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SPOTLIGHT REVIEW

The role of calpains in myocardial remodelling and heart failure

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Abstract Calpains are cytosolic calcium-activated cysteine proteases. Recently, they have been proposed to influence signal transduction processes leading to myocardial remodelling and heart failure. In this review, we will first describe some of these molecular mechanisms. Calpains may contribute to myocardial hypertrophy and inflammation, mainly through the activation of transcription factors such as NF-κB. They play an important role in the fibrosis process partly by activating transforming growth factor β. They are also implicated in cell death as they cause the breakdown of sarcolemma and sarcomeres. Nevertheless, a key to understanding the molecular basis of calpain-mediated myocardial remodelling likely lies in the identification of mechanisms involved in calpain secretion, since cytosolic and extracellular proteases would have different functions. Finally, we will provide an overview of the available evidence that calpains are indeed actively involved in the common causes of heart failure, including hypertension, diabetes, atherosclerosis, ischaemia-reperfusion, atrial fibrillation, congestive failure, and mechanical unloading.

Keywords Myocardial remodelling • Heart failure • Calpains • Extracellular matrix

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1. Introduction

Heart failure, a growing public health problem, develops in response to inherited or acquired abnormalities of myocardial structure and/or function. These changes are most commonly the consequence of a continuous cardiac stress such as pressure overload (e.g. due to hypertension) or energy metabolism alteration (e.g. hypoxia or hyperglycaemia).[1,2] They are characterized initially by an 'adapted' or physiological ventricular hypertrophy, then by an 'inadequate' pathological hypertrophy, associated with inflammatory/immune reactions, fibrosis, and ultimately by thinning of the ventricular walls with the death of cardiomyocytes, leading to functional decompensation. All these responses participate in cardiac plasticity or remodelling.

The development of cardiac remodelling implicates hormonal changes, including increased levels of circulating catecholamines and angiotensin II as a consequence of sympathetic nervous system stimulation and renin–angiotensin system activation, respectively. By producing oxidative stress and intracellular Ca^{2+} overload, these hormones activate proteases that play a role in the degradation of both intracellular (e.g. proteins involved in signal transduction and cell behaviour) and extracellular [e.g. proteins of the extracellular matrix (ECM)] targets.[3,4] Different proteases, such as matrix metalloproteinases (MMPs), cathepsins, caspases, and calpains, are thought to function cooperatively. This review will focus on the calpain system, describing successively some characteristics of this family of proteases, their molecular role in the pathophysiology of cardiac remodelling, and their involvement in the main causes of heart failure (summarized in *Tables 1 and 2*).

2. The calpain family

Calpains are calcium-activated neutral cysteine proteases.[5,6] Two major isoforms, calpain μ or 1, which requires micromolar Ca^{2+} concentrations for activity, and calpain m or 2, which requires millimolar Ca^{2+} concentrations, are ubiquitously expressed, whereas there are also tissue-specific forms of calpains. For instance, calpain3 (CAPN3) is a skeletal muscle-specific protease, although its expression appears transiently in the human embryonic heart.[7] Mutations in CAPN3 lead to a form of recessive limb-girdle muscular dystrophy (LGMD2A). In many cases, it is quite difficult to identify functional differences among all calpain species. In this review, the term calpain(s) will refer to μ- and m-calpain isoforms unless stated otherwise.

Both μ- and m-calpains form heterodimers with a common regulatory subunit, calpain 4/CAPNS1 (calpain small-1). Binding of Ca^{2+} to

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Table 1 Pathophysiological role of calpains in heart failure as evidenced by gene targeting

