Chapter 26
Intracellular Matrix Remodeling and Cardiac Function in Ischemia–Reperfusion Injury

Xiaohu Fan, Mohammad A.M. Ali, Bryan G. Hughes, Anna Laura B. Jacob-Ferreira, and Richard Schulz

Abstract The effects of ischemia on animal and human myocardium have been extensively studied during the last four decades. Myocardial ischemia followed by subsequent reperfusion can cause profound damage to cardiac myocytes through enhanced oxidative stress and intracellular Ca^{2+} overload. Ischemia–reperfusion (I/R) injury leads to structural and functional remodeling of multiple intracellular matrix components in the cardiac myocyte. The intracellular matrix of cardiac myocytes includes major cytosolic components that include the cytoskeleton, contractile myofibrils, and subcellular organelles such as mitochondria, sarcoplasmic reticulum, and the nucleus. There are several proteolytic pathways inside the cell which may participate in cell injury and/or cell repair upon reperfusion of ischemic heart muscle. These include matrix metalloproteinases, calpains, lysosomal proteases, and the proteasome system, which are a major focus of research in I/R injury. The discovery of intramyocyte matrix metalloproteinase-2 (MMP-2) and biologically relevant protein substrates of it in the intracellular matrix has shaped a new paradigm of the pathophysiological role of MMP-2 during myocardial I/R injury. Emerging evidence indicates that oxidative stress can efficiently activate intracellular MMP-2 which rapidly mediates intracellular matrix remodeling of injured myocytes. This chapter will focus on the structural and functional remodeling of the intracellular matrix including the sarcomere, cytoskeleton, mitochondria, and nucleus, by proteolytic and other processes, in the context of I/R injury, with a particular emphasis on the rapidly expanding knowledge of the biology of intracellular MMP-2.

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26.1 Sarcomeric Protein Degradation and Contractile Dysfunction

The cardiac sarcomere (Fig. 26.1) is the basic contractile unit of the heart which contains the contractile components of the thin (actin), thick (myosin), and third (titin) myofilaments essential for cardiac function. The acute contractile defect of the heart after I/R injury [1] has been shown to involve proteolysis and/or disorganization of myofilament proteins [2, 3] including troponin I, myosin, and titin [4, 5].

26.1.1 Calpain-Mediated Remodeling in Ischemic Heart Disease

Calpains are a family of non-lysosomal cysteine proteases consisting of several isoforms. The best characterized isoforms are calpain-1 (μ-calpain), calpain-2 (m-calpain), and calpain-3 (p94 calpain). The terms μ-calpain and m-calpain indicate the

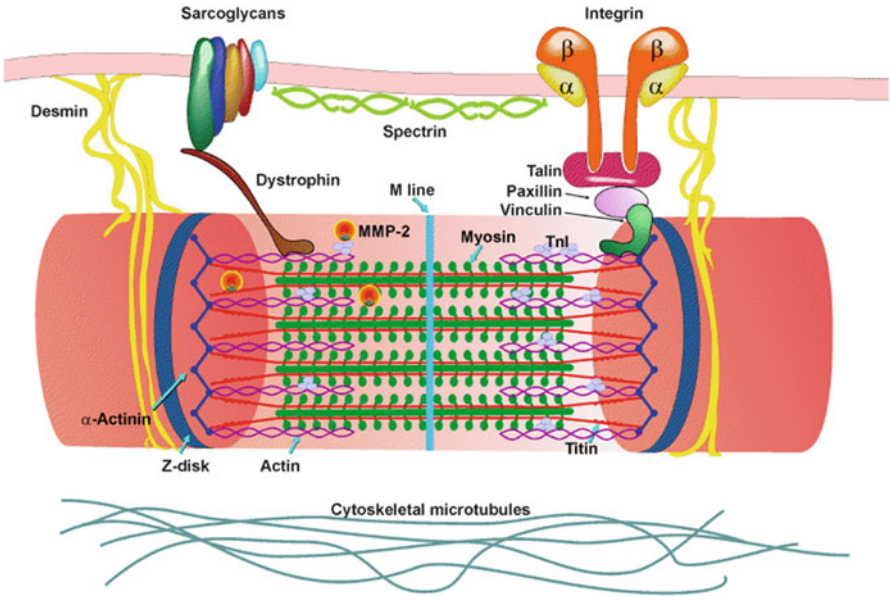

Fig. 26.1 Intracellular matrix and intracellular matrix metalloproteinase-2 (MMP-2). Schematic diagram shows the organization of sarcomeric and cytoskeletal proteins in relation to membrane anchor proteins in the cardiomyocyte. MMP-2 is localized to specific sarcomeric and cytoskeletal proteins and, upon myocardial oxidative stress injury, is able to proteolyze these substrates

required calcium concentration for in vitro activity (micromolar range for μ-calpain and millimolar range for m-calpain). Calpain-1 and -2 are considered to be ubiquitous, since they are expressed in nearly all tissues, whereas calpain-3 is expressed mainly in skeletal muscle [6]. Calpains participate in various cellular processes including remodeling of the sarcomere and cytoskeleton, signal transduction, and cell death [7]. Calpain-1, in particular, has been implicated in the pathogenesis of myocardial stunning injury, a reversible, sublethal injury of cardiac muscle which occurs upon reperfusion of the ischemic heart [8].

