krajewski, J.C. Reed, X.M. Yin, V. Turk, G.S. Salvesen, Lysosomal protease pathways to apoptosis: Cleavage of Bid, not pro-caspases, is the most likely route, J. Biol. Chem. 276 (2001) 3149–3157. https://doi.org/10.1074/jbc.M008944200.

[31] R. Guo, Y. Hua, J. Ren, K.E. Bornfeldt, S. Nair, Cardiomyocyte-specific disruption of Cathepsin K protects against doxorubicin-induced cardiotoxicity article, Cell Death Dis. 9 (2018) 692. https://doi.org/10.1038/s41419-018-0727-2.

[32] W. He, C. McCarroll, K. Nather, E. Elliott, S. Nicklin, C. Loughrey, 1 The cathepsin-L inhibitor CAA0225 protects against myocardial ischaemia-reperfusion injury, Heart. 101 (2015) A1.1-A1. https://doi.org/10.1136/heartjnl-2015-308734.1.

[33] M. Sun, M. Ouzounian, G. de Couto, M. Chen, R. Yan, M. Fukuoka, G. Li, M. Moon, Y. Liu, A. Gramolini, G.J. Wells, P.P. Liu, Cathepsin-L ameliorates cardiac hypertrophy through activation of the autophagy-lysosomal dependent protein processing pathways., J. Am. Heart Assoc. 2 (2013) e000191. https://doi.org/10.1161/JAHA.113.000191.

[34] E. Lutgens, S.P.M. Lutgens, B.C.G. Faber, S. Heeneman, M.M.J. Gijbels, M.P.J. De Winther, P. Frederik, I. Van Der Made, A. Daugherty, A.M. Sijbers, A. Fisher, C.J. Long, P. Saftig, D. Black, M.J.A.P. Daemen, K.B.J.M. Cleutjens, Disruption of the Cathepsin K gene reduces atherosclerosis progression and induces plaque fibrosis but accelerates macrophage foam cell formation, Circulation. 113 (2006) 98–107. https://doi.org/10.1161/CIRCULATIONAHA.105.561449.

[35] Q.-Q. Wu, M. Xu, Y. Yuan, F.-F. Li, Z. Yang, Y. Liu, M.-Q. Zhou, Z.-Y. Bian, W. Deng, L. Gao, H. Li, Q.-Z. Tang, Cathepsin B deficiency attenuates cardiac remodeling in response to pressure overload via TNF-α/ASK1/JNK pathway, Am. J. Physiol. Circ. Physiol. 308 (2015) H1143–H1154. https://doi.org/10.1152/ajpheart.00601.2014.

[36] M. Sun, M. Chen, Y. Liu, M. Fukuoka, K. Zhou, G. Li, F. Dawood, A. Gramolini, P.P. Liu, Cathepsin-L contributes to cardiac repair and remodelling post-infarction, Cardiovasc. Res. 89 (2011) 374–383. https://doi.org/10.1093/cvr/cvq328.

[37] H. Chen, J. Wang, M.X. Xiang, Y. Lin, A. He, C.N. Jin, J. Guan, G.K. Sukhova, P. Libby, J.A. Wang, G.P. Shi, Cathepsin S-mediated fibroblast trans-differentiation contributes to left ventricular remodelling after myocardial infarction, Cardiovasc. Res. 100 (2013) 84–94. https://doi.org/10.1093/cvr/cvt158.

[38] Y. Hua, X. Xu, G.P. Shi, A.J. Chicco, J. Ren, S. Nair, Cathepsin K knockout alleviates pressure overload-induced cardiac hypertrophy, Hypertension. 61 (2013) 1184–1192. https://doi.org/10.1161/HYPERTENSIONAHA.111.00947.

[39] Q. Tang, J. Cai, D. Shen, Z. Bian, L. Yan, Y.X. Wang, J. Lan, G.Q. Zhuang, W.Z. Ma, W. Wang, Lysosomal cysteine peptidase cathepsin L protects against cardiac hypertrophy through blocking AKT/GSK3β signaling, J. Mol. Med. 87 (2009) 249–260. https://doi.org/10.1007/s00109-008-0423-2.

[40] E. Harada, O. Nakagawa, M. Yoshimura, M. Harada, M. Nakagawa, Y. Mizuno, Y. Shimasaki, M. Nakayama, H. Yasue, K. Kuwahara, Y. Saito, K. Nakao, Effect of interleukin-1β on cardiac hypertrophy and production of natriuretic peptides in rat cardiocyte culture, J. Mol. Cell. Cardiol. 31 (1999) 1997–2006. https://doi.org/10.1006/jmcc.1999.1030.

[41] C.M. Thaik, A. Calderone, N. Takahashi, W.S. Colucci, Interleukin-1β modulates the growth and phenotype of neonatal rat cardiac myocytes, J. Clin. Invest. 96 (1995) 1093–1099. https://doi.org/10.1172/JCI118095.

[42] Q. Tang, J. Cai, D. Shen, Z. Bian, L. Yan, Y.-X. Wang, J. Lan, G.-Q. Zhuang, W.-Z. Ma, W. Wang, Lysosomal cysteine peptidase cathepsin L protects against cardiac hypertrophy through blocking AKT/GSK3β signaling, J. Mol. Med. 87 (2009) 249–260. https://doi.org/10.1007/s00109-008-0423-2.

[43] J. Reiser, B. Adair, T. Reinheckel, Specialized roles for cysteine cathepsins in health and disease, J. Clin. Invest. 120 (2010) 3421–3431. https://doi.org/10.1172/JCI42918.

[44] B. Wiederanders, G. Kaulmann, K. Schilling, Functions of propeptide parts in cysteine proteases., Curr. Protein Pept. Sci. 4 (2003) 309–26.

[45] G.W. Mellor, E.W. Thomas, C.M. Topham, K. Brocklehurst, Ionization characteristics of the Cys-25/His-159 interactive system and of the modulatory group of papain: resolution of ambiguity by electronic perturbation of the quasi-2-mercaptopyridine leaving group in a new pyrimidyl disulphide reactivity probe., Biochem. J. 290 (Pt 1 (1993) 289–96.

[46] M.E. McGrath, THE LYSOSOMAL CYSTEINE PROTEASES, Annu. Rev. Biophys. Biomol. Struct. 28 (1999) 181–204. https://doi.org/10.1146/annurev.biophys.28.1.181.

[47] A. Hasilik, C. Wrocklage, B. Schröder, Intracellular trafficking of lysosomal proteins and lysosomes., Int. J. Clin. Pharmacol. Ther. 47 Suppl 1 (2009) S18-33.

[48] M. Cygler, J. Sivaraman, P. Grochulski, R. Coulombe, A.C. Storer, J.S. Mort, Structure of rat procathepsin B: model for inhibition of cysteine protease activity by the proregion., Structure. 4 (1996) 405–16.

[49] M. Podobnik, R. Kuhelj, V. Turk, D. Turk, Crystal structure of the wild-type human procathepsin B at 2.5 Å resolution reveals the native active site of a papain-like cysteine protease zymogen, J. Mol. Biol. 271 (1997) 774–788. https://doi.org/10.1006/jmbi.1997.1218.

[50] R. Coulombe, P. Grochulski, J. Sivaraman, R. Ménard, J.S. Mort, M. Cygler, Structure of human procathepsin L reveals the molecular basis of inhibition by the prosegment., EMBO J. 15 (1996) 5492–503.

[51] J. Sivaraman, M. Lalumière, R. Ménard, M. Cygler, Crystal structure of wild-type human procathepsin K, Protein Sci. 8 (2008) 283–290. https://doi.org/10.1110/ps.8.2.283.

[52] J. Sivaraman, D.K. Nägler, R. Zhang, R. Ménard, M. Cygler, Crystal structure of human procathepsin X: a cysteine protease with the proregion covalently linked to the active site cysteine, J. Mol. Biol. 295 (2000) 939–951. https://doi.org/10.1006/jmbi.1999.3410.

[53] R. Jerala, E. Zerovnik, J. Kidric, V. Turk, pH-induced conformational transitions of the propeptide of human cathepsin L. A role for a molten globule state in zymogen activation., J. Biol. Chem. 273 (1998) 11498–504.

[54] Y. Nishimura, T. Kawabata, K. Furuno, K. Kato, Evidence that aspartic proteinase is involved in the proteolytic processing event of procathepsin L in lysosomes., Arch. Biochem. Biophys. 271 (1989) 400–6.

[55] A.D. Rowan, P. Mason, L. Mach, J.S. Mort, Rat procathepsin B. Proteolytic processing to the mature form in vitro., J. Biol. Chem. 267 (1992) 15993–9.

[56] J. Liu, G.K. Sukhova, J.-S. Sun, W.-H. Xu, P. Libby, G.-P. Shi, Lysosomal Cysteine Proteases in Atherosclerosis, Arterioscler. Thromb. Vasc. Biol. 24 (2004) 1359–1366. https://doi.org/10.1161/01.ATV.0000134530.27208.41.

[57] A. Punturieri, S. Filippov, E. Allen, I. Caras, R. Murray, V. Reddy, S.J. Weiss, Regulation of elastinolytic cysteine proteinase activity in normal and cathepsin K-deficient human macrophages., J. Exp. Med. 192 (2000) 789–99.

[58] M. Novinec, R.N. Grass, W.J. Stark, V. Turk, A. Baici, B. Lenarčič, Interaction between Human Cathepsins K, L, and S and Elastins, J. Biol. Chem. 282 (2007) 7893–7902. https://doi.org/10.1074/jbc.M610107200.

[59] G.P. Shi, J.S. Munger, J.P. Meara, D.H. Rich, H.A. Chapman, Molecular cloning and expression of human alveolar macrophage cathepsin S, an elastinolytic cysteine protease., J. Biol. Chem. 267 (1992) 7258–62.

[60] M.P. Nanut, J. Sabotič, A. Jewett, J. Kos, Cysteine cathepsins as regulators of the cytotoxicity of nk and t cells, Front. Immunol. 5 (2014) 616. https://doi.org/10.3389/fimmu.2014.00616.