Gene alteration	Condition	Result
Calpastatin transgenic[17]	Physiological conditions	Progressive dilated cardiomyopathy
Calpastatin transgenic[31]	AII-induced hypertension	↓ Myocardial hypertrophy
Calpastatin transgenic[43]	AII-induced endothelium dysfunction	↓ Leucocyte adherence to endothelium
Calpastatin transgenic[62]	Mechanical unloading	No effect
Calpastatin transgenic[54]	Diabetes	↓ Myocardial hypertrophy and fibrosis
Calpastatin transgenic[64]	Endotoxaemia	↓ Myocardial dysfunction
μ-Calpain knockout[43]	AII-induced endothelium dysfunction	↓ Leucocyte adherence to endothelium
m-Calpain knockout[56]	Atherosclerosis	↓ Vascular hyper-permeability
Calpain small-1 knockout[50]	Pressure overload	↑ Contractile dysfunction
Calpain small-1 knockout[47]	Pulmonary hypertension	↓ Myocardial hypertrophy
Calpain small-1 knockout[54]	Diabetes	↓ Myocardial hypertrophy and fibrosis

μ- or m-calpain induces the release of constraints that are imposed by domain interactions and results in activation process.[5] In the absence of cytosolic calcium flux, calpains can be activated after direct phosphorylation of serine 50 by extracellular signal-regulated kinases (ERK). Interestingly, during myocardial ischaemia, calcium overload induces calpain translocation to the sarcolemma but intracellular acidosis prevents its activation.[8]

Calpain activity, which is tightly controlled by calpastatin, a specific endogenous inhibitor, is responsible for the limited proteolysis of specific substrates. As they are expressed in the cytosol, these targets are thought to be intracellular. Structural determinants of calpain substrates are still not well defined. Several of them (e.g. IκBα which binds to dimeric nuclear factor-κB (NF-κB) complex, and ATP-binding cassette transporter A1, ABCA1) contain a sequence rich in proline, glutamic acid, serine, and threonine (PEST domain) enhancing both calpain-binding and calpain-dependent proteolysis.[9,10] Other possible sequence determinants of calpain cleavage have been defined as well.[11] Finally, it appears that many of the calpain substrate proteins are also MMP-2-degratative targets, suggesting that the two proteases share identical substrates.[12]

Alternatively, because the identification of protein substrates common to calpains and MMP-2 has been based on the response to non-selective calpain inhibitors (i.e. affecting MMP-2 in addition to calpains), calpain efficiency could have been overestimated. Nevertheless, the physiological functions of calpains have been assessed also using loss-of-function studies. Disruption of the mouse μ-calpain gene (*Capn1*) provides evidence that μ-calpain is essential only for normal platelet function.[13] Homozygous disruption of the mouse m-calpain gene (*Capn2*) results in pre-implantation embryonic lethality

Table 2 Targets of calpains in the pathogenesis of heart failure

Target	Condition	Calpain role	Result
ABCA1[56]	Atherosclerosis	ABCA1 degradation	↓ HDL formation
AKT-associated HSP90[38,39]	Cardiac remodelling	AKT inactivation	↓ Cardiac hypertrophy
Calcineurin inhibitory domain, cain/cabin1[36,37]	Cardiac remodelling	NFAT activation	↑ Cardiac hypertrophy
Cytoskeleton actin[51,52]	Cardiac ischaemia/reperfusion	↓ Resistance to membrane resealing	Plasma membrane repair
Fibronectin[20]	Cardiac ischaemia/reperfusion	Endothelial cell migration and growth	Endothelium repair
Fodrin and ankyrin[49]	Cardiac ischaemia/reperfusion	Sarcolemmal rupture	Loss of cardiac sarcomere structure
IκBα[30,31]	Cardiac remodelling	NF-κB activation	↑ Cardiac hypertrophy and inflammation
L-type Ca^{2+} channel protein[29]	Atrial fibrillation	L-type Ca^{2+} channel cleavage	↓ Excitation—contraction coupling
Talin and ezrin[31]	Cardiac remodelling	Cytoskeletal changes → Cell migration	↑ Cardiac inflammation
TGF-β-associated LAP[45-47]	Cardiac remodelling	↑ TGF-β-dependent collagen synthesis	↑ Cardiac fibrosis
Troponin T[49]	Cardiac ischaemia/reperfusion	Myofibrillar degradation	Loss of cardiac sarcomere structure
VE-cadherin[56]	Atherosclerosis	Loss of adherence junctions	Pro-atherogenic hyperpermeability