It has been suggested that activation of calpain-1 may lead to proteolytic degradation of sarcomeric proteins such as troponin I [9], titin, and α-actinin [10] which contributes to impaired contractile function (Fig. 26.2). In addition, calpain-1 activated during I/R injury has been shown to target various proteins involved in excitation–contraction coupling, thereby attenuating cardiac contractility. These targets include the SERCA2 pump [11], ryanodine receptor [12], and α-fodrin [13]. However, the conclusions of most of these studies rested on the use of pharmacological inhibitors of calpain, many of which were recently found to also inhibit MMP-2, another key protease in I/R injury [14]. In view of this, the relative contribution of calpain in I/R injury of the heart and other organs may need to be systemically revisited in comparison with MMP-2.

26.1.2 Intracellular Substrates of MMP-2 in Ischemic Heart Disease

Since their first description in amphibian metamorphosis [15], it has been recognized that MMPs play an active role in remodeling extracellular matrix proteins accompanying both physiological and pathological processes. Being originally described as secreted proteases, most researchers have focused on the long-term effects of MMPs on extracellular matrix remodeling following myocardial infarction (irreversible cellular injury resulting in myocyte death), hypertensive cardiac diseases, and other cardiomyopathies. In fact, MMPs have been recognized as proteases playing a pivotal role in matrix remodeling in such heart diseases (for review see [16]).

However, it has been recognized more recently that MMPs may also act on non-extracellular matrix substrates both outside [17] and inside the cell [4, 18]. This may occur within seconds to minutes rather than hours to days as occurs with so many of the extracellular matrix actions of MMPs. For example, MMP-2 was found to contribute to acute cardiac mechanical dysfunction in stunning injury before changes in extracellular matrix proteins [19]. Emerging evidence has shown that MMPs, and in particular MMP-2, are closely associated with subcellular compartments within cardiac myocytes, including the sarcomere [5, 20], cytoskeleton [21, 22], nuclei

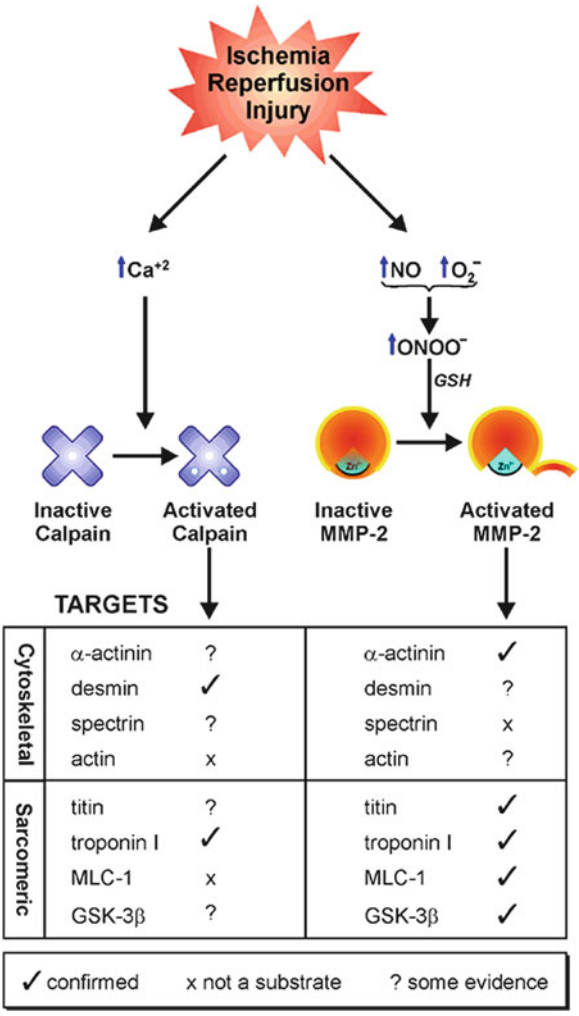

Fig. 26.2 Activation of intracellular proteases and their substrates. Mechanisms of enhanced intracellular proteolytic stress in acute myocardial ischemia/reperfusion injury include increased intracellular calcium, which leads to activation of calpains, and enhanced oxidative stress, particularly in the form of peroxynitrite biosynthesized from nitric oxide (NO) and superoxide, causing the direct activation of MMP-2. The table below summarizes potential targets for both proteases in ischemia/reperfusion injury

[23, 24], and mitochondria [20, 25]. Recently, distinct intracellular moieties of MMP-2 have been identified [25, 26], which physically confirm the concept that MMP-2 is indeed an intracellular protease [4, 18, 20].

