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Nuclear matrix metalloproteinase-2 in the cardiomyocyte and the ischemic-reperfused heart

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ABSTRACT

Matrix metalloproteinases (MMPs) are zinc-dependent proteases involved in intra- and extra-cellular matrix remodeling resulting from oxidative stress injury to the heart. MMP-2 was the first MMP to be localized to the nucleus; however, its biological functions there are unclear. We hypothesized that MMP-2 is present in the nucleus under normal physiological conditions but increases during myocardial ischemia-reperfusion (I/R) injury-induced oxidative stress, proteolyzing nuclear structural proteins. Lamins are intermediate filament proteins that provide structural support to the nucleus and are putative targets of MMP-2. To identify lamin susceptibility to MMP-2 proteolysis, purified lamin A or B was incubated with MMP-2 in vitro. Lamin A, but not lamin B, was proteolysed by MMP-2 into an approximately 50 kDa fragment, which was also predicted by in silico cleavage site analysis. Immunofluorescent confocal microscopy and subcellular fractionation showed MMP-2 both in the cytosol and nuclei of neonatal rat ventricular myocytes. Rat hearts were isolated and perfused by the Langendorff method aerobically, or subjected to I/R injury in the presence or absence of o-phenanthroline, an MMP inhibitor. Nuclear fractions extracted from I/R hearts showed increased MMP-2 activity, but not protein level. The level of troponin I, a known sarcomeric target of MMP-2, was rescued in I/R hearts treated with o-phenanthroline, demonstrating the efficacy of MMP inhibition. However, lamin A or B levels remained unchanged in I/R hearts. MMP-2 has a widespread subcellular distribution in cardiomyocytes, including a significant presence in the nucleus. The increase in nuclear MMP-2 activity seen during stunning injury here, indicates yet unknown biological actions, other than lamin proteolysis, which may require more severe ischemia to effect.

1. Introduction

MMPs are a family of zinc proteases involved in a variety of physiological and pathological processes both extra- and intra-cellularly [1,2]. MMP-2 is a ubiquitous enzyme that proteolyses extracellular matrix proteins as well as several intracellular proteins [3]. Intracellular localization of MMP-2 is due to the fact that canonical MMP-2 has a signal sequence that only inefficiently targets it to the endoplasmic reticulum for secretion. Furthermore, cardiomyocytes have been shown to express a splice variant of MMP-2 that is entirely lacking the signal sequence for secretion [4].

MMP-2 is transcribed as an inactive zymogen of 72 kDa, with the N-terminus autoinhibitory propeptide containing a conserved cysteine sulfhydryl residue that forms a hydrogen bond with the zinc ion in the active site [3]. The activation happens by the "cysteine switch" mechanism which requires the cysteine to either be proteolytically removed or chemically altered [5]. Reactive oxygen-nitrogen species (RONS) such as peroxynitrite, an oxidizing agent formed by the reaction of nitric oxide with superoxide, activates 72 kDa MMP-2 [6]. Peroxynitrite, in combination with glutathione, causes the S-glutathiolation of the cysteine residue, resulting in the formation of a disulfide S-oxide in the prodomain which alters the tertiary structure of MMP-2, allowing substrates access to the active site [6,7].

Intracellular MMP-2 plays a central role in the pathophysiology of acute cardiac ischemia and reperfusion (I/R) injury as it proteolyses sarcomeric proteins such as troponin I [8], myosin light chain 1 [9], α-actinin [10] and titin [11], thereby contributing to acute contractile dysfunction. Injury to the tissue happens in the first few minutes of reperfusion with the biosynthesis of RONS including peroxynitrite [12,13]. The activated intracellular 72 kDa MMP-2 proteolyses these sarcomeric proteins, leading to reduced contractile function as a result of I/R [2]. The MMP inhibitors o-phenanthroline or doxycycline improved the recovery of contractile performance [14] and reduced infarct size [15,16] along with reduced degradation of sarcomeric proteins in hearts that underwent acute I/R injury [8,9,11].

In cardiomyocytes MMP-2 has thus far been localized to the sarcomere [8], cytoskeleton [10], caveolae [17], mitochondria [8,18], mitochondria-associated membrane [19] and nuclei [20]. The discovery

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of nuclear MMP-2 sparked many other studies investigating the possible nuclear functions of these proteases. MMPs are suggested to play a role in nuclear processes, where they may proteolyse DNA repair proteins [20–22], act as transcription factors [23,24], play a role in apoptosis [25] and participate in mitotic events leading to cellular proliferation [26]. An improved understanding of the localization and functions of MMP-2 under conditions of both homeostasis and stress is important because it will allow the development of improved and specific MMP inhibitors targeted to specific subcellular compartments.

Analysis of the MMP-2 sequence showed that it possesses a nuclear localization sequence at the C-terminal domain, which is exposed on the surface of the protein [20]. MMP-2 levels and activity were previously described in nuclear extracts from human hearts and rat liver. Poly-ADP-ribose polymerase-1 (PARP-1), a protein that detects and repairs oxidized DNA, was shown to be proteolysed by MMP-2 in vitro [20]. Nuclear MMP-2 levels were shown to be increased in pulmonary artery endothelial cells that were subjected to cigarette smoke. It was suggested that the increased activity of MMP-2 in nuclei was due to RONS activating MMP-2 [27]. Furthermore, increased nuclear MMP-2 activity was seen in rat brains subjected to 90 min middle cerebral artery occlusion followed by reperfusion. The levels of PARP-1 and X-ray cross-complementary factor 1, a DNA base excision repair protein, were reduced in ischemic rat brains, and the MMP inhibitor BB1101 attenuated this, reducing the early oxidative DNA damage in that tissue [22]. However, the physiological and pathological roles of nuclear MMP-2 in the heart remain unclear.

