microRNA-144: the ‘what’ and ‘how’ of remote ischemic conditioning?

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Introduction

Remote ischemic preconditioning (RIPC) is the intriguing phenomenon whereby brief episodes of ischemia–reperfusion applied in a distant organ or tissue render the heart resistant to infarction [29, 37]. Most notably, there is compelling evidence that robust cardioprotection can be triggered by transient 5-min periods of limb ischemia, achieved in a simple and cost-effective manner by inflation–deflation of a standard blood pressure cuff [19]—a discovery that has spurred the clinical investigation of RIPC in patients undergoing cardiac surgery and percutaneous intervention [2, 5, 12, 14, 24, 25, 34]. However, despite the translational appeal and promise of this apparently simple strategy, two fundamental and not-so-simple questions continue to bedevil investigators in the field [27, 30, 31]: what is the identity of the protective factor(s), and how is the protective stimulus communicated from its source (presumably the remote tissue) to the heart?

miR-144: the ‘what’ and ‘how’ of RIPC

Among the multiple candidates and hypotheses that have been proposed, encompassing both neuronal and circulating humoral stimuli [4, 6, 7, 10, 11, 15, 17, 23, 27, 30, 31], a new player has

been proposed: Li and colleagues [21] report that release and transport of a microRNA (miR)—specifically, miR-144—plays a requisite role in the infarct-sparing effect of RIPC. The concept that miR-144 may be the elusive ‘what’ of RIPC is supported by three observations. First, using the mouse model, Li and colleagues [21] found that four 5-min episodes of limb ischemia evoked a significant increase in cardiac expression of miR-144, identified by microarray analysis in hearts harvested at 15 min after completion of the RIPC stimulus. Cause-and-effect was then established by demonstrating that in vivo pretreatment with an antisense oligonucleotide against miR-144 abolished the infarct-sparing effect of RIPC in isolated buffer-perfused hearts subjected to global ischemia–reperfusion, while cardioprotection achieved with RIPC in the isolated heart was mimicked by in vivo administration of an miR-144 homologue oligonucleotide given in lieu of brief limb ischemia. In addition, with regard to the ‘how’, Li and colleagues [21] also offer insight into the mechanism of humoral transport. Plasma concentrations of miR-144 were approximately doubled following limb ischemia in both mice and human volunteers. Interestingly, and contrary to expectations, mature miR-144 was not protected from RNase-mediated degradation by encapsulation in exosomes or microparticles. Although RIPC was associated with an increase in the content of miR-144 precursor oligonucleotide in circulating exosomes, mature miRNA was present in the exosome-poor plasma supernatant and was stabilized and protected against degradation via binding to the argonaute protein AGO-2.

The ‘miRNA revolution’, myocardial ischemia–reperfusion and cardioprotection

miRNAs are a class of small, non-coding RNAs that down-regulate gene expression by binding to the 3′

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untranslated region of target mRNAs and, thus, suppress protein translation and expression [16, 18, 22, 32, 33]. The discovery of miRNAs in 1993 [20] marked the first step of an ongoing ‘miRNA revolution’ [18]: >1,000 individual miRNAs have been identified to date, and >50% of the human genome is proposed to be regulated by miRNAs [18, 21].

It is therefore not surprising that miRNAs have been implicated as ubiquitous modulators of diverse biologic and physiologic functions including (but not limited to) cell proliferation, migration, differentiation, secretion, excitation and conduction, and, as a corollary, dysregulation of miRNAs has been hypothesized to contribute to a host of pathologies including cardiovascular disease. Indeed, up-regulation (or, in some instances, down-regulation) of specific miRNAs in heart has been proposed to play causal roles in hypertension, arrhythmia, heart failure, cardiac hypertrophy, fibrosis, as well as post-infarct healing and remodeling [16, 18, 22, 32, 33]. Of particular relevance: (1) changes in cardiac expression of miR-1, the most abundant miRNA in heart, as well as multiple other miRNAs, have been described in response to myocardial ischemia–reperfusion, and (2) the potential targets of these miRs include genes encoding key proteins involved in apoptosis, autophagy and survival signaling (i.e., Bcl-2, caspases, FAS ligand, heat shock proteins, mTOR, HIF-1 α , Akt, and PTEN) [13, 26, 35, 38]. Finally, considerable recent attention has focused on the concept that changes in circulating levels of miRNAs may serve as biomarkers for the prediction, diagnosis and prognosis of heart failure, hypertension, acute coronary syndromes, acute myocardial infarction and stroke [16, 18, 22, 32, 33].