Different mechanisms can activate and regulate MMP-2 activity, either extra- or intracellularly. MMP-2 is synthesized as a 72 kD zymogen protein which can be activated by proteolytic removal of the propeptide domain in pericellular and extracellular compartments, resulting in 64 kD MMP-2 [27]. However, 72 kD MMP-2 can also be activated without proteolysis as a direct result of oxidative stress. For example, MMP-2 is activated upon exposure to peroxynitrite, a prooxidant molecule implicated in various cardiac pathologies including I/R injury [28], via S-glutathiolation of a critical cysteine sulfhydryl moiety in the propeptide domain

[29] (Fig. 26.2). MMP-2 is also a phosphoprotein whose activity is increased by dephosphorylation [30]. MMP-2 activity can be inhibited by certain pharmacological agents, especially those which chelate the catalytic zinc found in its active site which is essential for MMP activity. Tetracyclines, especially doxycycline and minocycline, can inhibit MMPs independent of their antibacterial actions, most likely via chelation of zinc [31].

We will discuss below some cardiac sarcomeric and cytoskeletal proteins that have been shown to be targeted by MMP-2 in I/R injury (Fig. 26.2).

26.1.2.1 Troponin I

In 1999 Spinale's group showed a sarcomeric staining pattern of MMP-2 in pig heart muscle, yet did not provide an explanation of this unexpected result [21]. Our group then showed that MMP-2 is localized inside cardiac myocytes within the sarcomere and is responsible for the rapid degradation of troponin I in acute myocardial I/R injury [20]. Troponin I regulates actin–myosin interaction and is found in the thin myofilaments. Immunogold electron microscopy amongst other evidence showed that MMP-2 is an integral sarcomeric protein. Troponin I was highly susceptible to the proteolytic action of MMP-2 in vitro, and subjecting isolated rat hearts to acute I/R injury diminished myocardial troponin I content, an effect that was blocked by MMP inhibitors. In this study, we provided evidence that myocardial stunning injury is caused in part by MMP-2-mediated proteolysis of troponin I. This study was the first to recognize an intracellular biological role of a MMP as well as to identify the first intracellular target of MMP-2 in cardiac myocytes. Four years later, Lovett and Karliner's group reported that transgenic mice overexpressing a mutant, constitutively active MMP-2 in cardiomyocytes had marked derangements in the cardiac sarcomere, including troponin I degradation and reduced contractile function at the level of the myofilaments [32, 33].

26.1.2.2 Myosin Light Chain-1

Myosin light chain-1 was reported to undergo proteolytic degradation in hearts subjected to I/R injury [34]. MMP-2 activity was also found in preparations of thick myofilaments (which contain myosin light chain-1) from rat hearts, and MMP-2 was localized to the sarcomere in a pattern consistent with the known distribution of myosin light chain-1. Purified myosin light chain-1 was susceptible to proteolysis by MMP-2 in vitro. Two-dimensional gel electrophoresis followed by mass spectrometric analysis of myosin light chain-1 proteolysis products from I/R hearts identified a MMP-2 cleavage site within myosin light chain-1 at an accessible portion of the C terminus between tyrosine 189 and glutamate 190 [35].

26.1.2.3 Titin

Titin is the largest known mammalian protein (3,000–4,000 kD) and is found in cardiac and skeletal striated muscles. It spans nearly half the length of the sarcomere, from the Z-disk to the M-line region. It contains elastic segments formed by immunoglobulin-like repeats in the I band region, which allow it to act as a molecular spring, maintain the structural and functional stability of the myocyte and contribute to both active and passive stiffness of the myocyte [36].

Cardiac titin is expressed in two main isoforms: the shorter and stiffer N2B and the longer, more compliant N2BA isoforms. Hearts from adult small mammals (rats, mice, and rabbits) express predominately N2B titin, whereas large mammals including humans co-express N2BA and N2B titins at an approximate 1:1 ratio. The N2BA:N2B isoform ratio is increased in end-stage failing human hearts from chronically ischemic hearts of patients with coronary artery disease [37] and nonischemic dilated cardiomyopathy [38]. This titin remodeling decreases passive myocyte stiffness, most likely as a compensatory mechanism to counteract increased passive stiffness related to extracellular fibrosis [39]. Other forms of titin remodeling occur in heart disease, including reduced levels of titin phosphorylation, observed in dilated cardiomyopathy [40], and the formation of intramolecular disulfide bonds under oxidative stress [41], both of which could stiffen the titin spring function and contribute to impaired diastolic function following oxidative stress. The regulation of titin stiffness could affect various mechanical functions of the heart including diastolic filling, the Frank–Starling mechanism, and contractile performance in systole, the latter of which is also determined by titin [42].