MMPs were first described as proteases of tissue structural components — the extracellular matrix. The nucleus has a similar protein skeleton for support and organization known as the nuclear matrix. It is interconnected with the cytoskeleton and the extracellular matrix through complex structures [28]. Although our knowledge of the nuclear matrix proteins and their functions is incomplete, the so far known major components include the nuclear mitotic apparatus protein, heterogeneous nuclear ribonucleoprotein, matrins, actins and lamins [29,30]. The filamentous network of lamins act as a structural support of the nuclear membrane. Lamins are type V intermediate filament proteins further subdivided into A-type lamins (lamin A & C) and B-type lamins (lamin B1 & B2) [31]. Lamin A and C are alternative splice products of the same gene (LMNA) while lamin B1 & B2 are products of two genes (LMNB1,2) [32]. Lamin structure consists of a globular N-terminal domain with the central α-helical rod domain containing four coiled repeats and another C-terminal globular domain. The lamins dimerize and polymerize into more complex structures [33], incorporating lamin-binding proteins and supporting a wide range of functions [34], including chromatin organization [35], gene expression [36], cell cycle control [37], DNA repair mechanisms [38] and positioning of the nucleus in the cell [39]. Interestingly, lamin B as measured by immunohistochemistry was decreased in dog hearts subjected to I/R injury [40].

We hypothesized that MMP-2 is present in the nucleus under normal physiological conditions and increases during myocardial I/R induced oxidative stress, proteolyzing structural proteins including lamin A, B and C. In the present study, we visualized by immunofluorescence microscopy and measured by western blot the distribution of MMP-2 in the cytosol and nuclei of neonatal rat ventricular cardimyocytes. Using in silico and in vitro proteolysis methods we showed that lamin A or C but not B can be proteolyzed by MMP-2 in vitro. Using isolated rat hearts subjected to I/R injury we determined that nuclear MMP-2 activity, but not levels were increased by I/R injury. However, no changes in the putative targets of MMP-2, lamins A, B or C, were observed in I/R hearts.

2. Methods

Experiments involving animals were approved by the Institutional Animal Care and Use Committee at the University of Alberta and were done according to the guidelines given by the Guide to the Care and Use of Experimental Animals, published by the Canadian Council on Animal Care.

2.1. Materials

Conditioned media from human fibrosarcoma HT1080 cells (American Type Culture Collection, Manassas, VA) was used as a standard for gelatin zymography.

The cell culture reagents were all purchased from Gibco Life Technologies (Grand Island, NY, USA). Other reagents used in this study were purchased from Sigma-Aldrich (St. Louis, MO, USA), with the exception of recombinant human lamin A (PRO-690, Prospec Protein Specialists, East Brunswick, NJ, USA) and recombinant human lamin B (TP301604, Origene, Rockville, MD, USA), which were used in the in vitro proteolysis assay.

2.2. Neonatal rat cardiomyocytes

Neonatal rat ventricular cardiomyocytes (NRVM) from 1- to 2-day-old Sprague–Dawley rats were isolated and cultured as previously described [41].

2.3. Isolated rat heart perfusions

Male Sprague–Dawley rats (250 to 300 g), were anesthetized by an intraperitoneal injection of sodium pentobarbital (240 mg/kg), and the hearts were carefully excised and rinsed in Krebs-Henseleit solution (118 mM NaCl, 25 mM $NaHCO_3$, 4.7 mM KCl, 1.2 mM $MgSO_4$, 1.2 mM KH_2PO_4, 11 mM glucose, 0.5 mM EDTA and 3 mM $CaCl_2$, pH 7.4) at 4 °C. The heart was mounted via the aorta to the cannula of the heart perfusion apparatus (LH-04, Experimetria Ltd., Budapest, Hungary). The hearts were perfused in Langendorff mode at constant pressure of 60 mmHg with Krebs-Henseleit solution at 37 °C which was continuously gassed with 95% O_2/5% CO_2 [12].

A latex pressure balloon was inserted into the left ventricle and the balloon was filled with H_2O with a glass spindle syringe until left ventricular diastolic pressure was set at 10 mmHg. Hearts were perfused for 15 min to allow them to stabilize, at which point experiments were started. Coronary flow, heart rate and left ventricular pressure were recorded during the experiment using SPEL Advanced Haemosys software. All hearts were perfused for a total of 75 min. The aerobic (control) hearts were perfused for 75 min. Ischemic-reperfused (I/R) hearts were perfused aerobically for 20 min followed by 20 min of global, no flow ischemia, and 35 min aerobic reperfusion by restoring the flow of perfusate to the heart.

The hearts either received the vehicle, dimethyl sulfoxide (DMSO, 0.0625% v:v) or the MMP inhibitor o-phenanthroline (100 μM) through a drug infusion line feeding into the Krebs-Henseleit solution aortic delivery line, using a Gilson peristaltic minipump (MINIPULS 3, Mandel Scientific, Guelph, ON, Canada). The o-phenanthroline or vehicle solutions were infused at a flow rate of 50–100 μL/min, adjusted appropriately in relation to the coronary flow rate of each heart to give the final concentrations specified. The vehicle or drug was infused for a 10 min period immediately prior to ischemia, and for the first 10 min of reperfusion. Aerobic hearts received the vehicle 10 min after the start of perfusion until the 50 min time point, for a total of 40 min, as the perfusate flow was not interrupted in aerobic hearts.

2.4. Preparation of tissue subcellular fractions

The preparation of subcellular fractions from the ventricular heart tissue and the verification of their purity were performed as exactly as described [42].