[61] T. Nakanishi, Y. Ozaki, K. Blomgren, H. Tateyama, M. Sugiura-Ogasawara, K. Suzumori, Role of cathepsins and cystatins in patients with recurrent miscarriage, Mol. Hum. Reprod. 11 (2005) 351–355. https://doi.org/10.1093/molehr/gah172.

[62] V. Turk, W. Bode, The cystatins: protein inhibitors of cysteine proteinases., FEBS Lett. 285

(1991) 213–9.

[63] A.J. Barrett, The cystatins: a diverse superfamily of cysteine peptidase inhibitors., Biomed. Biochim. Acta. 45 (1986) 1363–74.

[64] R.A. DeLa Cadena, R.W. Colman, Structure and functions of human kininogens., Trends Pharmacol. Sci. 12 (1991) 272–5. https://doi.org/10.1016/0165-6147(91)90569-E.

[65] G. Salvesen, C. Parkes, M. Abrahamson, A. Grubb, A.J. Barrett, Human low-Mr kininogen contains three copies of a cystatin sequence that are divergent in structure and in inhibitory activity for cysteine proteinases., Biochem. J. 234 (1986) 429–34.

[66] H. Nagase, R. Visse, G. Murphy, Structure and function of matrix metalloproteinases and TIMPs., Cardiovasc. Res. 69 (2006) 562–73. https://doi.org/10.1016/j.cardiores.2005.12.002.

[67] X.W. Cheng, Z. Huang, M. Kuzuya, K. Okumura, T. Murohara, Cysteine Protease Cathepsins in Atherosclerosis-Based Vascular Disease and Its Complications, Hypertension. 58 (2011) 978–986. https://doi.org/10.1161/HYPERTENSIONAHA.111.180935.

[68] M.A. Konstam, D.G. Kramer, A.R. Patel, M.S. Maron, J.E. Udelson, Left ventricular remodeling in heart failure: Current concepts in clinical significance and assessment, JACC Cardiovasc. Imaging. 4 (2011) 98–108. https://doi.org/10.1016/j.jcmg.2010.10.008.

[69] X.W. Cheng, G.-P. Shi, M. Kuzuya, T. Sasaki, K. Okumura, T. Murohara, Role for Cysteine Protease Cathepsins in Heart Disease: Focus on Biology and Mechanisms With Clinical Implication, Circulation. 125 (2012) 1551–1562. https://doi.org/10.1161/CIRCULATIONAHA.111.066712.

[70] J.L. Samuel, C. Delcayre, Cardiac fibrosis, Bull. Acad. Natl. Med. 201 (2017) 775–784. https://doi.org/10.1161/circresaha.115.306565.

[71] J. Baum, H.S. Duffy, Fibroblasts and myofibroblasts: What are we talking about?, J. Cardiovasc. Pharmacol. 57 (2011) 376–379. https://doi.org/10.1097/FJC.0b013e3182116e39.

[72] S. Fredj, J. Bescond, C. Louault, A. Delwail, J.C. Lecron, D. Potreau, Role of interleukin-6 in cardiomyocyte/cardiac fibroblast interactions during myocyte hypertrophy and fibroblast proliferation, J. Cell. Physiol. 204 (2005) 428–436. https://doi.org/10.1002/jcp.20307.

[73] M. Litwiniuk, A. Krejner, T. Grzela, Hyaluronic Acid in Inflammation and Tissue Regeneration, Wounds. 28 (2016) 78–88.

[74] T.A. Wynn, T.R. Ramalingam, Mechanisms of fibrosis: Therapeutic translation for fibrotic disease, Nat. Med. 18 (2012) 1028–1040. https://doi.org/10.1038/nm.2807.

[75] K.K. Shalia, M.R. Mashru, V.K. Shah, S.L. Soneji, S. Payannavar, Levels of cathepsins in acute myocardial infarction, Indian Heart J. 64 (2012) 290–294. https://doi.org/10.1016/S0019-4832(12)60089-3.

[76] I. Abd-Elrahman, K. Meir, H. Kosuge, Y. Ben-Nun, T. Weiss Sadan, C. Rubinstein, Y. Samet, M. V. McConnell, G. Blum, Characterizing Cathepsin Activity and Macrophage Subtypes in Excised Human Carotid Plaques, Stroke. 47 (2016) 1101–1108. https://doi.org/10.1161/STROKEAHA.115.011573.

[77] D.H. Madsen, D. Leonard, A. Masedunskas, A. Moyer, H.J. Jürgensen, D.E. Peters, P. Amornphimoltham, A. Selvaraj, S.S. Yamada, D.A. Brenner, S. Burgdorf, L.H. Engelholm, N. Behrendt, K. Holmbeck, R. Weigert, T.H. Bugge, M2-like macrophages are responsible for collagen degradation through a mannose receptor-mediated pathway., J. Cell Biol. 202 (2013) 951–66. https://doi.org/10.1083/jcb.201301081.

[78] A.C. MacKinnon, S.L. Farnworth, P.S. Hodkinson, N.C. Henderson, K.M. Atkinson, H. Leffler, U.J. Nilsson, C. Haslett, S.J. Forbes, T. Sethi, Regulation of alternative macrophage activation by galectin-3., J. Immunol. 180 (2008) 2650–8. https://doi.org/10.4049/jimmunol.180.4.2650.

[79] D. Gong, W. Shi, S. Yi, H. Chen, J. Groffen, N. Heisterkamp, TGFβ signaling plays a critical role in promoting alternative macrophage activation, BMC Immunol. 13 (2012) 31. https://doi.org/10.1186/1471-2172-13-31.

[80] N.A. Turner, R.S. Mughal, P. Warburton, D.J. O'Regan, S.G. Ball, K.E. Porter, Mechanism of TNFα-induced IL-1α, IL-1β and IL-6 expression in human cardiac fibroblasts: Effects of statins and thiazolidinediones, Cardiovasc. Res. 76 (2007) 81–90. https://doi.org/10.1016/j.cardiores.2007.06.003.

[81] N. Suthahar, W.C. Meijers, H.H.W. Silljé, R.A. de Boer, From Inflammation to Fibrosis—Molecular and Cellular Mechanisms of Myocardial Tissue Remodelling and Perspectives on Differential Treatment Opportunities, Curr. Heart Fail. Rep. 14 (2017) 235–250. https://doi.org/10.1007/s11897-017-0343-y.

[82] G.C. Meléndez, J.L. McLarty, S.P. Levick, Y. Du, J.S. Janicki, G.L. Brower, Interleukin 6 mediates myocardial fibrosis, concentric hypertrophy, and diastolic dysfunction in rats, Hypertension. 56 (2010) 225–231. https://doi.org/10.1161/HYPERTENSIONAHA.109.148635.

[83] C.A. Fielding, G.W. Jones, R.M. McLoughlin, L. McLeod, V.J. Hammond, J. Uceda, A.S. Williams, M. Lambie, T.L. Foster, C. Te Liao, C.M. Rice, C.J. Greenhill, C.S. Colmont, E. Hams, B. Coles, A. Kift-Morgan, Z. Newton, K.J. Craig, J.D. Williams, G.T. Williams, S.J. Davies, I.R. Humphreys, V.B. O'Donnell, P.R. Taylor, B.J. Jenkins, N. Topley, S.A. Jones, Interleukin-6 signaling drives fibrosis in unresolved inflammation, Immunity. 40 (2014) 40–50. https://doi.org/10.1016/j.immuni.2013.10.022.

[84] M. Pauschinger, K. Chandrasekharan, J. Li, P.L. Schwimmbeck, M. Noutsias, H.-P. Schultheiss, Remodeling der extrazellulären Matrix bei dilatativer Kardiomyopathie, Herz. 27 (2002) 677–

682. https://doi.org/10.1007/s00059-002-2413-4.

[85] V. Talman, H. Ruskoaho, Cardiac fibrosis in myocardial infarction—from repair and remodeling to regeneration, Cell Tissue Res. 365 (2016) 563–581. https://doi.org/10.1007/s00441-016-2431-9.

[86] W. Fang, A. He, M.X. Xiang, Y. Lin, Y. Wang, J. Li, C. Yang, X. Zhang, C.L. Liu, G.K. Sukhova, N. Barascuk, L. Larsen, M. Karsdal, P. Libby, G.P. Shi, Cathepsin K-deficiency impairs mouse cardiac function after myocardial infarction, J. Mol. Cell. Cardiol. 127 (2019) 44–56. https://doi.org/10.1016/j.yjmcc.2018.11.010.

[87] M. Kasabova, A. Joulin-Giet, F. Lecaille, B.F. Gilmore, S. Marchand-Adam, A. Saidi, G. Lalmanach, Regulation of TGF-β1-driven differentiation of human Lung fibroblasts: Emerging roles of cathepsin B and cystatin C, J. Biol. Chem. 289 (2014) 16239–16251. https://doi.org/10.1074/jbc.M113.542407.

[88] J. Stypmann, K. Glaser, W. Roth, D.J. Tobin, I. Petermann, R. Matthias, G. Monnig, W. Haverkamp, G. Breithardt, W. Schmahl, C. Peters, T. Reinheckel, Dilated cardiomyopathy in mice deficient for the lysosomal cysteine peptidase cathepsin L, Proc. Natl. Acad. Sci. 99 (2002) 6234–6239. https://doi.org/10.1073/pnas.092637699.

[89] I. Petermann, C. Mayer, J. Stypmann, M.L. Biniossek, D.J. Tobin, M.A. Engelen, T. Dandekar, T. Grune, L. Schild, C. Peters, T. Reinheckel, Lysosomal, cytoskeletal, and metabolic alterations in cardiomyopathy of cathepsin L knockout mice, FASEB J. 20 (2006) 1266–1268. https://doi.org/10.1096/fj.05-5517fje.

[90] L. Pan, Y. Li, L. Jia, Y. Qin, G. Qi, J. Cheng, Y. Qi, H. Li, J. Du, Cathepsin S deficiency results in abnormal accumulation of autophagosomes in macrophages and enhances Ang II-induced cardiac inflammation., PLoS One. 7 (2012) e35315. https://doi.org/10.1371/journal.pone.0035315.