ABCA1, ATP-binding cassette transporter A1; HDL, high-density lipoprotein; HSP90, heat shock protein 90; NFAT, nuclear factor of activated T cells; IκBα, inhibitor of kappa B-alpha; NF-κB, nuclear factor-κB; TGF-β, transforming growth factorβ; LAP, latency-associated peptide.

between the morula and blastocyst stage, demonstrating that m-calpain is mandatory for the development of embryo and that μ- and m-calpain have distinct functions.[14] Finally, disruption of the mouse CAPNS1 gene (*Capns1*) eliminates both μ- and m-calpain activities and is responsible for the death of embryos at E11.5.[15] At E10.5, embryos display defects particularly in the cardiovascular system. Fibroblasts derived from those mice demonstrate a physiological role for calpains in cell migration, cytoskeleton organization, apoptosis, autophagy, plasma membrane repair, and membrane blebbing.[16] In a gain-of-function approach, conditional overexpression of μ-calpain, but not m-calpain, in heart of transgenic mice causes significant proteolytic activity in unstressed myocardium, increasing ubiquitination of cardiac proteins and proteasomal activity.[17] Conversely, conditional overexpression of calpastatin in the heart diminishes ubiquitination of myocardial proteins, resulting in a progressive dilated cardiomyopathy with pathological accumulation of specific non-ubiquitinated proteins that are shunted to autophagic destruction pathways.[17]

Even if calpains are mainly located in the intracellular compartment, a fraction of these proteases is detectable in the extracellular space of tissues. Indeed, calpains are actively secreted independently of cell destruction. This turns attention to another emerging aspect of calpains, the molecular mechanisms of their secretion and their functions when exteriorized. Calpains are secreted by lymphocytes, endothelial cells, chondrocytes, and osteoblasts, among other cells.[18–20] This secretion is thought to be in an unconventional way due to the lack of N-terminal classic secretion signal peptide. Nevertheless, in addition to associating with the cytosolic side of the endoplasmic reticulum and the Golgi apparatus, m-calpain is also contained in their lumen, allowing eventually their secretion.[21] Alternatively, calpains can be secreted in membrane microvesicles, as demonstrated in the environment of lymphocytes, endothelial, and parathyroid cells.[18,20,22] Serine phosphorylation by ERK and protein kinase Cι might be essential for this process.[23,24] Conversely, protein phosphatase 2A (PP2A), a physiological calpain phosphatase, decreases their secretion, a control amplified by C2-ceramide.[25] The role of calpains once outside the cell is still not well defined. They damage cell[26] and, as intracellular ones, promote migration and invasion of cells, e.g. by targeting ECM proteins.[23] Interestingly, a novel hypothesis proposes that unconventional secretion provides a mechanism through which the functions of a single enzymatic activity differ dramatically according to intracellular or extracellular localization.[27] It is, thus, expected that the functions of intracellular and extracellular calpains would differ. We will address this issue when highlighting specialized roles of calpains in pathophysiology of cardiac remodelling.

