Free Radical Biology and Medicine 83 (2015) 115–128

Contents lists available at ScienceDirect

Free Radical Biology and Medicine

journal homepage: www.elsevier.com/locate/freeradbiomed

Original Contribution

A novel function of nuclear nonmuscle myosin regulatory light chain in promotion of xanthine oxidase transcription after myocardial ischemia/reperfusion

Yi-Shuai Zhang [a,b], Bin Liu [a,b], Xiu-Ju Luo [a,b], Jie-Jie Zhang [a,b], Nian-Sheng Li [a,b], Qi-Lin Ma [c], Jun-Lin Jiang [a,b], Yuan-Jian Li [a,b], Qingjie Li [d,*], Jun Peng [a,b,*]

[a] Department of Pharmacology, School of Pharmaceutical Sciences, Central South University, Changsha 410078, China
[b] Hunan Provincial Key Laboratory of Cardiovascular Research, School of Pharmaceutical Sciences, Central South University, Changsha 410078, China
[c] Department of Cardiovascular Medicine, Xiangya Hospital, Central South University, Changsha 410008, China
[d] Department of Internal Medicine, The University of Texas Medical Branch, Galveston, TX 77555-1083, USA

ARTICLE INFO

Article history:
Received 24 August 2014
Received in revised form
3 February 2015
Accepted 5 February 2015
Available online 17 February 2015

Keywords:
Nonmuscle myosin regulatory light chain
Ischemia
Reperfusion
Xanthine oxidase
Transcription
Free radicals

ABSTRACT

Nuclear myosin regulates gene transcription and this novel function might be modulated through phosphorylation of the myosin regulatory light chain (p-MLC_{20}). Nonmuscle MLC_{20} (nmMLC_{20}) is also present in the nuclei of cardiomyocytes and a potential nmMLC_{20} binding sequence has been identified in the promoter of the xanthine oxidase (XO) gene. Thus, we investigated its function in the regulation of XO transcription after myocardial ischemia/reperfusion (IR). In a rat model of myocardial IR and a cardiomyocyte model of hypoxia/reoxygenation (HR) injury, the cardiac or cell injury, myosin light chain kinase (MLCK) content, XO expression and activity, XO-derived products, and level of nuclear p-nmMLC_{20} were detected. Coimmunoprecipitation (co-IP), chromatin immunoprecipitation, DNA pull-down, and luciferase reporter gene assays were used to decipher the molecular mechanisms through which nmMLC_{20} promotes XO expression. IR or HR treatment dramatically elevated nuclear p-nmMLC_{20} level, accompanied by increased XO expression, activity, and products (H_2O_2 and uric acid), as well as the IR or HR injury; these effects were ameliorated by inhibition of MLCK or knockdown of nmMLC_{20}. Our findings from these experiments demonstrated that nuclear p-nmMLC_{20} binds to the consensus sequence GTCGCC in the XO gene promoter, interacts with RNA polymerase II and transcription factor IIB to form a transcription preinitiation complex, and hence activates XO gene transcription. These results suggest that nuclear p-nmMLC_{20} plays an important role in IR/HR injury by transcriptionally upregulating XO gene expression to increase oxidative stress in myocardium. Our findings demonstrate nuclear nmMLC_{20} as a potential new therapeutic target to combat cardiac IR injury.
© 2015 Elsevier Inc. All rights reserved.

The conventional myosin (myosin II) is best known for its role in muscle contraction and actin-based motility. In the cytoplasm of the cardiomyocyte, myosin and actin are organized into cardiac filaments, and the sliding between the two filaments, when ATP is hydrolyzed, is the basis for cardiac muscle contraction. Although the presence of myosin family members in the nucleus was reported many years ago, their molecular and physiological functions remain largely unknown compared to their counterparts in the cytoplasm. To date, more than seven members of the myosin superfamily (myosins I, II, V, VI, X, XVI, and XVIII) have been identified in the nucleus [1]. Most of them do not form filaments in the nucleus. Nuclear myosin (referred to as myosin II here) was first reported in 2005 [2], but myosin filaments have not been found in the nucleus so far. These reports strongly suggest that nuclear myosin may have different functions from its cytoplasmic counterpart. Indeed, together with nuclear actin, nuclear myosin I was found to mediate the transcription of ribosomal RNA genes [3,4], whereas nuclear myosin (II) was found to function as a transcription factor to regulate the expression of protein-coding genes [5]. The transcriptional activity might be determined by the phosphorylation status of the nuclear myosin regulatory light chain (MLC_{20})[1] and is probably cell-type-specific.

Abbreviations: XO, xanthine oxidase; MLC_{20}, myosin regulatory light chain; smMLC_{20}, smooth muscle MLC_{20}; nmMLC_{20}, nonmuscle MLC_{20}; MYL2, cardiac muscle MLC_{20}; MLCK, myosin light chain kinase; co-IP, coimmunoprecipitation; ChIP, chromatin immunoprecipitation; ROS, reactive oxygen species; IR, ischemia/reperfusion; HR, hypoxia/reoxygenation; TUNEL, terminal deoxynucleotidyl transferase-mediated dUTP nick-end labeling; ICAM-1, intercellular adhesion molecule-1; siRNA, small interfering RNA; MUT, mutant; WT, wild type; GAPDH, glyceraldehyde-3-phosphate dehydrogenase.

* Correspondence to: Department of Pharmacology, School of Pharmaceutical Sciences, Central South University, Changsha 410078, China; or Department of Internal Medicine, The University of Texas Medical Branch, Galveston, TX 77555-1083, USA.

E-mail addresses: Junpeng@csu.edu.cn (J. Peng), quli@UTMB.EDU (Q. Li).

http://dx.doi.org/10.1016/j.freeradbiomed.2015.02.013
0891-5849/© 2015 Elsevier Inc. All rights reserved.

Myosin was originally thought to be restricted to muscle cells, but it also exists in nonmuscle cells, named nonmuscle myosin. Both muscle myosin and nonmuscle myosin molecules comprise three pairs of peptides: two heavy chains of 230 kDa, which constitute the head and tail domains; two 20- kDa regulatory light chains (MLC_{20}); and two essential light chains (17 kDa) [6]. Actually, myosin and nonmuscle myosin are encoded by different genes, and nonmuscle myosin is expressed in muscle cells, too [7,8]. Compared with myosin, the functions of nonmuscle myosin in muscle cells remain largely unknown. In a recent study, we have found that overexpression of nonmuscle MLC_{20} ($nmMLC_{20}$) in smooth muscle cells can significantly increase the transcription of the intercellular adhesion molecule-1 (ICAM-1) gene, suggesting that $nmMLC_{20}$ plays a role in the regulation of gene expression [5]. In cardiomyocytes, $nmMLC_{20}$ was found to be present not only in the cytoplasm but also in the nucleus (Supplementary Fig. S1). However, it is not known whether $nmMLC_{20}$ in cardiomyocytes could also function as a transcription factor to regulate gene expression.

It is well recognized that oxidative stress is one of the major mechanisms responsible for myocardial ischemia/reperfusion (IR) injury [9,10]. Oxidative stress refers to a steady-state level of oxidative damage in cells, tissues, or organs because of the accumulation of reactive oxygen species (ROS) [11]. Enzymatic and metabolic pathways are potential sources of ROS. In addition to NADPH oxidase, the xanthine oxidase (XO)-derived product H_2O_2 is another important source of ROS in the myocardium [12,13]. It has been reported that XO protein expression and activity were significantly increased in a mouse model of myocardial infarction and XO inhibitor was able to attenuate IR injury in different animal models and humans [14–16], suggesting that upregulation of XO expression and/or XO activity accounts for, at least in part, myocardial IR oxidative injury. However, the molecular mechanisms underlying the IR-induced XO expression remain poorly understood.

As we mentioned earlier, the transcriptional function of nuclear MLC_{20} might be related to its phosphorylation status, which is primarily regulated by myosin light chain kinase (MLCK) [5,17]. It has been demonstrated that MLCK is activated after myocardial IR, and ML-7, a specific inhibitor of MLCK, exerts a beneficial effect on myocardial IR injury [18], which might be due to, at least partially, the reduction in MLC_{20} phosphorylation level. Because of the positive correlation between MLC_{20} phosphorylation and XO expression after myocardial IR as well as the transcriptional function of $nmMLC_{20}$, we hypothesized that nuclear $nmMLC_{20}$ promotes IR-induced XO expression through its increased phosphorylation. By using bioinformatics analysis (Proscan version 1.7) [19], we predicted a potential recognition sequence (GTCGCC) for $nmMLC_{20}$ in the promoter region of the XO gene.

The main purpose of this study was to explore the role of nuclear $nmMLC_{20}$ in the regulation of XO expression after myocardial IR and to decipher the underlying mechanisms. By using a rat model of myocardial IR, we first investigated whether nuclear $nmMLC_{20}$ is involved in the regulation of XO expression in myocardium. Next, by using a cell model of hypoxia/reoxygenation (HR), we verified the transcriptional function of nuclear $nmMLC_{20}$ in the regulation of XO expression. Finally, by using strategies of coimmunoprecipitation (co-IP), chromatin immunoprecipitation (ChIP), DNA pull-down, and luciferase reporter gene assay, we identified the molecular mechanisms through which $nmMLC_{20}$ transcriptionally upregulates XO expression.