[91] N. Frey, H.A. Katus, E.N. Olson, J.A. Hill, Hypertrophy of the Heart: A New Therapeutic Target?, Circulation. 109 (2004) 1580–1589. https://doi.org/10.1161/01.CIR.0000120390.68287.BB.

[92] M. Samak, J. Fatullayev, A. Sabashnikov, M. Zeriouh, B. Schmack, M. Farag, A.F. Popov, P.M. Dohmen, Y.H. Choi, T. Wahlers, A. Weymann, Cardiac Hypertrophy: An Introduction to Molecular and Cellular Basis, Med. Sci. Monit. Basic Res. 22 (2016) 75–79. https://doi.org/10.12659/MSMBR.900437.

[93] B.A. Vakili, P.M. Okin, R.B. Devereux, Prognostic implications of left ventricular hypertrophy, Am. Heart J. 141 (2001) 334–341. https://doi.org/10.1067/mhj.2001.113218.

[94] C. Mihl, W.R.M. Dassen, H. Kuipers, Cardiac remodelling: Concentric versus eccentric

hypertrophy in strength and endurance athletes, Netherlands Hear. J. 16 (2008) 129–133. https://doi.org/10.1007/BF03086131.

[95] Y. Hua, Y. Zhang, J. Dolence, G.P. Shi, J. Ren, S. Nair, Cathepsin K knockout mitigates high-fat diet-induced cardiac hypertrophy and contractile dysfunction, Diabetes. 62 (2013) 498–509. https://doi.org/10.2337/db12-0350.

[96] S. Sciarretta, M. Volpe, J. Sadoshima, Mammalian target of rapamycin signaling in cardiac physiology and disease, Circ. Res. 114 (2014) 549–564. https://doi.org/10.1161/CIRCRESAHA.114.302022.

[97] C.-H. Lee, K. Inoki, K.-L. Guan, mTOR Pathway as a Target in Tissue Hypertrophy, Annu. Rev. Pharmacol. Toxicol. 47 (2007) 443–467. https://doi.org/10.1146/annurev.pharmtox.47.120505.105359.

[98] Y. Hua, T.J. Robinson, Y. Cao, G.-P. Shi, J. Ren, S. Nair, Cathepsin K knockout alleviates aging-induced cardiac dysfunction, Aging Cell. 14 (2015) 345–351. https://doi.org/10.1111/acel.12276.

[99] M. Sun, M. Ouzounian, G. de Couto, M. Chen, R. Yan, M. Fukuoka, G. Li, M. Moon, Y. Liu, A. Gramolini, G.J. Wells, P.P. Liu, Cathepsin-L ameliorates cardiac hypertrophy through activation of the autophagy-lysosomal dependent protein processing pathways., J. Am. Heart Assoc. 2 (2013). https://doi.org/10.1161/JAHA.113.000191.

[100] X.W. Cheng, K. Obata, M. Kuzuya, H. Izawa, K. Nakamura, E. Asai, T. Nagasaka, M. Saka, T. Kimata, A. Noda, K. Nagata, H. Jin, G.P. Shi, A. Iguchi, T. Murohara, M. Yokota, Elastolytic cathepsin induction/activation system exists in myocardium and is upregulated in hypertensive heart failure, Hypertension. 48 (2006) 979–987. https://doi.org/10.1161/01.HYP.0000242331.99369.2f.

[101] Y.W. Qin, P. Ye, J.Q. He, L. Sheng, L.Y. Wang, J. Du, Simvastatin inhibited cardiac hypertrophy and fibrosis in apolipoprotein E-deficient mice fed a Western-style diet by increasing PPAR α and γ expression and reducing TC, MMP-9, and Cat S levels, Acta Pharmacol. Sin. 31 (2010) 1350–1358. https://doi.org/10.1038/aps.2010.109.

[102] D.A. Eisner, J.L. Caldwell, K. Kistamás, A.W. Trafford, Calcium and Excitation-Contraction Coupling in the Heart, Circ. Res. 121 (2017) 181–195. https://doi.org/10.1161/CIRCRESAHA.117.310230.

[103] E.B. Elliott, A. Kelly, G.L. Smith, C.M. Loughrey, Isolated rabbit working heart function during progressive inhibition of myocardial SERCA activity, Circ. Res. 110 (2012) 1618–1627. https://doi.org/10.1161/CIRCRESAHA.111.262337.

[104] D.H. MacLennan, E.G. Kranias, Phospholamban: a crucial regulator of cardiac contractility,

Nat. Rev. Mol. Cell Biol. 4 (2003) 566–577. https://doi.org/10.1038/nrm1151.

[105] A. Belevych, Z. Kubalova, D. Terentyev, R.L. Hamlin, C.A. Carnes, S. Györke, Enhanced ryanodine receptor-mediated calcium leak determines reduced sarcoplasmic reticulum calcium content in chronic canine heart failure, Biophys. J. 93 (2007) 4083–4092. https://doi.org/10.1529/biophysj.107.114546.

[106] K. SIPIDO, D. EISNER, Something old, something new: Changing views on the cellular mechanisms of heart failure, Cardiovasc. Res. 68 (2005) 167–174. https://doi.org/10.1016/j.cardiores.2005.08.011.

[107] S.R. Houser, K.B. Margulies, Is Depressed Myocyte Contractility Centrally Involved in Heart Failure?, Circ. Res. 92 (2003) 350–358. https://doi.org/10.1161/01.RES.0000060027.40275.A6.

[108] G. Hasenfuss, B. Pieske, Calcium Cycling in Congestive Heart Failure, J. Mol. Cell. Cardiol. 34 (2002) 951–969. https://doi.org/10.1006/jmcc.2002.2037.

[109] C. Fattah, K. Nather, C.S. McCarroll, M.P. Hortigon-Vinagre, V. Zamora, M. Flores-Munoz, L. McArthur, L. Zentilin, M. Giacca, R.M. Touyz, G.L. Smith, C.M. Loughrey, S.A. Nicklin, Gene Therapy With Angiotensin-(1-9) Preserves Left Ventricular Systolic Function After Myocardial Infarction, J. Am. Coll. Cardiol. 68 (2016) 2652–2666. https://doi.org/10.1016/j.jacc.2016.09.946.

[110] G. Hasenfuss, H. Reinecke, R. Studer, M. Meyer, B. Pieske, J. Holtz, C. Holubarsch, H. Posival, H. Just, H. Drexler, Relation between myocardial function and expression of sarcoplasmic reticulum Ca2+-ATPase in failing and nonfailing human myocardium, Circ. Res. 75 (1994) 434–442. https://doi.org/10.1161/01.RES.75.3.434.

[111] E. Kiss, N.A. Ball, E.G. Kranias, R.A. Walsh, Differential changes in cardiac phospholamban and sarcoplasmic reticular Ca2+-ATPase protein levels: Effects on Ca2+ transport and mechanics in compensated pressure-overload hypertrophy and congestive heart failure, Circ. Res. 77 (1995) 759–764. https://doi.org/10.1161/01.RES.77.4.759.

[112] E.B. Elliott, H. Hasumi, N. Otani, T. Matsuda, R. Matsuda, N. Kaneko, G.L. Smith, C.M. Loughrey, K201 (JTV-519) alters the spatiotemporal properties of diastolic Ca 2+ release and the associated diastolic contraction during β-adrenergic stimulation in rat ventricular cardiomyocytes, Basic Res. Cardiol. 106 (2011) 1009–1022. https://doi.org/10.1007/s00395-011-0218-4.

[113] J. Blondelle, S. Lange, B.H. Greenberg, R.T. Cowling, Cathepsins in heart disease-chewing on the heartache?, Am. J. Physiol. Heart Circ. Physiol. 308 (2015) H974-6. https://doi.org/10.1152/ajpheart.00125.2015.

[114] Y. Wang, S. Tandan, J.A. Hill, Calcineurin-dependent ion channel regulation in heart, Trends Cardiovasc. Med. 24 (2014) 14–22. https://doi.org/10.1016/j.tcm.2013.05.004.

[115] E.B. Elliott, D. McCarroll, H. Hasumi, C.E. Welsh, A.A. Panissidi, N.G. Jones, C.L. Rossor, A. Tait, G.L. Smith, J.C. Mottram, L.J. Morrison, C.M. Loughrey, Trypanosoma brucei cathepsin-L increases arrhythmogenic sarcoplasmic reticulum-mediated calcium release in rat cardiomyocytes, Cardiovasc. Res. 100 (2013) 325. https://doi.org/10.1093/CVR/CVT187.

[116] C.S. McCarroll, C.L. Rossor, L.R. Morrison, L.J. Morrison, C.M. Loughrey, A Pre-clinical Animal Model of Trypanosoma brucei Infection Demonstrating Cardiac Dysfunction, PLoS Negl. Trop. Dis. 9 (2015) e0003811. https://doi.org/10.1371/journal.pntd.0003811.

[117] J.F.R. Kerr, A.H. Wyllie, A.R. Currie, Apoptosis: A basic biological phenomenon with wide-ranging implications in tissue kinetics, Br. J. Cancer. 26 (1972) 239–257. https://doi.org/10.1038/bjc.1972.33.

[118] P. Xia, Y. Liu, Z. Cheng, Signaling Pathways in Cardiac Myocyte Apoptosis, Biomed Res. Int. 2016 (2016). https://doi.org/10.1155/2016/9583268.

[119] A. Haunstetter, S. Izumo, Apoptosis: Basic mechanisms and implications for cardiovascular disease, Circ. Res. 82 (1998) 1111–1129. https://doi.org/10.1161/01.RES.82.11.1111.

[120] R. Singh, A. Letai, K. Sarosiek, Regulation of apoptosis in health and disease: the balancing act of BCL-2 family proteins, Nat. Rev. Mol. Cell Biol. 20 (2019) 175–193. https://doi.org/10.1038/s41580-018-0089-8.