In addition, earlier studies of ischemic and failing human hearts showed that titin is hydrolyzed [43] and appears highly disorganized in cardiac myocytes when analyzed by immunofluorescence microscopy [44]. We showed in rat and human myocardium that MMP-2 colocalizes with titin mainly near the Z-disk region of the cardiac sarcomere. Cleavage of titin in perfused rat hearts subjected to I/R injury, or in skinned cardiac myocytes incubated with MMP-2, was prevented by the MMP inhibitors *o*-phenanthroline or ONO-4817. Titin proteolysis in hearts was abolished in MMP-2 knockout mice subjected to I/R in vivo [5]. Thus MMP-2 appears to play an important role in titin homeostasis, which directly affects the contractile function of the heart at the sarcomeric level. Taken together, these studies reveal that MMP-2 may be a crucial protease which targets specific sarcomeric proteins as a result of oxidative stress injury to the heart.

26.1.3 MMP-2 and Calpains: A Case of Misattributed Function?

In light of the above studies, there appears to be overlap in the substrates and/or biological actions of MMP-2 and calpains in various cellular pathways (Fig. 26.2). It is now becoming evident that MMP-2 either targets a similar subset of proteins as calpain or calpain has been incorrectly identified as the protease responsible for

some intracellular proteolytic activities [1, 45]. Indeed, much of the evidence for calpain degradation of substrates in cardiac cells rests on the use of pharmacological calpain inhibitors, including ALLN and PD-150606, which we recently found to efficiently inhibit MMP-2 activity at commonly used micromolar concentrations [14]. Furthermore, the exact role of calpain in acute myocardial I/R injury (stunning) is controversial as many of the earlier studies dating back several decades did not provide evidence for the subcellular co-localization of calpain with its putative substrates [1]. In a more recent study, myocardial-specific overexpression of calpain-1 in transgenic mice showed no evidence of troponin I degradation in the heart [46], whereas as mentioned above, troponin I levels were reduced in hearts from transgenic mice with myocardial-specific overexpression of constitutively active MMP-2 [33]. It is possible that MMP-2 and calpains share similar substrates. However, given the points raised above, it would be prudent to reevaluate suggested calpain substrates in the myocardium for their susceptibility to cleavage by MMP-2 and to critically evaluate the co-localization of calpains with their putative substrates. Moreover, caution is necessary in interpreting tissue calpain activity solely on the use of peptide-based enzyme assays which may not only measure calpain but also MMP activity.

26.1.4 Proteasomal and Lysosomal Degradation of the Intracellular Matrix

Proteasomes and lysosomes play important roles in the proteolysis of cardiac proteins. Lysosomes degrade the majority of endocytosed proteins when digesting expired organelles or cell debris. However, the proteasome system removes and recycles most unneeded or damaged intracellular proteins [47]. Oxidative modification of proteins affects their secondary and tertiary structures, resulting in protein unfolding, which may lead to a loss of function and enhanced proteolysis of these proteins [48]. Damaged, oxidized, and/or misfolded proteins are removed by the ubiquitin–proteasome system (UPS). The UPS is the main non-lysosomal protease complex involved in the proteolysis of intracellular proteins and therefore plays a key role in protein quality control [49].

As ATP is required for activation of the proteasome and for proper function of the UPS, ATP depletion during ischemia could be partially responsible for decreased proteasome activity in the ischemic heart. Also, accumulation of misfolded or mutated proteins as a result of oxidative stress can inhibit the cardiac UPS and may result in cardiomyopathy [48]. On the other hand, it was also reported in animal models of myocardial I/R injury that inhibition of the proteasome system significantly reduced infarct size by more than 50% and preserved ventricular contractility suggesting a role of proteasomes in the process of I/R injury [47]. Although myosin, actin, troponin C, and tropomyosin purified from skeletal muscle can be hydrolyzed by the proteasome pathway in vitro, these proteins are much less susceptible to proteasomal degradation when present in intact myofibrils or as soluble actomyosin

complexes [47]. Thus, the rate-limiting step in their degradation seems to be their dissociation from the contractile filaments, where intracellular MMP-2 or calpains may play an important role in the scenario of I/R injury [47].

26.2 Cytoskeletal Protein Remodeling in Cardiac Ischemia and Reperfusion

The cardiac cytoskeleton (Fig. 26.1) consists of microfilaments, intermediate filaments (such as desmin), and the microtubular network. The cytoskeleton preserves cellular shape and enables cell migration and intracellular transport. It also maintains the proper localization of subcellular organelles such as the mitochondria, Golgi apparatus, nucleus, and sarcomere. The cardiac myocyte cytoskeleton is also specialized to transmit mechanical and electronic stimuli between cells. Investigation of early changes to cytoskeletal proteins in ischemic human myocardium suggest that they undergo degenerative alterations earlier than subcellular organelles [2]. Furthermore, disruption of the localization and integrity of the filamentous network of the cytoskeleton during prolonged ischemia accompanies I/R injury [50] (Fig. 26.3).