Nascent efforts have also been made to extend the ‘miRNA revolution’ to conditioning-induced cardioprotection. Among the small number of studies conducted to date, attention has focused largely on miR-1, and contradictory results have been obtained: for example, increased cardiac expression has been described with ischemic preconditioning [8], while, with postconditioning and RIPC, both up-regulation and down-regulation of miR-1 (possibly in a time-dependent manner) have been reported [1, 3, 8, 13]. Indeed, in a recent study conducted in the in vivo swine model, differences in expression of miR-1 (and other candidate miRNAs) following application of a postconditioning stimulus were considered to be ‘false positives’, playing no causal role in cardioprotection [1]. miR-144 (or, more precisely, the miR-144/451 cluster) has been the specific topic of investigation in two previous publications, and, in contrast to miR-1, there is an emerging consensus: overexpression of the miR-144/451 cluster reduces cell death in isolated cardiomyocytes subjected to simulated ischemia–

reperfusion [40], in vivo cardiac expression of miR-144/451 was increased in response to brief preconditioning ischemia [36], and preconditioning failed to reduce infarct size in miR-144/451 knockout mice [36].

New contributions and future challenges

Li and colleagues [21] make three contributions that advance both the ‘miRNA revolution’ and our understanding of the ‘what’ and ‘how’ of RIPC: they are the first to identify miR-144 as being necessary and sufficient for the infarct-sparing effect of RIPC, the first to suggest that delivery of an miR-144 homologue may represent a novel cardioprotective therapy, and the first to propose that increased plasma concentrations of miR-144 may serve as a biomarker to document the successful implementation of an RIPC stimulus. However, the results obtained by Li et al. [21] raise five questions and controversies that await resolution. First, it is important to note that all conclusions regarding miR-144, RIPC and cardioprotection were derived from a protocol in which the RIPC stimulus, the miR-144 homologue or the anti-sense oligonucleotide were administered in vivo, the hearts were then excised, and infarct size was assessed following global ischemia–reperfusion. Corroboration in an in vivo model of coronary artery occlusion–reperfusion is clearly required. Two additional and arguably tangential issues that warrant investigation are the observations that the favorable effects of miR-144 were not associated with transport via exosomes (a finding that appears to conflict with the emerging concept that extracellular vesicles serve as vectors for the RIPC-initiated humoral communication of cardioprotective signals to the heart [9, 28, 39]), and the apparent lack of involvement of miR-451, the component of the miR-144/451 cluster that was implicated as being largely responsible for preconditioning-induced cardioprotection [36]. A fourth question, the resolution of which will be challenging, is the cellular source of the circulating miR-144 released in response to the RIPC stimulus: is miR-144 liberated from skeletal muscle, or from a more typical, blood-borne source [33]? Finally, although the established role of miRNAs is gene silencing and suppression of protein translation [16, 18, 22, 32, 33], Li et al. [21] make the provocative observation that in vivo administration of the miR-144 homologue oligonucleotide was associated with a rapid (within 1 h) increase in expression of phosphorylated Akt, ERK and GSK3 β . If miR-144 is confirmed to be capable of inducing rapid post-translational protein modification, this would be a paradigm shift in our knowledge of miRNAs, survival kinase signaling and cardioprotection.

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