[121] A. Abbate, J. Narula, Role of apoptosis in adverse ventricular remodeling., Heart Fail. Clin. 8 (2012) 79–86. https://doi.org/10.1016/j.hfc.2011.08.010.

[122] P. Boya, K. Andreau, D. Poncet, N. Zamzami, J.L. Perfettini, D. Metivier, D.M. Ojcius, M. Jäättelä, G. Kroemer, Lysosomal membrane permeabilization induces cell death in a mitochondrion-dependent fashion, J. Exp. Med. 197 (2003) 1323–1334. https://doi.org/10.1084/jem.20021952.

[123] M. Heinrich, J. Neumeyer, M. Jakob, C. Hallas, V. Tchikov, S. Winoto-Morbach, M. Wickel, W. Scheider-Brachert, A. Trauzold, A. Hethke, S. Schütze, Cathepsin D links TNF-induced acid sphingomyelinase to Bid-mediated caspase-9 and -3 activation, Cell Death Differ. 11 (2004) 550–563. https://doi.org/10.1038/sj.cdd.4401382.

[124] R. Blomgran, L. Zheng, O. Stendahl, Cathepsin-cleaved Bid promotes apoptosis in human neutrophils via oxidative stress-induced lysosomal membrane permeabilization, J. Leukoc. Biol. 81 (2007) 1213–1223. https://doi.org/10.1189/jlb.0506359.

[125] M. Pauschinger, K. Chandrasekharan, J. Li, P.L. Schwimmbeck, M. Noutsias, H.-P. Schultheiss, [Mechanisms of extracellular matrix remodeling in dilated cardiomyopathy]., Herz. 27 (2002)

677–682. https://doi.org/10.1007/s00059-002-2413-4.

[126] V. Roldán, F. Marín, J.R. Gimeno, F. Ruiz-Espejo, J. González, E. Feliu, A. García-Honrubia, D. Saura, G. de la Morena, M. Valdés, V. Vicente, Matrix metalloproteinases and tissue remodeling in hypertrophic cardiomyopathy., Am. Heart J. 156 (2008) 85–91. https://doi.org/10.1016/j.ahj.2008.01.035.

[127] Q.-Q. Wu, M. Xu, Y. Yuan, F.-F. Li, Z. Yang, Y. Liu, M.-Q. Zhou, Z.-Y. Bian, W. Deng, L. Gao, H. Li, Q.-Z. Tang, Cathepsin B deficiency attenuates cardiac remodeling in response to pressure overload via TNF-α/ASK1/JNK pathway, Am. J. Physiol. Circ. Physiol. 308 (2015) H1143–H1154. https://doi.org/10.1152/ajpheart.00601.2014.

[128] Y. Wang, L. Jia, J. Shen, Y. Wang, Z. Fu, S.A. Su, Z. Cai, J.A.A. Wang, M. Xiang, Cathepsin B aggravates coxsackievirus B3-induced myocarditis through activating the inflammasome and promoting pyroptosis, PLoS Pathog. 14 (2018) e1006872. https://doi.org/10.1371/journal.ppat.1006872.

[129] T. Hamid, S.Z. Guo, J.R. Kingery, X. Xiang, B. Dawn, S.D. Prabhu, Cardiomyocyte NF-κB p65 promotes adverse remodelling, apoptosis, and endoplasmic reticulum stress in heart failure, Cardiovasc. Res. 89 (2011) 129. https://doi.org/10.1093/CVR/CVQ274.

[130] G. Mitchell, M.I. Cheng, C. Chen, B.N. Nguyen, A.T. Whiteley, S. Kianian, J.S. Cox, D.R. Green, K.L. McDonald, D.A. Portnoy, Listeria monocytogenes triggers noncanonical autophagy upon phagocytosis, but avoids subsequent growth-restricting xenophagy, Proc. Natl. Acad. Sci. 115 (2018) E210–E217. https://doi.org/10.1073/PNAS.1716055115.

[131] P.-F. Wei, L. Zhang, S.K. Nethi, A.K. Barui, J. Lin, W. Zhou, Y. Shen, N. Man, Y.-J. Zhang, J. Xu, C.R. Patra, L.-P. Wen, Accelerating the clearance of mutant huntingtin protein aggregates through autophagy induction by europium hydroxide nanorods, Biomaterials. 35 (2014) 899–907. https://doi.org/10.1016/J.BIOMATERIALS.2013.10.024.

[132] Y.A. Lee, L.A. Noon, K.M. Akat, M.D. Ybanez, T.-F. Lee, M.-L. Berres, N. Fujiwara, N. Goossens, H.-I. Chou, F.P. Parvin-Nejad, B. Khambu, E.G.M. Kramer, R. Gordon, C. Pfleger, D. Germain, G.R. John, K.N. Campbell, Z. Yue, X.-M. Yin, A.M. Cuervo, M.J. Czaja, M.I. Fiel, Y. Hoshida, S.L. Friedman, Autophagy is a gatekeeper of hepatic differentiation and carcinogenesis by controlling the degradation of Yap, Nat. Commun. 9 (2018) 4962. https://doi.org/10.1038/s41467-018-07338-z.

[133] Y.-M. Lim, H. Lim, K.Y. Hur, W. Quan, H.-Y. Lee, H. Cheon, D. Ryu, S.-H. Koo, H.L. Kim, J. Kim, M. Komatsu, M.-S. Lee, Systemic autophagy insufficiency compromises adaptation to metabolic stress and facilitates progression from obesity to diabetes, Nat. Commun. 5 (2014) 4934. https://doi.org/10.1038/ncomms5934.

[134] K.F. Cooper, Till death do us part: The marriage of autophagy and apoptosis, Oxid. Med. Cell. Longev. 2018 (2018) 1–13. https://doi.org/10.1155/2018/4701275.

[135] N. Mizushima, B. Levine, A.M. Cuervo, D.J. Klionsky, Autophagy fights disease through cellular self-digestion, Nature. 451 (2008) 1069–1075. https://doi.org/10.1038/nature06639.

[136] L. Bonapace, B.C. Bornhauser, M. Schmitz, G. Cario, U. Ziegler, F.K. Niggli, B.W. Schäfer, M. Schrappe, M. Stanulla, J.P. Bourquin, Induction of autophagy-dependent necroptosis is required for childhood acute lymphoblastic leukemia cells to overcome glucocorticoid resistance, J. Clin. Invest. 120 (2010) 1310–1323. https://doi.org/10.1172/JCI39987.

[137] A. Chiramel, N. Brady, R. Bartenschlager, Divergent Roles of Autophagy in Virus Infection, Cells. 2 (2013) 83–104. https://doi.org/10.3390/cells2010083.

[138] Y. Feng, D. He, Z. Yao, D.J. Klionsky, The machinery of macroautophagy., Cell Res. 24 (2014) 24–41. https://doi.org/10.1038/cr.2013.168.

[139] J.B. Kunz, H. Schwarz, A. Mayer, Determination of four sequential stages during microautophagy in vitro., J. Biol. Chem. 279 (2004) 9987–96. https://doi.org/10.1074/jbc.M307905200.

[140] M. Xie, C.R. Morales, S. Lavandero, J.A. Hill, Tuning flux: Autophagy as a target of heart disease therapy, Curr. Opin. Cardiol. 26 (2011) 216–222. https://doi.org/10.1097/HCO.0b013e328345980a.

[141] S. Sciarretta, P. Zhai, D. Shao, Y. Maejima, J. Robbins, M. Volpe, G. Condorelli, J. Sadoshima, Rheb is a critical regulator of autophagy during myocardial ischemia: Pathophysiological implications in obesity and metabolic syndrome, Circulation. 125 (2012) 1134–1146. https://doi.org/10.1161/CIRCULATIONAHA.111.078212.

[142] H. Kanamori, G. Takemura, K. Goto, R. Maruyama, A. Tsujimoto, A. Ogino, T. Takeyama, T. Kawaguchi, T. Watanabe, T. Fujiwara, H. Fujiwara, M. Seishima, S. Minatoguchi, The role of autophagy emerging in postinfarction cardiac remodelling, Cardiovasc. Res. 91 (2011) 330–339. https://doi.org/10.1093/cvr/cvr073.

[143] S. Sciarretta, D. Yee, N. Nagarajan, F. Bianchi, T. Saito, V. Valenti, M. Tong, D.P. Del Re, C. Vecchione, L. Schirone, M. Forte, S. Rubattu, A. Shirakabe, V.S. Boppana, M. Volpe, G. Frati, P. Zhai, J. Sadoshima, Trehalose-Induced Activation of Autophagy Improves Cardiac Remodeling After Myocardial Infarction, J. Am. Coll. Cardiol. 71 (2018) 1999–2010. https://doi.org/10.1016/j.jacc.2018.02.066.

[144] H. Zhu, P. Tannous, J.L. Johnstone, Y. Kong, J.M. Shelton, J.A. Richardson, V. Le, B. Levine, B.A. Rothermel, J.A. Hill, Cardiac autophagy is a maladaptive response to hemodynamic stress, J. Clin. Invest. 117 (2007) 1782–1793. https://doi.org/10.1172/JCI27523.

[145] J. Stypmann, K. Gläser, W. Roth, D.J. Tobin, I. Petermann, R. Matthias, G. Mönnig, W. Haverkamp, G. Breithardt, W. Schmahl, C. Peters, T. Reinheckel, Dilated cardiomyopathy in mice deficient for the lysosomal cysteine peptidase cathepsin L., Proc. Natl. Acad. Sci. U. S. A. 99 (2002) 6234–9. https://doi.org/10.1073/pnas.092637699.

[146] J. Dennemärker, T. Lohmüller, S. Müller, S.V. Aguilar, D.J. Tobin, C. Peters, T. Reinheckel, Impaired turnover of autophagolysosomes in cathepsin L deficiency, Biol. Chem. 391 (2010). https://doi.org/10.1515/bc.2010.097.

[147] T. Ueno, K. Takahashi, A cathepsin L-specific inhibitor preferentially inhibits degradation of autophagosomal LC3 and GABARAP in HeLa and Huh-7 cells, (2009). https://doi.org/10.4161/auto.9097.