26.2.1 α-Actinin

α-Actinin connects actin filaments of adjacent sarcomeres and plays a substantial role in transmitting force generated by actin–myosin interaction. MMP-2 was found to colocalize with α-actinin in cardiac myocytes [21, 22]. We found that α-actinin is susceptible to degradation by MMP-2 in vitro and infusion of peroxynitrite into isolated, perfused rat hearts caused activation of MMP-2 with concomitant loss of myocardial α-actinin content. This was prevented by a selective MMP inhibitor, PD-166793 [22].

26.2.2 Desmin

Desmin is a cardiac-specific cytoskeletal protein with an average molecular weight of 53 kD. Desmin monomers assemble to form intermediate filaments 10 nm in diameter by polymerization which form in turn a transverse network that links the Z-bands of adjacent myofibrils. This maintains the integrity of cardiac myocytes and allows force transmission and mechanochemical signaling between cells [51]. In addition, mitochondria and T-tubules appear to be attached to the intermediate filament network [52].

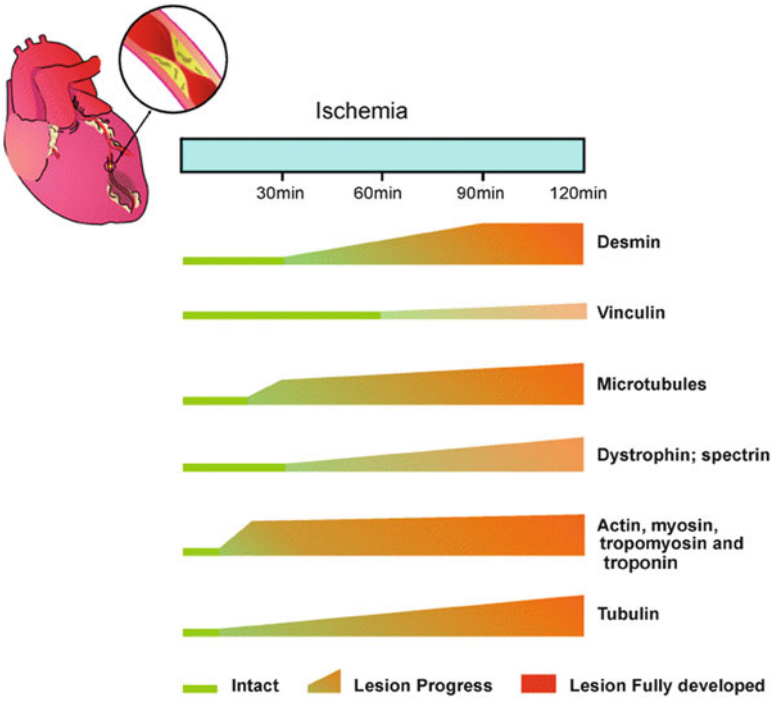

Fig. 26.3 Sarcomeric and cytoskeletal proteins have different sensitivities towards ischemic damage ("lesion"). For example, tropomyosin and troponins are among the most sensitive targets to proteolysis during myocardial ischemia, whereas vinculin resists this proteolytic damage. Reperfusion following ischemia accelerates damage to these proteins

Interestingly, although desmin is not considered to be directly involved in the generation of contractile force or the maintenance of tension, vascular smooth muscle cells from desmin knockout mice generate only 40% of the contractile force than that of wild-type controls [53]. Severe cardiac ischemia followed by reperfusion leads to intracellular Ca^{2+} overload and subsequent activation of calpain, which has the ability to proteolyze desmin [6]. Desmin content in the whole heart was shown to be decreased in myocardial I/R injury, and desmin hydrolysis by activated calpain reduces maximal force production and Ca^{2+} sensitivity in isolated cardiac myofilaments [54]. Hein et al. studied the effects of global ischemia on various cytoskeletal and contractile proteins in human left ventricles obtained from transplant recipients. Desmin was found to be affected by ischemia at later time points than the contractile filaments (Fig. 26.3). Disappearance of desmin from the cross striation pattern began after at 30 min of ischemia whereas, and the fully developed pattern of changes occurred after 90 min of ischemic injury [2]. Disruption of desmin damages the link between myofibrils and the sarcolemma and was found to

contribute to increased fragility of the myofibrils [55]. Since calpain was also found to be colocalized with desmin in the Z-band of skeletal myoblast cells [56], it was postulated that calpain-mediated degradation of desmin may contribute to the increase of cell fragility which may increase the chance for rupture of the cell during reperfusion. Note that desmin is susceptible to hydrolysis by MMP-2 in vitro [22] but whether this accompanies ischemic heart injury is unknown.

26.2.3 Vinculin

Vinculin is a membrane-associated cytoskeletal protein required for the attachment of the actin-based microfilaments to the plasma membrane (Fig. 26.1). In the cardiac myocyte, vinculin participates in the formation of the attachment complex between the plasma membrane and the Z-line of myofibrils [57]. Vinculin was also localized in the intercalated disk and in the lateral sarcolemma [2].