3. Molecular roles of calpains in pathophysiology of cardiac remodelling

3.1 Hypertrophy

As mentioned above, cardiac remodelling is characterized by hypertrophy. This process is defined by an increase in the individual cardiomyocyte size in length and/or width, that is initiated by stretch-sensitive mechanisms (mechanical deformation detected partly by integrins) and neurohumoral mechanisms (release of catecholamines, endothelin-1, angiotensin II, cytokines, chemokines, and growth factors).[28] Intracellular signalling pathways shared by these different stimuli and responsible for an imbalance between protein synthesis and degradation would be a target for calpain activity.[29] Nuclear factor-κB has recently emerged as a key transcription factor in this process. Calpains degrade the inhibitor IκBα, a necessary step in nuclear translocation of NF-κB, and mice lacking the p50 subunit of NF-κB or expressing an NF-κB super-repressor show limited cardiac hypertrophy in response to chronic angiotensin II infusion.[30] In addition, we demonstrated that infusion of angiotensin II leads to a marked increase in the nuclear expression of NF-κB p65 subunit within the heart of wild-type mice, and significant blunting of these angiotensin II-mediated effects in mice expressing high levels of calpastatin.[31] The signalling cascade downstream angiotensin II binding to the G protein-coupled angiotensin II type 1 receptor (AT_1R) would include successively inositol 1,4,5-trisphosphate ($InsP_3$) release from the cardiomyocyte membrane, $InsP_3$ binding to $InsP_3$ receptors of the endoplasmic reticulum, and calcium release from this storage structure into the cytosol, allowing eventually calpain activation (*Figure 1*).[29,32–34] Calcium release in response to $InsP_3$ receptor engagement is thought to be amplified by the local expression of the calcium storage protein chromogranin B (CGB). In turn, NF-κB promotes the transcription of numerous genes that code for apoptosis inhibitory molecules (iAP1, bcl-2, bcl-xL) and mediators of cardiomyocyte hypertrophy.[29,30] However, NF-κB-binding sites in the promoter regions of genes involved in ventricular hypertrophy and associated foetal gene expression have not been identified.[35] Alternatively, transcription control by NF-κB might involve its association with other transcription factor(s) and/or its indirect role through the expression of different signalling pathways.

Calpains also activate the serine/threonine PP calcineurin via the proteolysis of its autoinhibitory domain or via the cleavage of the endogenous calcineurin inhibitor cain/cabin1,[36] thus leading to the dephosphorylation of conserved serine residues at the N terminus of nuclear factor of activated T cells (NFAT), a transcription factor (*Figure 1*). As a consequence, dephosphorylated NFAT translocates to the nucleus in tissues including the heart, and activates pro-hypertrophic gene expression.[28] *In vivo*, NFAT transcription factors would be involved in cardiac hypertrophy since NFATc3-null mice have blunted cardiac hypertrophy following angiotensin II infusion.[37] However, based on our results, calpain activity mediates angiotensin II-dependent left ventricular hypertrophy through an NFATc3-independent process.[31] Similarly, CGB amplification loop of calcium signalling does not affect NFAT response.[33,34]

Additional signalling cascades initiated by calpains may paradoxically limit cardiac hypertrophy (*Figure 1*). Engagement of G-protein-coupled receptors (GPCR) activates phosphatidylinositol 3-kinase, thereby promoting the recruitment of the serine-threonine kinase Akt/protein kinase B (AKT) and phosphoinositide-dependent kinase-1 (PDK1). As a consequence, PDK1 phosphorylates and activates AKT.[28] AKT-mediated phosphorylation of the glycogen synthetase kinase 3β limits its antihypertrophic effects, thereby inducing pathological cardiac hypertrophy.[28,38] Because calpains negatively regulate the AKT pathway [e.g. by degrading AKT-associated heat shock protein (HSP) 90], they would blunt those hypertrophy mechanisms.[29,38,39] Thus, calpain activity affects many of the signalling effectors that are implicated in remodelling. The balance between these pathways exerting opposite effects, ends in cardiac hypertrophy.