[148] S.M. Man, T.D. Kanneganti, Regulation of lysosomal dynamics and autophagy by CTSB/cathepsin B, Autophagy. 12 (2016) 2504–2505. https://doi.org/10.1080/15548627.2016.1239679.

[149] S. Aydin, K. Ugur, S. Aydin, İ. Sahin, M. Yardim, Biomarkers in acute myocardial infarction: current perspectives, Vasc. Health Risk Manag. 15 (2019) 1–10. https://doi.org/10.2147/VHRM.S166157.

[150] G. Zhao, Y. Li, L. Cui, X. Li, Z. Jin, X. Han, E. Fang, Y. Gao, D. Zhou, H. Jiang, X. Jin, G. Piao, X. Li, G. Yang, J. Jin, E. Zhu, M. Piao, L. Piao, K. Yuan, Y. Lei, D. Ding, C. Jin, Y. Nan, X. Cheng, Increased Circulating Cathepsin K in Patients with Chronic Heart Failure, PLoS One. 10 (2015) e0136093. https://doi.org/10.1371/journal.pone.0136093.

[151] S. Mehra, M. Kumar, M. Manchanda, R. Singh, B. Thakur, N. Rani, S. Arava, R. Narang, D.S. Arya, S.S. Chauhan, Clinical significance of cathepsin L and cathepsin B in dilated cardiomyopathy, Mol. Cell. Biochem. 428 (2017) 139–147. https://doi.org/10.1007/s11010-016-2924-6.

[152] E. Fernlund, T. Gyllenhammar, R. Jablonowski, M. Carlsson, A. Larsson, J. Ärnlöv, P. Liuba, Serum Biomarkers of Myocardial Remodeling and Coronary Dysfunction in Early Stages of Hypertrophic Cardiomyopathy in the Young, Pediatr. Cardiol. 38 (2017) 853–863. https://doi.org/10.1007/s00246-017-1593-x.

[153] E. Jobs, E. Ingelsson, U. Risérus, E. Nerpin, M. Jobs, J. Sundström, S. Basu, A. Larsson, L. Lind, J. Ärnlöv, Association between serum cathepsin S and mortality in older adults., JAMA. 306 (2011) 1113–1121. https://doi.org/10.1001/jama.2011.1246.

[154] T. Feldreich, A.C. Carlsson, U. Risérus, A. Larsson, L. Lind, J. Ärnlöv, The association between serum cathepsin L and mortality in older adults., Atherosclerosis. 254 (2016) 109–116. https://doi.org/10.1016/j.atherosclerosis.2016.09.062.

[155] Y. Liu, X. Li, D. Peng, Z. Tan, H. Liu, Y. Qing, Y. Xue, G.-P. Shi, Usefulness of serum cathepsin L as an independent biomarker in patients with coronary heart disease., Am. J. Cardiol. 103 (2009) 476–481. https://doi.org/10.1016/j.amjcard.2008.10.011.

[156] J. Liu, L. Ma, J. Yang, A. Ren, Z. Sun, G. Yan, J. Sun, H. Fu, W. Xu, C. Hu, G.-P. Shi, Increased serum cathepsin S in patients with atherosclerosis and diabetes., Atherosclerosis. 186 (2006) 411–419. https://doi.org/10.1016/j.atherosclerosis.2005.08.001.

[157] B. Mirjanic-Azaric, J. Vekic, A. Zeljkovic, Z. Jelic-Ivanovic, M. Djeric, T. Milivojac, U. Pecar Fonovic, J. Marc, J. Kos, D. Cerne, Interrelated cathepsin S-lowering and LDL subclass profile improvements induced by atorvastatin in the plasma of stable angina patients., J. Atheroscler. Thromb. 21 (2014) 868–877. https://doi.org/10.5551/jat.21410.

[158] Y. Qin, Y. Yang, R. Liu, X. Cao, O. Liu, J. Liu, M. Wang, Y. Yang, Z. Chen, H. Zhang, J. Du, Combined Cathepsin S and hs-CRP predicting inflammation of Abdominal Aortic Aneurysm, Clin. Biochem. 46 (2013) 1026–1029. https://doi.org/https://doi.org/10.1016/j.clinbiochem.2013.05.065.

[159] M. Sarhene, Y. Wang, J. Wei, Y. Huang, M. Li, L. Li, E. Acheampong, Z. Zhengcan, Q. Xiaoyan, X. Yunsheng, M. Jingyuan, G. Xiumei, F. Guanwei, Biomarkers in heart failure: the past, current and future, Heart Fail. Rev. 24 (2019) 867–903. https://doi.org/10.1007/s10741-019-09807-z.

[160] M. Adamcova, F. Šimko, Multiplex biomarker approach to cardiovascular diseases, Acta Pharmacol. Sin. 39 (2018) 1068–1072. https://doi.org/10.1038/aps.2018.29.

[161] P.S. Burrage, K.S. Mix, C.E. Brinckerhoff, Matrix metalloproteinases: role in arthritis., Front. Biosci. 11 (2006) 529–43.

[162] J. Cathcart, A. Pulkoski-Gross, J. Cao, Targeting matrix metalloproteinases in cancer: Bringing new life to old ideas, Genes Dis. 2 (2015) 26–34. https://doi.org/10.1016/J.GENDIS.2014.12.002.

[163] B. Turk, Targeting proteases: successes, failures and future prospects, Nat. Rev. Drug Discov. 5 (2006) 785–799. https://doi.org/10.1038/nrd2092.

[164] G.S. Butler, C.M. Overall, Updated Biological Roles for Matrix Metalloproteinases and New “Intracellular” Substrates Revealed by Degradomics, Biochemistry. 48 (2009) 10830–10845. https://doi.org/10.1021/bi901656f.

[165] C.J. Morrison, G.S. Butler, D. Rodríguez, C.M. Overall, Matrix metalloproteinase proteomics: substrates, targets, and therapy, Curr. Opin. Cell Biol. 21 (2009) 645–653. https://doi.org/10.1016/J.CEB.2009.06.006.

[166] E. Vidak, U. Javoršek, M. Vizovišek, B. Turk, Cysteine Cathepsins and their Extracellular Roles:

Shaping the Microenvironment, Cells. 8 (2019) 264. https://doi.org/10.3390/cells8030264.

[167] Y. Qin, G.-P. Shi, Cysteinyl cathepsins and mast cell proteases in the pathogenesis and therapeutics of cardiovascular diseases, Pharmacol. Ther. 131 (2011) 338–350. https://doi.org/10.1016/j.pharmthera.2011.04.010.

[168] H. Chen, J. Wang, M.-X. Xiang, Y. Lin, A. He, C.-N. Jin, J. Guan, G.K. Sukhova, P. Libby, J.-A. Wang, G.-P. Shi, Cathepsin S-mediated fibroblast trans-differentiation contributes to left ventricular remodelling after myocardial infarction, (n.d.). https://doi.org/10.1093/cvr/cvt158.

[169] M.-H. Abdulla, K.-C. Lim, M. Sajid, J.H. McKerrow, C.R. Caffrey, Schistosomiasis mansoni: novel chemotherapy using a cysteine protease inhibitor, PLoS Med. 4 (2007) e14–e14. https://doi.org/10.1371/journal.pmed.0040014.

[170] J.D. Chaparro, T. Cheng, U.P. Tran, R.M. Andrade, S.B.T. Brenner, G. Hwang, S. Cohn, K. Hirata, J.H. McKerrow, S.L. Reed, Two key cathepsins, TgCPB and TgCPL, are targeted by the vinyl sulfone inhibitor K11777 in in vitro and in vivo models of toxoplasmosis., PLoS One. 13 (2018) e0193982. https://doi.org/10.1371/journal.pone.0193982.

[171] S.C. Barr, K.L. Warner, B.G. Kornreic, J. Piscitelli, A. Wolfe, L. Benet, J.H. McKerrow, A cysteine protease inhibitor protects dogs from cardiac damage during infection by Trypanosoma cruzi., Antimicrob. Agents Chemother. 49 (2005) 5160–5161. https://doi.org/10.1128/AAC.49.12.5160-5161.2005.

[172] A. LIU, X. GAO, Q. ZHANG, L. CUI, Cathepsin B inhibition attenuates cardiac dysfunction and remodeling following myocardial infarction by inhibiting the NLRP3 pathway, Mol. Med. Rep. 8 (2013) 361–366. https://doi.org/10.3892/mmr.2013.1507.

[173] P.P. Shah, M.C. Myers, M.P. Beavers, J.E. Purvis, H. Jing, H.J. Grieser, E.R. Sharlow, A.D. Napper, D.M. Huryn, B.S. Cooperman, A.B. 3rd Smith, S.L. Diamond, Kinetic characterization and molecular docking of a novel, potent, and selective slow-binding inhibitor of human cathepsin L., Mol. Pharmacol. 74 (2008) 34–41. https://doi.org/10.1124/mol.108.046219.

[174] K. Takahashi, T. Ueno, I. Tanida, N. Minematsu-Ikeguchi, M. Murata, E. Kominami, Characterization of CAA0225, a Novel Inhibitor Specific for Cathepsin L, as a Probe for Autophagic Proteolysis, Biol. Pharm. Bull. 32 (2009) 475–479. https://doi.org/10.1248/bpb.32.475.

[175] W. He, D. McCarroll, E.B. Elliott, C.M. Loughrey, The Cathepsin-L Inhibitor CAA0225 Improves Cardiac Function During Ischaemia-Reperfusion, Biophys. J. 106 (2014) 729a. https://doi.org/https://doi.org/10.1016/j.bpj.2013.11.4021.

[176] C.-L. Liu, J. Guo, X. Zhang, G.K. Sukhova, P. Libby, G.-P. Shi, Cysteine protease cathepsins in

cardiovascular disease: from basic research to clinical trials, Nat. Rev. Cardiol. 15 (2018) 351–370. https://doi.org/10.1038/s41569-018-0002-3.