The effects of different durations of ischemia on cardiac vinculin were assessed by immunofluorescence, and it was found to be more resistant to the effects of ischemia than desmin, with effects not being detectable until 60 min post-ischemia [2]. Similarly, in a canine heart ischemia model, Steenbergen et al. reported an unchanged pattern of vinculin staining in longitudinal sections of myocardium subjected to 60 min of total ischemia and 60 min of reperfusion. When subject to more severe myocardial ischemia (120 min or longer), there was a progressive loss of vinculin staining and increase in inulin permeability, which likely contributes to the detachment of actin from the sarcolemma, leading to the formation of blebs and rupture of the sarcolemmal membrane [58].

26.2.4 Microtubules

Microtubules are hollow tubes 25 nm in diameter that exist as a ubiquitous filamentous structure of the cytoskeleton. Polymerized α- and β-tubulin form the microtubes which surround the nucleus and spread throughout the entire cell [59]. In myocytes, microtubules are distributed along their longitudinal axis as an irregular network.

In an in situ canine heart model [50], 15 min of cardiac ischemia caused no detectable changes in the filamentous staining pattern. After 20 min of ischemia, however, small patchy lesions appeared in some myocytes in which the immunoreactivities of microtubules began to decrease in intensity. Progressive loss of microtubular staining continued to be observed over 120 min of cardiac ischemia.

Microtubules contribute significantly to the stability of cell morphology by supporting cellular architecture, plasma membranes, myofibrils, and other cellular organelles. Protection of microtubule integrity during I/R injury could therefore be

a means of protecting against I/R-induced damage. For example, paclitaxel, a microtubule stabilizer, reduced myocardial I/R injury, myocardial infarct size, and the incidence of ischemic ventricular arrhythmias in perfused rat hearts [60].

26.2.5 Other Cytoskeleton Proteins: Talin, Dystrophin, and Spectrin

Prolonged cardiac I/R injury damages a wide array of cytoskeletal protein structures. Talin, dystrophin, and spectrin are membrane-associated cytoskeletal proteins that link the structural components of the intracellular milieu with those of the extracellular matrix via the integrins (Fig. 26.1). Dystrophin connects intracellular actin and extracellular laminin and acts as a stabilizing force and mechanotransducer for the sarcolemmal membrane [61]. Spectrin forms the backbone of the membrane skeleton providing an elastic support to the sarcolemmal membrane. Ischemia-induced loss of membrane dystrophin and spectrin was found following 30–45 min of coronary artery ligation in rabbit hearts. Loss of sarcolemmal dystrophin and spectrin seems to contribute to subsarcolemmal bleb formation and membrane fragility during the transition from reversible to irreversible ischemic myocardial injury [62]. Spectrin was not susceptible to proteolysis by MMP-2 in vitro [22].

26.2.6 Mechanisms of Cytoskeletal Damage in I/R Injury

Beside the direct degenerative effects of ischemia on the cytoskeleton, the process of restoring circulation to the ischemic heart, usually by acute intervention therapy, can also induce additional injury to the myocardium. The initial mechanism underlying reperfusion injury had been attributed to the generation of reactive oxygen/nitrogen species including peroxynitrite [22] and elevated intracellular Ca^{2+}. Beyond this, a variety of other biochemical abnormalities have been proposed to explain the myocardial contractile dysfunction that occurs after reperfusion, including excitation–contraction uncoupling due to dysfunction of the sarcoplasmic reticulum, altered fuel metabolism in mitochondria, inefficient energy use by myofibrils, altered ion channel activities, and decreased sensitivity of myofilaments to calcium. Importantly, these potential mechanisms are not mutually exclusive [1].

In I/R injury, the activity of cytosolic proteases such as calpain and MMPs is increased in response to increased Ca^{2+} and reactive oxygen/nitrogen species, respectively. The proteolysis of their cytoskeletal and sarcomeric substrates was found to be a major mechanism of intracellular pathology in I/R injury [4, 45]. These alterations to contractile and cytoskeletal proteins (Fig. 26.2), in addition to damaged subcellular organelles, play a key role in impairing cardiac function during I/R injury.

26.2.7 Cytoskeletal Injury as an Indicator of Irreversible Cell Injury

Myocardial injury is a dynamic process which, if mild, results in reversible cell injury, but if severe enough causes irreversible damage. Action to protect (or salvage) the ischemic myocardium should therefore preferably be performed before irreversible damage of myocardium occurs. Many biochemical and metabolic changes have been observed early after the onset of ischemia, but the precise cause of the transition to irreversibility is not known.

A number of hypotheses have been proposed to account for this transition to irreversible myocardial damage, including mitochondrial dysfunction, depletion of antioxidant reserves, leakage of lysosomal enzymes [63], the toxicity of metabolic end products, and lipid peroxidation caused by reactive oxygen/nitrogen species [64]. None of these postulated theories completely account for all features of the irreversible damage to cardiac myocytes. However, cytoskeletal damage can possibly explain many important biological phenomena associated with irreversible damage.