Materials and methods

Animals

Male Sprague–Dawley rats weighing 220–280 g were purchased from the Laboratory Animal Center, Xiang-Ya School of Medicine (Central South University, Changsha, China). All animals received humane care in compliance with the *Guide for the Care and Use of Laboratory Animals'* published by the National Institutes of Health (NIH Publication No. 85-23, revised 1996). Experimental procedures were approved by the Central South University Veterinary Medicine Animal Care and Use Committee.

Experiments in animals

The first set of animal experiments was designed to explore the correlation between phosphorylation of $nmMLC_{20}$ and XO expression after IR. The animals were randomly allocated to four groups ($n=8$ per group): (1) the control group (without surgery), (2) the sham group (underwent surgical procedures but without ischemic insult), (3) the IR group (subjected to 1 h of ischemia followed by 3 h of reperfusion), and (4) the ML-7 group (pretreated with 1 mg/kg ML-7 water solution ip 1 h before IR). ML-7 (hexahydro-1-[(5-iodo-1-naphthalenyl)sulfonyl]-1 *H*-1,4-diazepine hydrochloride) is a cell-permeative, potent, and selective inhibitor of MLCK ($K_i=0.3$ μM) [20,21]. Anesthetized rats (sodium pentobarbital, 60 mg/kg, ip) were mechanically ventilated with room air using a positive-pressure ventilator. The ventilation rate was maintained at 30–35 strokes/min with a tidal volume of approximately 15 ml/kg body wt. Electrocardiography was used to continuously monitor changes in the electric activity of the heart throughout the experiment. The surgical procedure for IR was carried out as described previously [22].

In the second set of animal experiments, $nmMLC_{20}$ expression was knocked down by small interfering RNA (siRNA) to demonstrate the cause-and-effect relationship between $nmMLC_{20}$ and XO expression. Animals were divided into five groups ($n=6$ per group): (1) the control group, (2) the $nmMLC_{20}$ siRNA group (animals were transfected with $nmMLC_{20}$ siRNAs but did not undergo IR), (3) the IR group, (4) the IR+$nmMLC_{20}$ siRNA group (animals were transfected with $nmMLC_{20}$ siRNAs 48 h before IR), and (5) the IR+siRNA negative control (siRNA NC) group (animals transfected with scrambled siRNAs 48 h before IR).

Experiments in cell cultures

H9c2 cells derived from rat heart were seeded at a density of 1×10^6 cells/cm^2 and grown to 70–80% confluence in Dulbecco's modified Eagle's medium containing 10% fetal bovine serum. Cells were washed with phosphate-buffered saline (PBS) and then made to be quiescent in serum free DMEM for 24 h before experiments.

The first set of in vitro experiments was designed to verify the correlation between the phosphorylation of $nmMLC_{20}$ and XO expression under the condition of HR, which mimics IR in vivo. H9c2 cells were divided into three groups (six individual experiments per group): (1) the control group (H9c2 cells cultured under normal conditions), (2) the HR group (H9c2 cells subjected to 24 h of hypoxia ($O_2/N_2/CO_2$, 1/94/5) followed by 12 h of reoxygenation), and (3) the ML-7 group (ML-7 was added into the culture medium at 2 μM final concentration at the beginning of HR).

To demonstrate the cause-and-effect relationship between $nmMLC_{20}$ and XO expression in vitro, $nmMLC_{20}$ was knocked down by using siRNA. H9c2 cells were divided into five groups (six individual experiments per group): (1) the control group, (2) the $nmMLC_{20}$ siRNA group (cells transfected with $nmMLC_{20}$ siRNAs, cultured under normal conditions), (3) the HR group, (4) the HR+$nmMLC_{20}$ siRNA group (cells transfected with $nmMLC_{20}$ siRNAs, subjected to HR), and (5) the HR+siRNA NC group (cells transfected with scrambled siRNAs, subjected to HR).

To prove that HR-induced H_2O_2 production is primarily due to increased XO activity, allopurinol, a well-known XO inhibitor [23], was applied in the third set of in vitro experiments. H9c2 cells

Download English Version:

https://daneshyari.com/en/article/1907958

Download Persian Version:

https://daneshyari.com/article/1907958

Daneshyari.com