[177] C.S. Li, D. Deschenes, S. Desmarais, J.-P. Falgueyret, J.Y. Gauthier, D.B. Kimmel, S. Léger, F. Massé, M.E. McGrath, D.J. McKay, M.D. Percival, D. Riendeau, S.B. Rodan, M. Thérien, V.-L. Truong, G. Wesolowski, R. Zamboni, W.C. Black, Identification of a potent and selective non-basic cathepsin K inhibitor., Bioorg. Med. Chem. Lett. 16 (2006) 1985–1989. https://doi.org/10.1016/j.bmcl.2005.12.071.

[178] J.Y. Gauthier, N. Chauret, W. Cromlish, S. Desmarais, L.T. Duong, J.-P. Falgueyret, D.B. Kimmel, S. Lamontagne, S. Léger, T. LeRiche, C.S. Li, F. Massé, D.J. McKay, D.A. Nicoll-Griffith, R.M. Oballa, J.T. Palmer, M.D. Percival, D. Riendeau, J. Robichaud, G.A. Rodan, S.B. Rodan, C. Seto, M. Thérien, V.-L. Truong, M.C. Venuti, G. Wesolowski, R.N. Young, R. Zamboni, W.C. Black, The discovery of odanacatib (MK-0822), a selective inhibitor of cathepsin K., Bioorg. Med. Chem. Lett. 18 (2008) 923–928. https://doi.org/10.1016/j.bmcl.2007.12.047.

[179] S.A. Stoch, J.A. Wagner, Cathepsin K inhibitors: a novel target for osteoporosis therapy., Clin. Pharmacol. Ther. 83 (2008) 172–176. https://doi.org/10.1038/sj.clpt.6100450.

[180] H.G. Bone, M.R. McClung, C. Roux, R.R. Recker, J.A. Eisman, N. Verbruggen, C.M. Hustad, C. DaSilva, A.C. Santora, B.A. Ince, Odanacatib, a cathepsin-K inhibitor for osteoporosis: a two-year study in postmenopausal women with low bone density., J. Bone Miner. Res. Off. J. Am. Soc. Bone Miner. Res. 25 (2010) 937–947. https://doi.org/10.1359/jbmr.091035.

[181] A. Mullard, Merck & Co. drops osteoporosis drug odanacatib, Nat. Rev. Drug Discov. 15 (2016) 669. https://doi.org/10.1038/nrd.2016.207.

[182] T.M. Rünger, S. Adami, C.-L. Benhamou, E. Czerwiński, J. Farrerons, D.L. Kendler, L. Mindeholm, G. Realdi, C. Roux, V. Smith, Morphea-like skin reactions in patients treated with the cathepsin K inhibitor balicatib, J. Am. Acad. Dermatol. 66 (2012) e89–e96. https://doi.org/https://doi.org/10.1016/j.jaad.2010.11.033.

[183] K. Engelke, S. Nagase, T. Fuerst, M. Small, T. Kuwayama, S. Deacon, R. Eastell, H.K. Genant, The effect of the cathepsin K inhibitor ONO-5334 on trabecular and cortical bone in postmenopausal osteoporosis: the OCEAN study., J. Bone Miner. Res. Off. J. Am. Soc. Bone Miner. Res. 29 (2014) 629–638. https://doi.org/10.1002/jbmr.2080.

Figure(s)

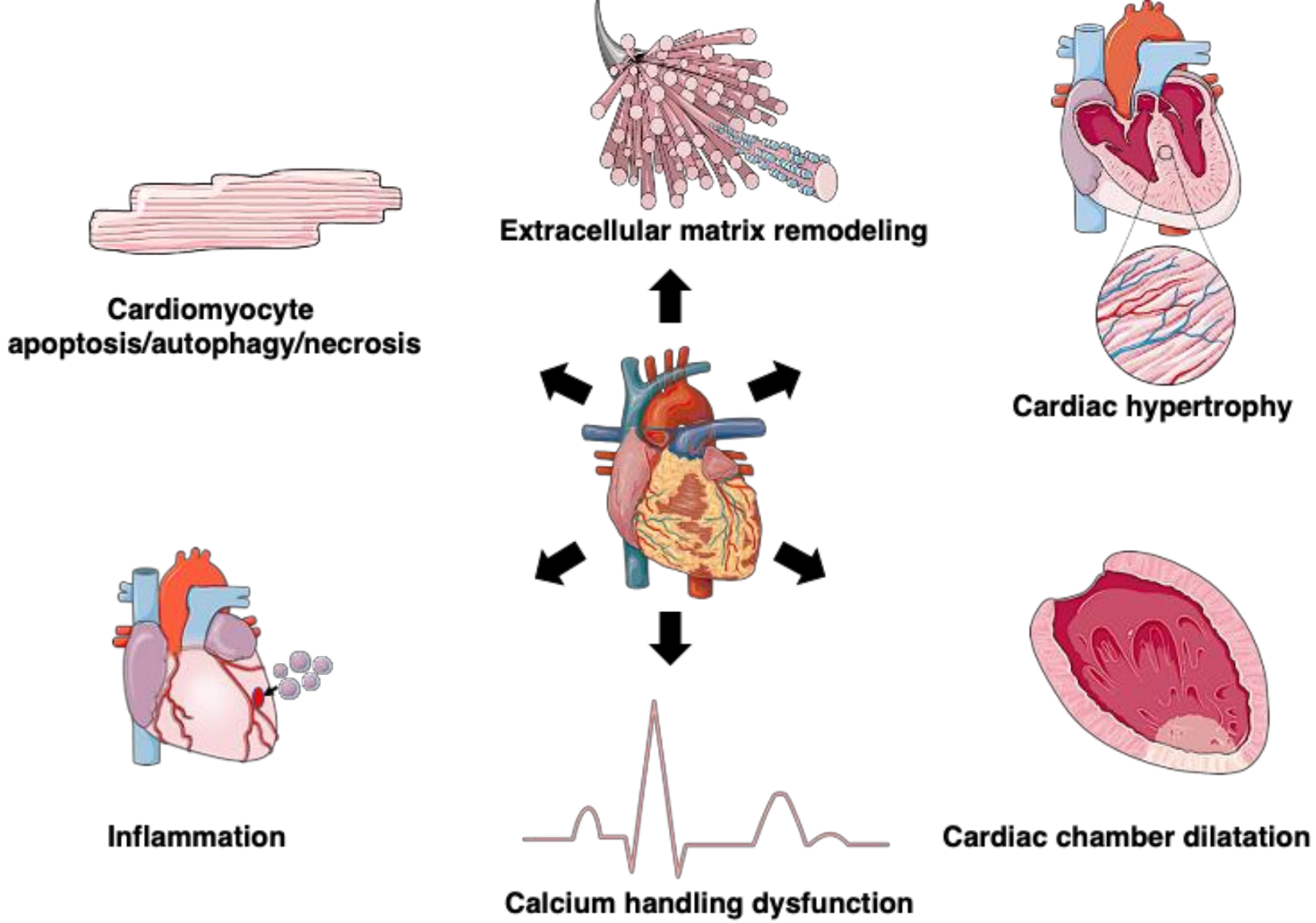

Figure 1. Adverse cardiac remodelling is characterized by structural and morphological abnormalities to the myocardium and extracellular matrix (ECM). These changes present clinically as ventricular wall thinning, impaired contractility and, chamber dilatation which are driven by molecular and cellular alterations such as ECM remodelling, inflammation, hypertrophy,, impaired calcium handling, apoptosis, necrosis, and autophagy.