When ATP is severely depleted during ischemia, the connection between the cytoskeletal components is weakened resulting in an increased potential to dissociate under mechanical force. During the early reperfusion phase, the influx of calcium leads to calcium overload and hypercontraction [65]. Hypercontraction of the ischemia-injured myocyte will lead to cytoskeletal deformation to an extent beyond that seen under normal contraction. This will result in "cytoskeletal fracture". Since the cytoskeleton is required to maintain integrity of the cell membrane and cytosolic organelles, cytoskeletal injury is accompanied by osmotic swelling which eventually leads to rupture of the cell membrane. The membrane rupture hypothesis of irreversibility is supported by the observation that "irreversibly injured" cells can indeed recover if membrane rupture and necrosis during reperfusion are prevented [66].

26.3 Cardiac Mitochondria Remodeling in Ischemic Heart Disease

Current models of I/R injury feature mitochondria as important arbiters of cardiac myocyte survival or death [67]. The pivotal event is the opening of the mitochondrial permeability transition pore (MPTP), which results in the permeabilization of the inner mitochondrial membrane. This collapses mitochondrial membrane potential, rendering mitochondria unable to produce ATP. Responding to an osmotic gradient between the mitochondrial matrix and cytosol, water rushes into the matrix resulting in the characteristic "swollen" appearance of mitochondria in electron

micrographs of cardiac tissue subject to I/R injury. This may lead to the rupture of the outer mitochondrial membrane, releasing cytochrome c and other pro-apoptotic proteins into the cytosol.

26.3.1 A Potential Role for Intracellular Matrix Remodeling in Post-I/R Mitochondrial Dysfunction

The existence of a physical relationship between mitochondria and the cytoskeleton is well established. Mitochondria have been shown to interact with microtubules, intermediate filaments, and microfilaments, with cytoskeletal elements believed to play a role in their intracellular localization and movement and possibly morphology [52]. The possibility that intracellular matrix remodeling may play a role in I/R-induced mitochondrial dysfunction remains mostly unexplored, although there are tantalizing hints that this is the case. For instance, pharmaceutical manipulation of the cytoskeleton has been shown to affect mitochondrial functions relevant to I/R. Pharmaceutical agents that either depolymerized or stabilized microtubules prevented closure of the MPTP [68], and pharmaceutical disruption of the actin cytoskeleton impaired the effectiveness of certain drugs that decrease the vulnerability of cardiac tissue to I/R injury by manipulating mitochondria [69]. It has been suggested that the cytoskeleton plays a role in the translocation of signaling molecules to the mitochondria during ischemic preconditioning, perhaps via the endosomal system [70]. Furthermore, cardiac mitochondrial function in desmin-null mice is impaired, with mitochondria exhibiting ultrastructural changes—such as swelling—strikingly similar to those observed in I/R injury [71].

More generally, it is hypothesized that the cytoskeleton modulates mitochondrial function, although the mechanisms remain unclear [52]. One possible mechanism could be via the cytoskeletal control of the subcellular localization of mitochondria. This may be particularly relevant in adult cardiac myocytes, in which most mitochondria are arranged in highly structured linear arrays between myofibrils [72]. After I/R injury, mitochondria have been observed as appearing to be detached from myofibrils [73], suggestive of a mechanism, possibly cytoskeletal in nature, that maintains this association. Indeed, disrupting cytoskeletal structure by knocking out desmin or enzymatic digestion in vitro results in a loss of the normal mitochondrial position and neat arrangement between myofibrils [71, 74]. Mitochondria appear to be localized near sites of Ca^{2+} release from the sarcoplasmic reticulum, which may facilitate Ca^{2+} movements between the two cellular subcompartments [75, 76]; it has been proposed that the cytoskeleton may play a role in maintaining this physical orientation [77]. Likewise, the close association of mitochondria with sarcomeres and the sarcoplasmic reticulum may facilitate the channeling of ADP to mitochondria; this is supported by the finding that proteolytic digestion of cytoskeletal elements in permeabilized cardiac myocytes in vitro altered the apparent binding affinity of ADP [74].

26.3.2 *Mitochondrial Localization of Modifiers of the Intracellular Matrix*

As described above, calpains and MMP-2 have both been shown to actively remodel the intracellular matrix in response to I/R. MMP-2 and calpain-1 have both been found in cardiac mitochondria [20, 25, 78, 79]. Mitochondrial calpain-1 is activated by I/R injury and cleaves apoptosis-inducing factor, allowing it to be released into the cytosol [78]. Overexpression of constitutively active MMP-2 in the mouse heart does not affect baseline mitochondrial function; however, the response to I/R is more severe, with mitochondrial respiration and structure being affected to a greater extent than in hearts from wild-type mice [73]. Mitochondria appear to accumulate a constitutively active, N-truncated isoform of MMP-2, overexpression of which triggers the nuclear activation of several pro-inflammatory transcriptional pathways [25]. The fact that these proteases of the sarcomere and cytoskeleton also appear to be active in mitochondria suggests that the remodeling of the intracellular matrix may actually be part of a wider program of response to I/R injury.