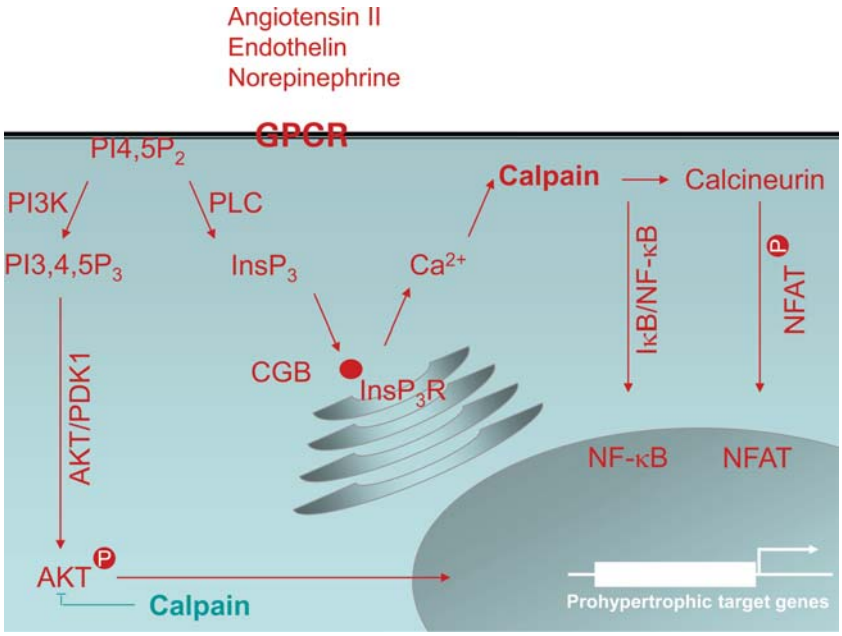

Figure 1 Schematic representation of cellular signalling pathways involved in calpain-dependent cardiomyocyte hypertrophy. Hormone binding to G-protein-coupled receptors (GPCR) activates phospholipase C (PLC), leading to the production of inositol-1,4,5-triphosphate ($InsP_3$) from phosphatidylinositol-4,5-biphosphate ($PI4,5P_2$). In turn, binding of $InsP_3$ to its receptor ($InsP_3R$) at the surface of the endoplasmic reticulum induces the release of calcium through a mechanism amplified by chromogranin B (CGB). Thereby, calpains become activated and degrades IκB, allowing the release of nuclear factor-κB (NF-κB), a transcription factor involved in the expression of pro-hypertrophic genes. Calpains may also activate calcineurin, a serine phosphatase responsible for the translocation of nuclear factor of activated T cells (NFAT) to the nucleus where it induces the expression of pro-hypertrophic genes. However, NFAT involvement in hypertrophy response to angiotensin II has been excluded. Finally, activated calpain may paradoxically limit the expression of pro-hypertrophic genes by degrading the heat shock protein 90 that stabilizes AKT phosphorylated by phosphoinositide-dependent kinase-1 (PDK1).

3.2 Inflammatory/immune reaction

Continuous cardiac stress, irrelevant to its cause, initiates an inflammatory and immune response.[40–42] Following an initial insult to the myocardium, the expression of endogenous stress proteins, such as HSP10, HSP60, and HSP70 increases, being both associated to the cells and secreted. These stress proteins, the so-called damage-associated molecular patterns or 'alarmins', represent a ligand for toll-like receptors (TLRs) expressed at the surface of inflammatory/immune cells and cardiac cells themselves. Binding of HSPs to TLRs causes the expression of cell adhesion molecules, chemokines, and chemokine receptors, leading to both the recruitment and the activation of a range of inflammatory cells, including monocytes. These cells release cytotoxic compounds, worsening endothelial cell damage and, hence, amplifying alarmin expression.[41]

That calpains play a key mechanistic role in these processes has been well demonstrated in experimental models of angiotensin II-induced cardiovascular remodelling.[40–43] Mice expressing high levels of calpastatin have an impaired (neutrophils) and delayed (monocytes and lymphocytes) ability to recruit inflammatory cells.[31] This is attributable to a defect in NF-κB-dependent chemotaxis process (as reflected by a decrease in chemokine release and inflammatory cell response to chemokine gradient), and/or to a limited cleavage of cytoskeletal linkage molecules, such as talin and ezrin, which are responsible for the extravasation and the migration of leucocytes.[31] Similarly, endothelium adhesiveness to circulating leucocytes in response to angiotensin II infusion appears limited in μ-calpain deficient or calpastatin overexpressing mice.[43] Additional mechanistic studies demonstrated the contribution of endothelial-expressed rather than leucocyte-expressed calpain in that process.[43]

3.3 Fibrosis

Interstitial fibrosis participates in myocardial remodelling and contributes to increase myocardial stiffness, to alter diastolic and systolic functions, and to exaggerate the risk of arrhythmias.[3,44] These changes constitute either a 'reparative' response to tissue injury (e.g. ischaemia and inflammation) or a 'reactive' response to stimulation of fibroblasts (e.g. in hypertension).[44] Fibrosis development requires the transformation of fibroblasts into active myofibroblasts that express α-smooth muscle actin, migrate, and secrete both pro-inflammatory cytokines and collagens. Obviously, accumulation of type I and III collagens and of other constituents of ECM in the interstitium and perivascular regions of the myocardium depend mainly on the balance between deposition and degradation of ECM proteins. Calpain activity would affect this equilibrium. In our experimental model of angiotensin II-mediated cardiovascular remodelling, we demonstrated that overexpression of calpastatin limited fibrosis around aorta and tissue arteries, as evidenced by polarized light microscopy analysis of Sirius red staining and immunohistochemical analysis of type I collagen.[31] This change was mirrored paradoxically by a decrease in MMP activity. Thus, the antifibrotic action of calpastatin would be explained by a decrease in collagen deposition rather than an increase in collagen degradation. This hypothesis is consistent

with our observation that vascular smooth muscle cells derived from the aorta of mice overexpressing calpastatin produced much less collagen in response to angiotensin II.[31]

Binding of angiotensin II to its AT1 receptor is thought to promote a transactivation of epidermal growth factor receptor (EGFR). In turn, EGFR engagement activates calpains via increased intracellular calcium and mitogen-activated protein kinase. We confirmed these molecular mechanisms in our experimental model of angiotensin II-mediated cardiovascular remodelling.[31] Downstream of EGFR-dependent calpain activation, the signalling pathways involved in ECM protein synthesis appear under the main control of transforming growth factor β (TGF-β).[44] Its expression, which is induced by angiotensin II, appears up-regulated in the pressure-overloaded heart. Mice deficient in TGF-β1 exhibit attenuated fibrosis of the ageing heart. Conversely, mice overexpressing TGF-β1 develop heart interstitial fibrosis. Signalling through TGF-β receptors, expressed in both cardiomyocytes and heart fibroblasts, involves the phosphorylation of Smad proteins, which act as transcription factors (*Figure* 2). The promoter of type I collagen includes Smad-binding sites. In addition, TGF-β, through signalling cascade involving Smads, induces the expression of connective tissue growth factor β (CTGF), which is essential for collagen synthesis. Notably, extracellular calpains activate the latent form of TGF-β by cleaving latency-associated peptide (LAP).[45] In addition, recent publications demonstrate that intracellular calpains as well may activate the latent form of TGF-β, e.g. in the Golgi of pulmonary arteriole smooth muscle cells.[46,47] This intracrine TGF-β signalling would be involved in subsequent CTGF expression and collagen deposition.[46,47]

3.4 Apoptosis/necrosis... and repair

Calpains also play an important role in cell death, particularly well identified during myocardial ischaemia-reperfusion.[48] During the reperfusion phase, Ca^{2+} concentration increases in cardiac sarcomere, as a consequence of a reverse activity of the sarcolemmal Na^+/Ca^{2+} exchanger. Indeed, at that time, inactivity of Na^+-pump increases cytosolic Na^+ concentration. Calcium-activated calpains hydrolyse proteins in the sarcolemma and the cytoskeleton, including α-fodrin and ankyrin, both injuries leading to sarcolemmal rupture. In addition, degradation of α-fodrin and ankyrin speeds up detachment of the Na^+-pump, increasing again cytosolic Na^+ concentration, Ca^{2+} influx through reverse Na^+/Ca^{2+} exchanger and, hence, sarcolemmal injury by calcium-activated calpains. Finally, calpain activity is responsible for the proteolysis of troponin, promoting myofibrillar degradation and thereby the loss of cardiac sarcomere structure.[49]

Two strategies designed to limit cardiomyocyte death during the reperfusion phase affect calpain activity. First, ischaemic preconditioning (brief episodes of myocardial ischaemia) limits cardiomyocyte death induced by subsequent prolonged myocardial ischaemia-reperfusion. This protection, which is characterized by a decrease in calpain activation, could be due to an attenuation of Ca^{2+} signal, and/or a protein kinase A-dependent phosphorylation of calpain.[48] Second, postconditioning (prolongation of acidosis during the first minutes of cardiac reperfusion) protects cardiomyocytes, partly by limiting calpain activity.[8]