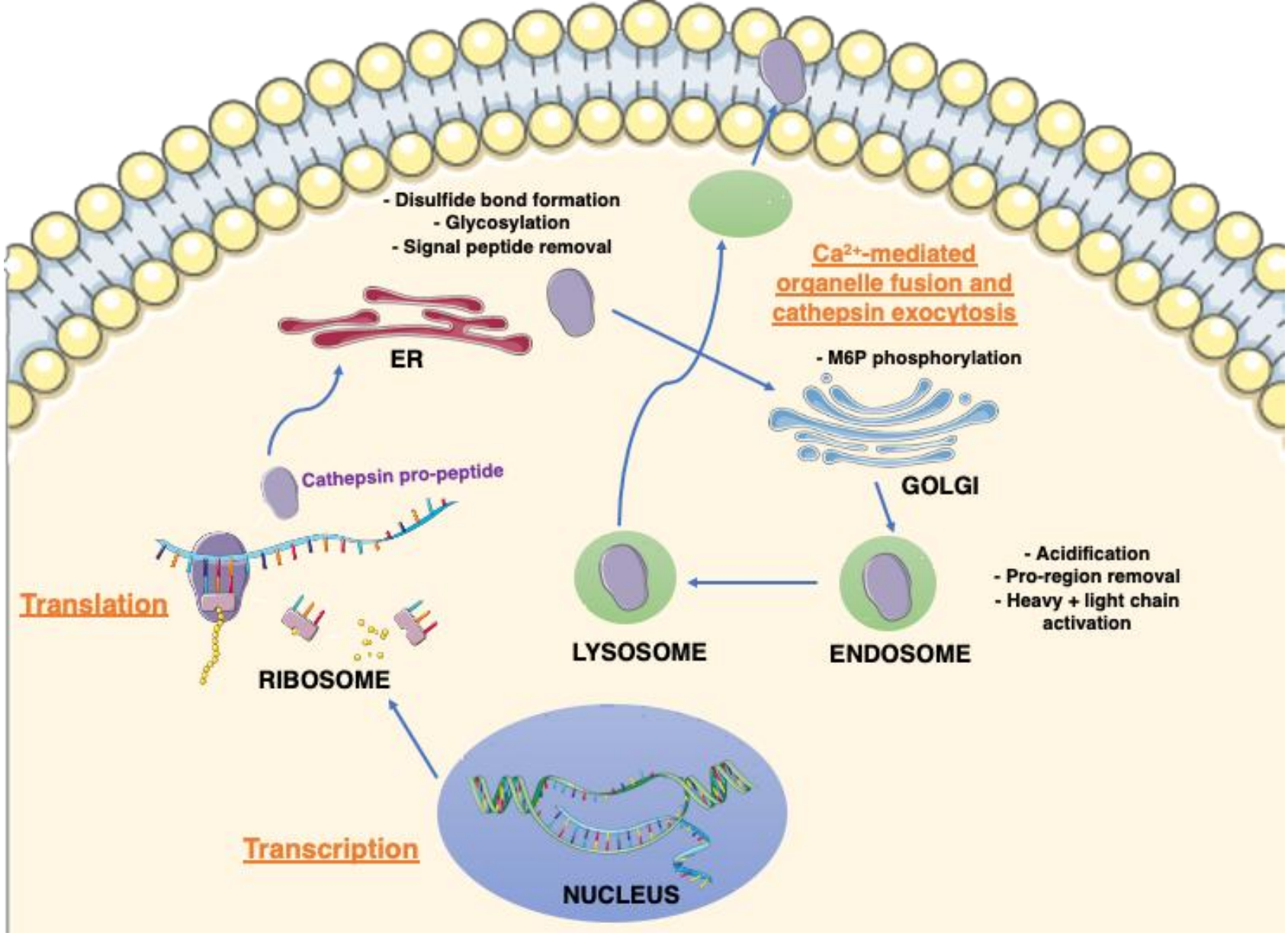

Figure 2. Cathepsin maturation process. Following nuclear transcription and ribosomal translation, the newly synthesised cathepsin pro-peptide undergoes a series of modifications in the endoplasmic reticulum (ER), including disulphide bond formation, glycosylation and signal peptide removal. The mannose-6 phosphate residue undergoes phosphorylation in the Golgi apparatus. This is followed by acidification, pro-region removal and heavy and light chain activation in the early and late endosome. Calcium (Ca^{2+})-mediated organelle fusion subsequently results in exocytosis of cathepsins into the extracellular space.

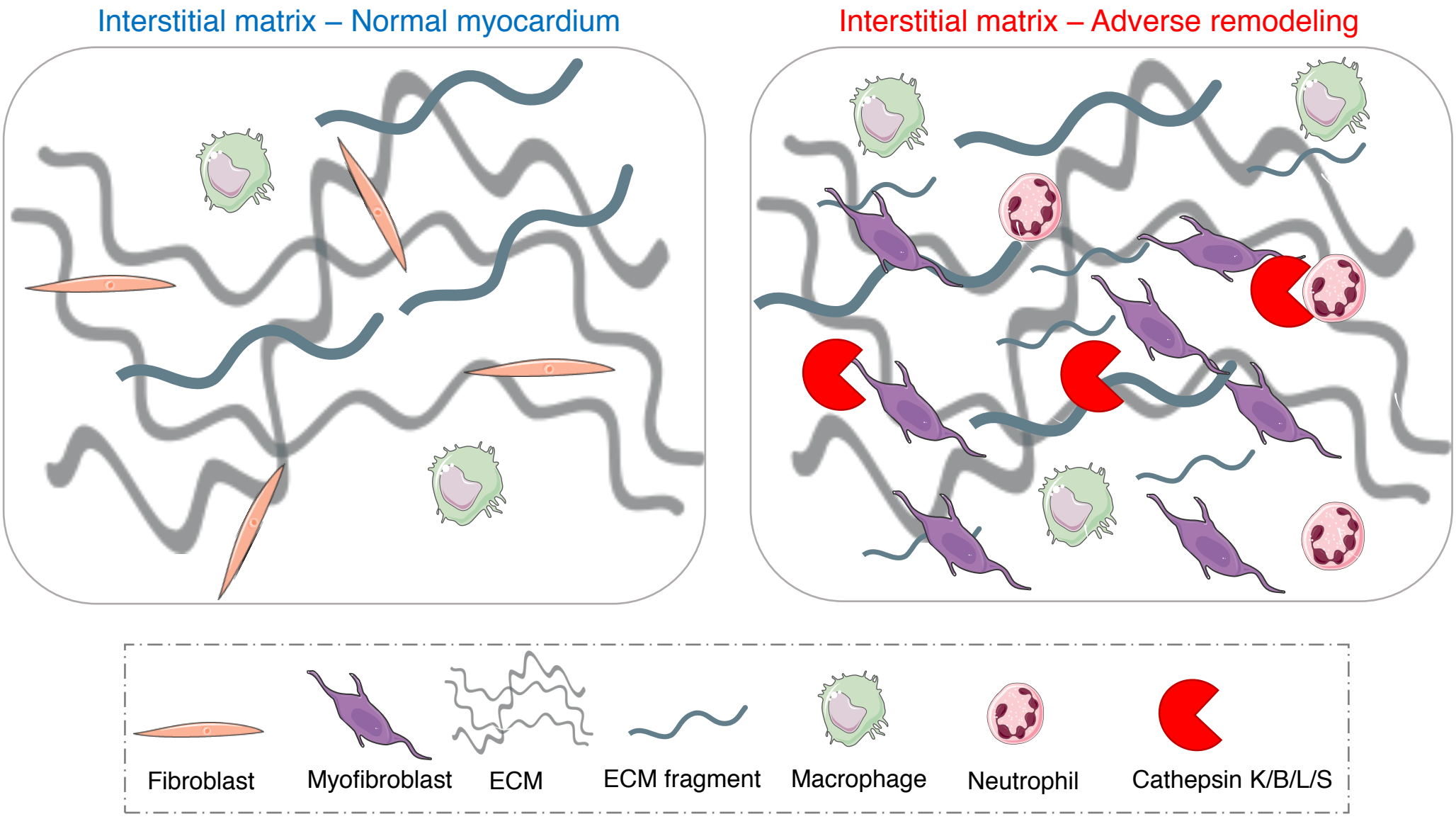

Figure 3. Cathepsins and ECM remodelling. Following myocardial insult, such as infarction, remodelling of the ECM ensues. The cysteine cathepsins K [84,85,27], B [39], L [87,88,40] and S [89,41], have all been shown to be involved in this process including; fibroblast differentiation to myofibroblasts, degradation of ECM proteins, increasing inflammatory cell infiltration and inflammatory signalling.

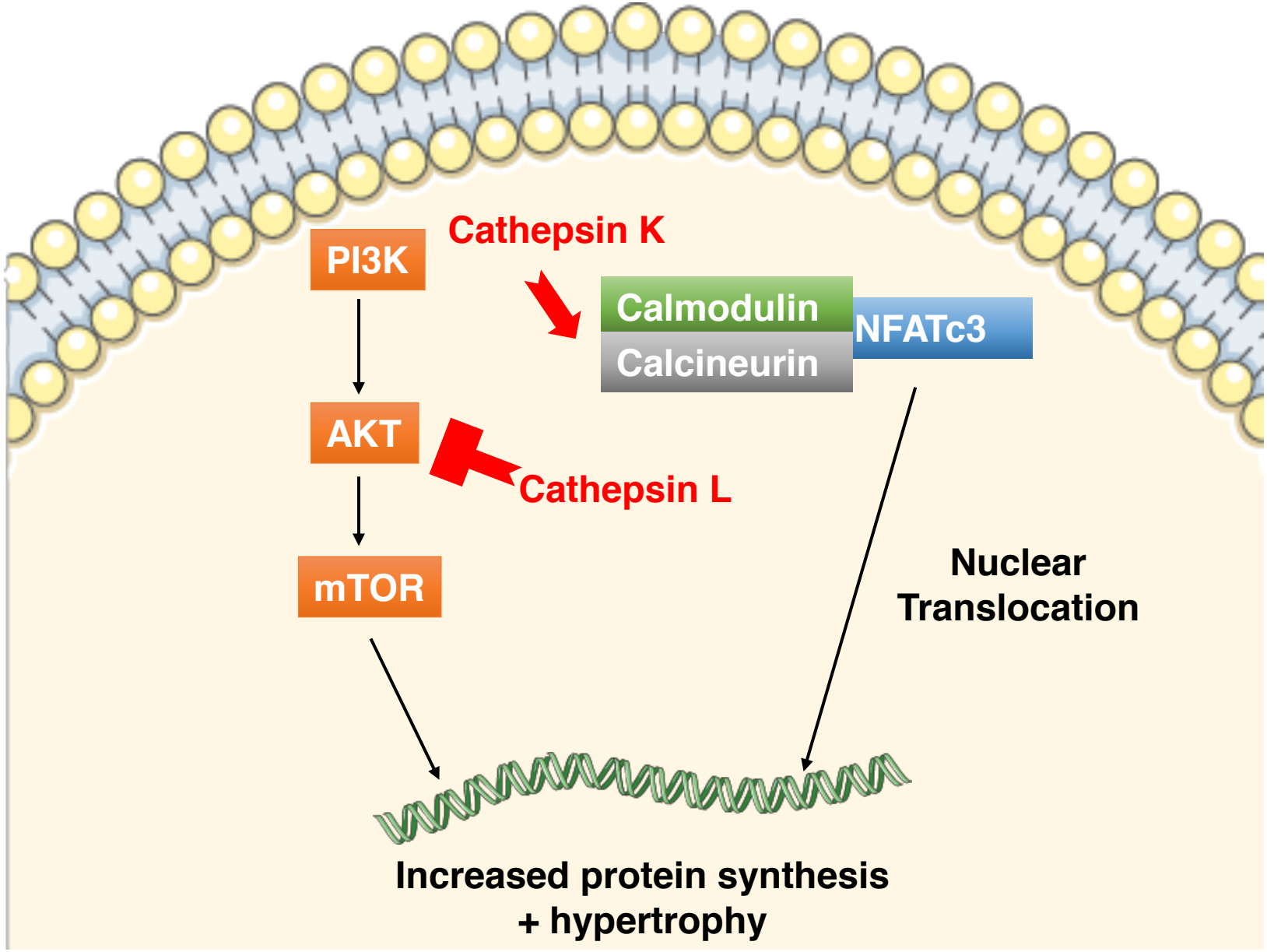

Figure 4. Cathepsins and cardiac hypertrophy. Cathepsin L has been shown to inhibit AKT phosphorylation [30], in such acting as a negative regulator of the PI3K/AKT/mTOR pathway. This leads to a decrease in protein synthesis and directly inhibits cardiomyocyte hypertrophy. It has also been shown that cathepsin K can activate calcineurin, resulting in NFATc3 dephosphorylation and subsequent nuclear translocation [27,97]. NFATc3 augments hypertrophic and pro-apoptotic gene transcription which can contribute to the development of cardiac disease particularly cardiomyopathies