26.4 Nuclear Matrix Remodeling in Cardiac Disease

The nuclear matrix is the network of fibers found throughout the inside of the cell nucleus. It is analogous to the cellular cytoskeleton and provides structural and organizational support for various nuclear processes. Proteolytic cleavage of the nuclear matrix occurs in processes such as apoptosis [80], regulation of the cell cycle [81], and nuclear matrix degradation [82].

Irreversible I/R injury may lead to cellular apoptosis. During apoptosis, morphological changes such as chromatin condensation, nuclear shrinkage, and the formation of apoptotic bodies occur in the nucleus [83]. These changes are associated with numerous molecular alterations, such as DNA and RNA cleavage, posttranslational modifications of nuclear proteins, and proteolysis of several polypeptides of the nuclear matrix including topoisomerase IIa, NuMA, SAF-A, lamin B1, lamins A and C, and SATB1 [83].

Nuclear MMP-2 and/or MMP-9 activities may also contribute to the I/R injury-induced apoptotic process by processing poly-ADP-ribose polymerase [18] and X-ray cross complementary factor 1, hence, interfering with the DNA repair system [84]. Indeed, MMP-2 [23] and MMP-3 [85] carry a putative nuclear localization sequence. An active, truncated fragment of MMP-3 was localized to the nucleus of several human cancer cell lines, and it is associated with the onset of apoptosis [85]. MMP-2 and MMP-9 were found in the nucleus of human cardiac myocytes, and although MMP-2 was able to proteolyze the nuclear DNA repair enzyme, poly (ADP-ribosyl) polymerase in vitro, its precise role in the nucleus remains to be discovered [23].

26.5 Future Prospects: The Intracellular Matrix as Therapeutic Target

Ischemic heart disease is the most common cause of death in developed and, ever more so, in developing countries. Studies of the pathogenesis of myocardial I/R injury reveal structural and functional remodeling of intracellular matrix components of the cardiac myocyte. Protection of the intracellular matrix may represent a novel strategy to prevent or reduce the impact of ischemic heart disease. Many cytoskeletal and sarcomeric proteins were found to be susceptible to proteolysis by intracellularly localized MMP-2; indeed, there is substantial evidence that the cleavage of these intracellular targets by this crucial protease mediates several important pathogenic processes in myocardial I/R injury. Oxidative stress generated in I/R injury can efficiently activate intracellular MMP-2 which then mediates intracellular matrix remodeling of injured myocytes. Given that MMP-2 is readily activated by prooxidant stress, such as peroxynitrite generated in I/R injury [29], MMP-2 may represent one of the earliest mediators of the detrimental actions of oxidative stress to the heart.

Doxycycline, one of the tetracycline antibiotics, has been shown to act as an MMP inhibitor at a plasma concentration below that required for its antimicrobial action [86]. Indeed, a retrospective epidemiological study found a significant reduction in the risk of first-time acute myocardial infarction for patients who had taken tetracycline class antibiotics for prior infection. This effect was not observed in patients who had received any other classes of antibiotics [87]. Furthermore, doxycycline was also found to protect against streptozotocin-induced diabetic cardiomyopathy [88] and cardiac mechanical dysfunction triggered by endotoxic shock in rats [89]. Increased activity of cardiac matrix MMP-2 and MMP-9 was found during the acute phase of Chagasic cardiomyopathy in mice, an inflammatory heart disease triggered by infection with *Trypanosoma cruzi*. This increased MMP activity was associated with mortality following infection, and doxycycline treatment significantly improved survival [90]. Based on our current understanding of MMP-2 as an intracellular protease, the cardiovascular benefits associated with tetracycline use may be reasonably attributed to the inhibition of pathological MMP activity. These findings suggest that doxycycline may be useful as a possible therapeutic regimen for ischemic heart disease. Of note, doxycycline has already been approved by the Food and Drug Administration and Health Canada in the therapeutic treatment of periodontitis. Doxycycline may therefore emerge as a promising drug in the near future for the treatment or prevention of cardiac disease.

Increasing evidence suggests that blocking MMP-2 activity can alleviate I/R-mediated cardiac injury in animal models. It is important to consider the fact that MMP-2 plays very diverse roles in various pathological and physiological circumstances other than cell injury, such as cell cycle control, cell death, inflammation, cancer, development, and tissue remodeling. Universal MMP inhibitors could therefore have detrimental or undesirable side effects due to a lack of selectivity and/or specificity [91]. Development of pathway-specific or subcellular location “selective”

MMP-2 inhibitors, based on the advancing knowledge in this field, may help to alleviate acute myocardial I/R injury or detrimental chronic cardiac remodeling, while spare physiological MMP-2 activities inside and outside the cell.

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