Although the role of calpains in cardiomyocyte death is quite well established, emerging evidence indicates that these proteases could also be protective. Using a model of pressure overload by transverse aortic constriction, Taneike *et al.*[50] recently demonstrated that cardiac-specific deletion of *Capns1* limits local expression of μ- and m-calpain and paradoxically worsens heart failure. Molecular mechanisms would involve defective repair of cardiomyocyte membrane, leading to cardiomyocyte loss and replacement by fibrosis. The precise role of calpain in the membrane repair process is still not elucidated, but mechanistic hypotheses are envisaged.[16,51] Calpain

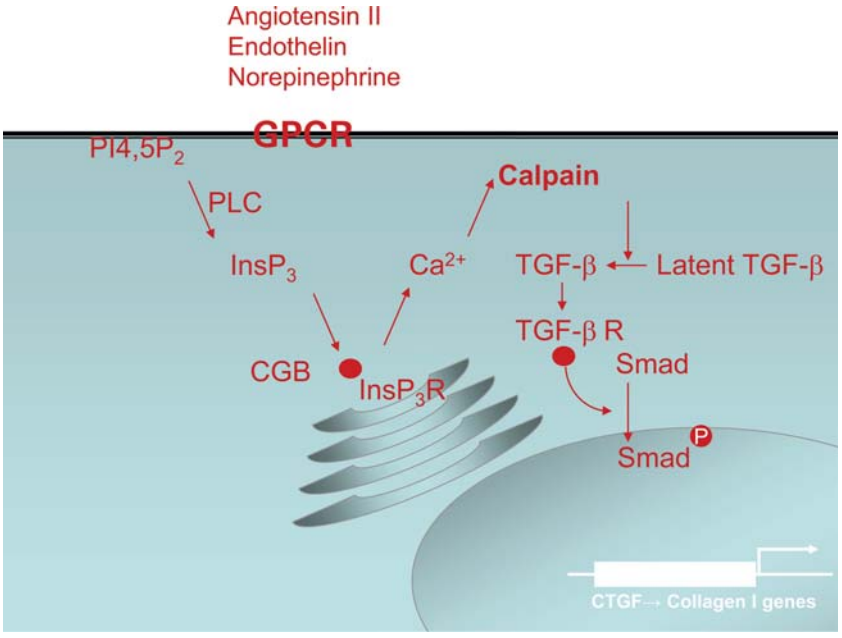

Figure 2 Schematic representation of cellular signalling pathways involved in calpain-dependent myocardial fibrosis. Calpains activate transforming growth factor β (TGF-β), which binds its specific receptors TGF-βRI and TGF-βRII. In turn, TGF-βR intracrine signalling allows Smad phosphorylation and translocation into the nucleus where it promotes the expression of connective tissue growth factor β (CTGF) and collagen I genes.

activity is involved in cytoskeletal remodelling after membrane disruption and in the release of membrane microvesicles targeted to and fusing with damaged membrane. This forms a membrane patch that seals the membrane disruption. Besides intracellular calpains, exteriorized calpains could play a major role, since fetuin A, an extracellular protein, facilitates plasma membrane repair by stabilizing m-calpain.[52] Given these data and our report that exteriorized calpains speed up endothelium repair,[20] extracellular calpains could be considered as mainly protective.

4. Calpains in the common causes of heart failure

4.1 Hypertension

Hypertension is characterized by increased arteriole pressure and total peripheral resistance, leading to haemodynamic overload, cardiac remodelling, and ultimately heart failure. In Milan hypertensive rats, calpastatin levels in the heart decrease markedly as a function of ageing.[53] Loss of calpastatin is completely reversed by antihypertensive drugs affecting intracellular calcium homeostasis, suggesting that calpastatin is degraded by calcium-activated calpains. Thus, alteration of the balance calpain/calpastatin could be considered as a risk factor in essential hypertension.

4.2 Diabetes

Diabetes worsens ischaemic damage of the heart and promotes *per se* cardiomyopathic changes, such as hypertrophy, fibrosis, and cardiomyocyte apoptosis. In the streptozotocin-induced model of type 1 diabetes in mice, overexpression of calpastatin or cardiac-specific deletion of *Capns1* reduces myocardial hypertrophy and fibrosis, leading to improvement of myocardial function.[54] Interestingly, cardiac-specific deletion of *Capns1* and calpastatin overexpression inhibits the activities of both MMP-2 and MMP-9 through up-regulation of tissue inhibitors of metalloproteinases (TIMP-1 and -2). This illustrates again coordinated activities between calpains and other proteases. Finally, treatment of ZDF rats, a genetic model of type 2 diabetes, with a pharmacological inhibitor of calpains limits vascular inflammation by preserving endothelial nitric oxide synthase.[55]

4.3 Atherosclerosis

The proatherogenic role of μ-calpain has been attributed to its involvement in the degradation of ABCA1, which participates in cholesterol clearance through the formation of high-density lipoprotein. Furthermore, the endothelial cell-specific expression of m-calpain is induced in atherosclerotic lesions by the action of modified low-density lipoprotein. In turn, m-calpain cleaves vascular endothelial-cadherin, a protein responsible for homophilic associations between adjacent endothelial cells.[56] This cleavage leads to the disorganization of adherence junctions and, hence, proatherogenic hyperpermeability. Thus, as expected, calpain inhibition by a specific inhibitor attenuates angiotensin II-induced atherosclerosis development in LDL receptor $^{-/-}$ mice.[57]

4.4 Ischaemia reperfusion injury

The expression of m-calpain increases 3 days after myocardial infarction, mainly in interventricular septum, whereas μ-calpain expression peaks later on, i.e. only after 2 weeks, and rather in left ventricular free wall.[58] The level of calpastatin remains unchanged.[4] The imbalance between calpains and calpastatin would explain cardiac tissue damage in the early phase and cardiac remodelling in the late phase, respectively. Accordingly, limiting calpain activity with pharmacological inhibitors in experimental myocardial infarction both reduces cardiomyocyte loss and improves ventricular function.[59]

4.5 Atrial fibrillation

Calpain activity could be responsible for atrial fibrillation through the cleavage of specific L-type Ca^{2+} channel protein and thereby the disruption of the excitation-contraction coupling.[29] These changes in fibrillating atria are associated with reduced amounts of troponin T and degradation of myofilaments.[60]

4.6 Congestive heart failure

There is also evidence for calpain involvement in congestive heart failure. Its expression increases in ventricular tissue, limited to μ-calpain in milder cases of congestive heart failure (class II on the New York Heart Association scale) and including both μ- and m-calpains after heart failure progression (classes III and IV on the New York Heart Association scale).[29] Calpain-induced regulatory pathways involved in the transduction of cardiac remodelling, including increase in cain/cabin1 cleavage and calcineurin activation, have been evidenced under these conditions.[61]

4.7 Mechanical unloading

In the failing heart, expression and activity levels of μ- and m-calpains appear elevated.[29,62] However, unexpectedly, overexpression of calpastatin did not attenuate unloading-induced cardiac remodelling, suggesting the compensatory role of other proteases.[62]

5. Conclusion

Data are emerging that associate calpains with the remodelling process, including ventricular hypertrophy, inflammation, and fibrosis, and ultimately thinning of the ventricular walls. Thus, targeting the calpain pathway would be a novel therapeutic approach for patients with heart failure. The strategies previously tested in animal models include the suppression of calpain expression or activity and the overexpression of calpastatin. For instance, continuous perfusion of a pharmacologic inhibitor of calpains (e.g. MDL-28170) at the initial period of reperfusion after myocardial ischaemia appears efficient to reduce infarct size.[63] Similarly, overexpression of calpastatin in a model of endotoxaemia inhibits calpain activation and improves myocardial function.[64] Finally, taking into account the capacity of extracellular calpains to repair the plasma membrane of injured myotubes[52] and to speed up the regeneration of capillary endothelium,[20] strategies aimed at increasing calpain externalization can be envisaged. Identification of molecular mechanisms involved in calpain externalization will be essential before taking advantage of such a therapeutic approach.

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