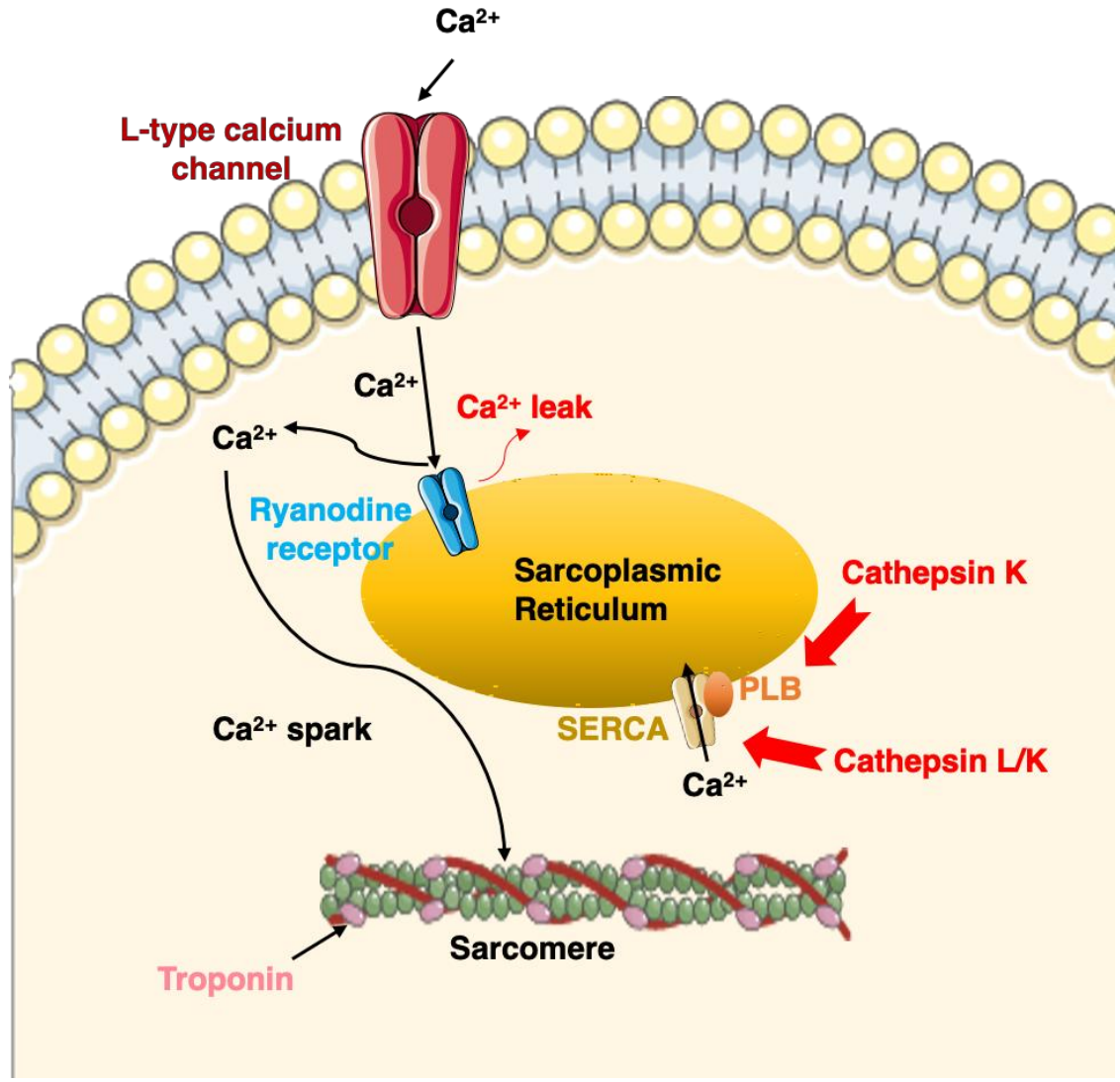

Figure 5. Cathepsins and cardiomyocyte calcium handling. Calcium (Ca^{2+}) enters the cardiomyocyte via L-type calcium channels. Ca^{2+} stimulates further Ca^{2+} release from the sarcoplasmic reticulum (SR) by interacting with ryanodine receptors. A Ca^{2+}-spark is triggered and Ca^{2+} binds to troponin in the sarcomere to initiate cardiomyocyte contractility. Ca^{2+} unbinds from troponin resulting in cardiomyocyte relaxation and calcium is pumped back into the SR through SR-ATPase (SERCA2a) where it is stored. Cathepsin L has been shown to reduce calcium influx from L-type calcium channels [114,115,36]. Cathepsin K has been shown to reduce the expression and activity of SERCA2a and phospholamban (PLB) [27,113,27,35,94] which results in impaired SR calcium translocation and contributes to cytosolic calcium overload.

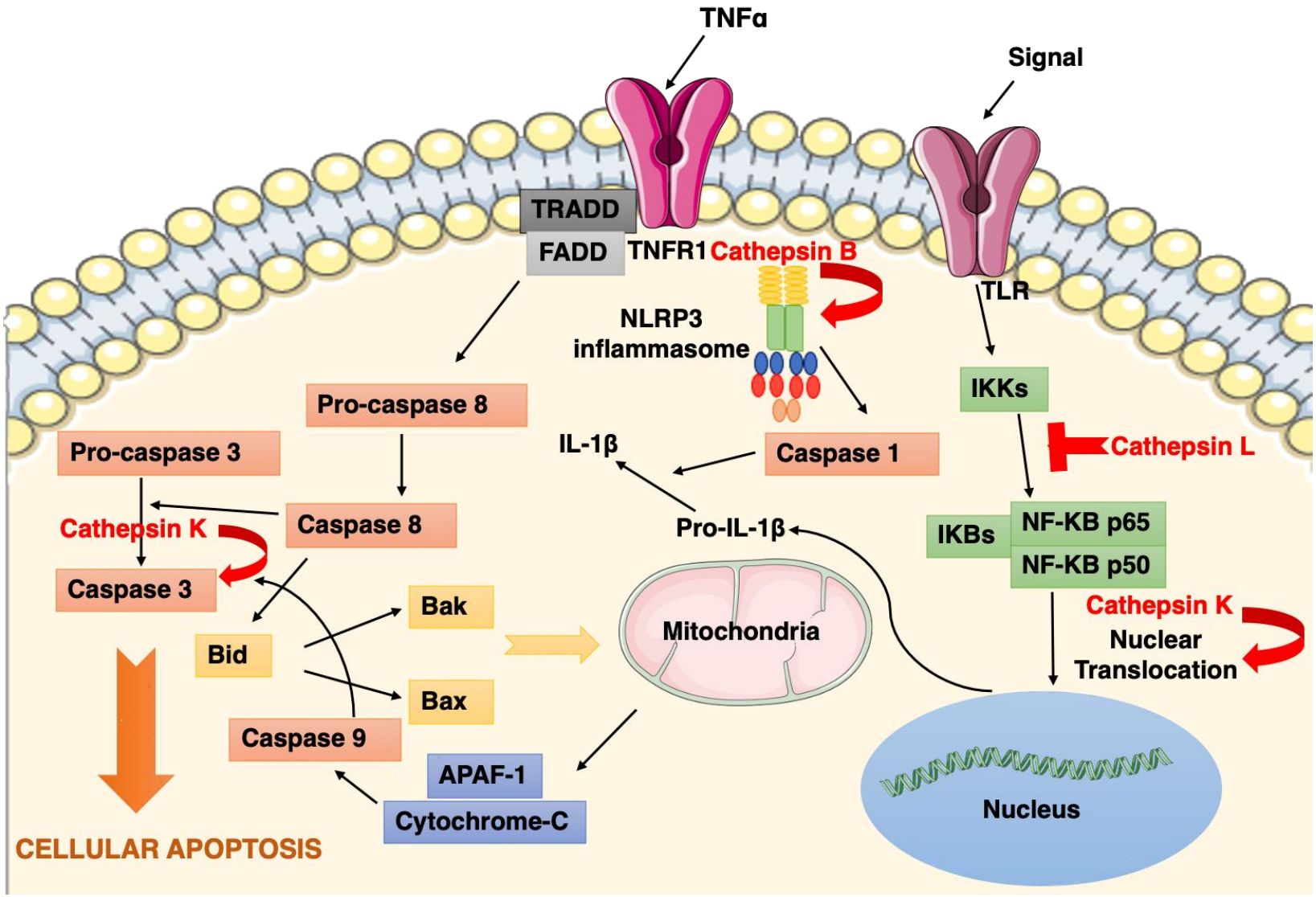

Figure 6. Cathepsins and apoptosis. Tumor necrosis factor alpha (TNF- α) activates both survival and proliferation pathways along with apoptotic pathways via TNFR1. Caspase 8 activation in the extrinsic apoptotic pathway activates cleaved caspase 3 to initiate the caspase cascade which culminates in cellular apoptosis. The mitochondrial pro-apoptotic proteins, Bid, Bax and Bak stimulate mitochondrial cytochrome c release which in association with apoptosis protein activating factor 1 (APAF-1) activates caspase-9 to further activate caspase 3. Cathepsin K can increase cardiomyocyte protein expression of cleaved caspase 3 leading to cellular apoptosis [34]. Additionally, toll like receptor (TLR) signalling which induces nuclear translocation of NF-kB can also be increased by cathepsin K [35], whereas it is inhibited by Cathepsin L [33,128]. Finally, the activation of caspase-1 by the NLRP3 inflammasome leads to mature IL-1β activation. Cathepsin B has been shown to increase the cellular expression of NLRP3 [127] and thus, increased expression of IL-1β.

***Declaration of Interest Statement**

Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: