EXPERIMENTAL CELL RESEARCH 317 (2011) 2938–2949

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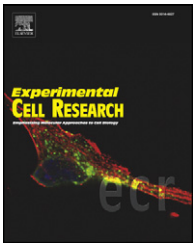

Research Article

A novel cardioprotective p38-MAPK/mTOR pathway

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ARTICLE INFORMATION

Article Chronology:
Received 15 June 2011
Revised version received 22 September 2011
Accepted 23 September 2011
Available online 2 October 2011

Keywords:
Ischemia/reperfusion
oxidant stress
mTOR
p38 MAPK
cell survival

ABSTRACT

Despite intensive study, the mechanisms regulating activation of mTOR and the consequences of that activation in the ischemic heart remain unclear. This is particularly true for the setting of ischemia/reperfusion (I/R) injury. In a mouse model of I/R injury, we observed robust mTOR activation, and its inhibition by rapamycin increased injury. Consistent with the in-vivo findings, mTOR activation was also protective in isolated cardiomyocytes exposed to two models of I/R. Moreover, we identify a novel oxidant stress-activated pathway regulating mTOR that is critically dependent on p38-MAPK and Akt. This novel p38-regulated pathway signals downstream through REDD1, Tsc2, and 14-3-3 proteins to activate mTOR and is independent of AMPK. The protective role of p38/Akt and mTOR following oxidant stress is a general phenomenon since we observed it in a wide variety of cell types. Thus we have identified a novel protective pathway in the cardiomyocyte involving p38-mediated mTOR activation. Furthermore, the p38-dependent protective pathway might be able to be selectively modulated to enhance cardio-protection while not interfering with the inhibition of the better-known detrimental p38-dependent pathways.

Introduction

Reperfusion is the definitive treatment for acute coronary syndromes including myocardial infarction (MI), but reperfusion injury is, at this point, largely unavoidable [1]. Reactive oxygen species (ROS), which activate a host of signaling pathways including, among others, the stress-activated protein kinases, are key mediators of I/R injury. In an attempt to reduce reperfusion injury, pre-clinical studies have identified a large number of putative targets of ROS, but very few have been validated and much remains to be done to better understand the consequences of modulating their activity in the ischemic heart. The p38 MAPKs are clear examples of this. p38s are members of the stress-activated protein kinase family [2] and are activated by various stresses including I/R in the heart [3]. Although several studies report that p38 activation enhances injury in hearts subjected to I/R, other studies suggest that p38 activation may confer protection in some circumstances [4] and reviewed in [5]. There are many reasons for these disparate results. Most notably, different animal models and different protocols have been employed and this likely leads to different magnitudes and time courses of activity.

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Abbreviations: I/R, ischemia/ reperfusion injury; MI, myocardial infarction; H/R, hypoxia/reoxigenation; ROS, reactive oxygen species; AAR, area at risk; IA, infarct area; NRVM, neonatal rat ventricular myocytes.

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doi:10.1016/j.yexcr.2011.09.011

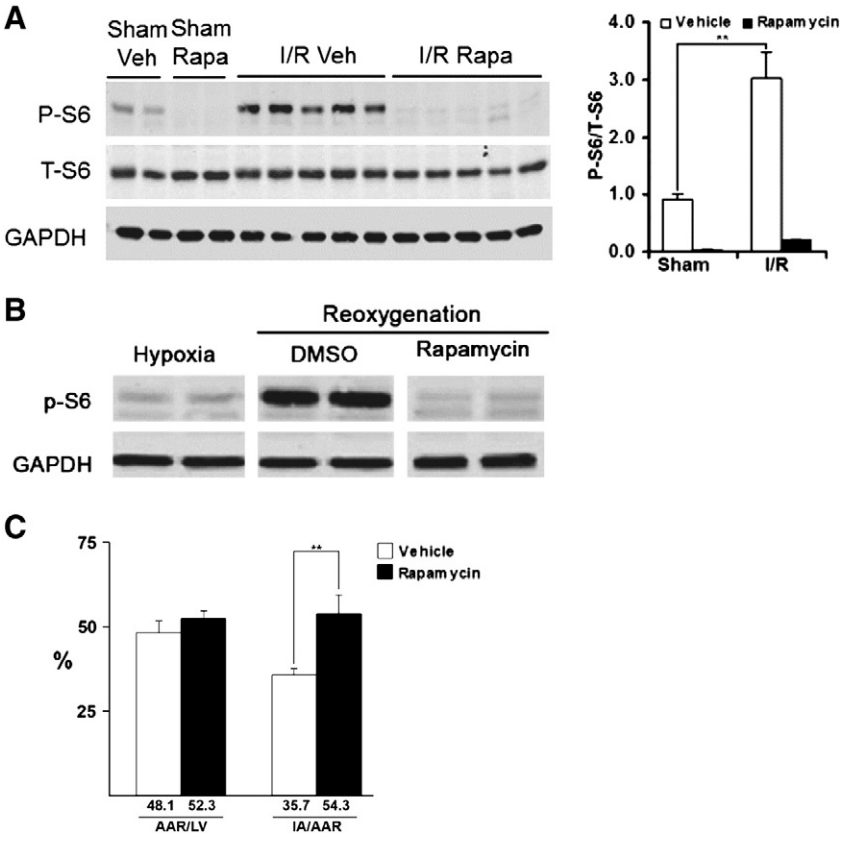

Fig. 1 – Activation of mTOR in reperfused hearts is cardioprotective. A. Anesthetized C57/Bl6 mice were subject to left coronary artery ischemia (30 min) followed by reperfusion for 24 h. Mice were either previously pretreated with rapamycin (2 mg/kg, 24 h and again at 3 h before ischemia, n = 20), or left untreated (vehicle, DMSO 2%, EtOH 48%, PBS 50%, n = 17). Hearts were processed for immunoblotting as described in Methods. Immunoblot shows S6 phosphorylation (a readout of mTOR activity) in hearts subjected to sham I/R or I/R, either following rapamycin treatment or vehicle treatment (left panel with quantification shown in right panel). B. Reoxygenation following hypoxia activates mTOR as determined by p-S6. NRVMs in modified KRH medium were subjected to hypoxia (45 min) followed by reoxygenation for 30 min, in the presence of 0.1% DMSO or 20 nM rapamycin. Immunoblot shows levels of phosphorylated S6 (p-S6). C, mTOR inhibition increases infarct size. Anesthetized C57/Bl6 mice were treated as in A. Tissue was processed as described in Methods. Quantification of area at risk of infarction (AAR) as a percentage of total left ventricular area (LV; AAR/LV)), and infarct area (IA) as percentage of the AAR (IA/AAR) are shown. ** p < 0,01.

This culminates in different profiles of activation of downstream targets. Importantly, some of the downstream targets of p38 are protective, while others are inducers of cell death, and the overall result of p38 activation may depend on the balance between these [5].

Recently, Downward and co-workers reported the surprising finding that p38 activates mTOR in response to oxidant stress in the A547 cancer cell line [6]. This was surprising on two fronts: 1) mTOR is typically inhibited by stressors [7,8], and 2) p38 had never been implicated in regulating mTOR. Herein we examine whether this novel pathway is active in non-transformed cells, whether it plays an important biological role, and what are the mechanisms regulating activity of the pathway. We chose to focus on cardiomyocytes, since oxidant stress injury plays such a major role in the cell death seen in the setting of ischemia/reperfusion (I/R). We now report that activation of mTOR is protective in the setting of I/R in vivo and H/R in vitro. Furthermore, we delineate an extensive signaling cascade regulated primarily by p38 but also by Akt, that recruits multiple factors that converge on mTOR. We believe that many of these factors could serve as novel targets to limit I/R injury. Our studies significantly advance understanding of I/R injury and the factors regulating it.

Materials and methods

Ischemia/reperfusion model

C57/Bl6 mice were utilized in accordance with the *Guide for the Care and Use of Laboratory Animals*. These studies were approved by the Institutional Animal Care and Use Committee of Thomas Jefferson University. 11 week old male mice, were injected intraperitoneally with vehicle (DMSO 2%, EtOH 48%, PBS 50%) or rapamycin (2 mg/kg in DMSO 2%, EtOH 48%, PBS 50%) 24 h and 3 h before surgery. They were then anesthetized with 2.0% isofluorane and their heart was exposed using a vertical pericardiotomy. Ischemia was induced by occluding the left anterior descending coronary artery for 30 min, followed by release of the occlusion [9]. 24 h after reperfusion, the animals were anesthetized with 2.0% isofluorane