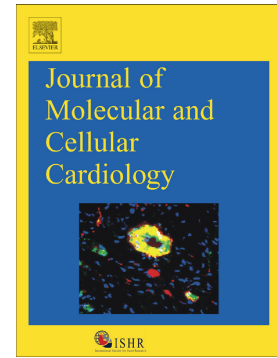


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Zoltán V. Varga, Bence Ágg, Péter Ferdinandy



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miR-125b is a protectomiR: a rising star for acute cardioprotection

¹Zoltán V. Varga, MD, PhD; ^{1,2,3}Bence Ágg, MD; ^{1,3}Péter Ferdinandy, MD, PhD, MBA

¹Department of Pharmacology and Pharmacotherapy, Semmelweis University, Budapest, Hungary

²Heart and Vascular Center, Semmelweis University, Budapest, Hungary

³Pharmahungary Group, Szeged, Hungary

Address of correspondence: Péter Ferdinandy - Department of Pharmacology and Pharmacotherapy, Semmelweis University, Budapest, Hungary, H-1089, Nagyvárad tér 4., phone: +36 1 2104416, fax: +36 1 2104412; E-mail: peter.ferdinandy@pharmahungary.com

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Myocardial infarction and resulting heart failure remain the leading causes of death worldwide. Therefore, novel therapies are required to protect the heart against the detrimental effects of acute ischemia/reperfusion injury. Micro-RNAs are promising novel targets for cardioprotection as highlighted by recent seminal position papers and reviews [1-3].

The miR-125 family

The miR-125 family is highly conserved mammalian miR family, implicated in a variety of physiological and pathological processes, including embryonic development, immune responses, cancer development (as either a repressor or promoter), and in ischemia/reperfusion injury [4-6]. miR-125 family in humans is composed of three homologs miR-125a, miR-125b-1 and miR-125-2. The mature miR-125b, alternatively called miR-125b-5p is a result of the maturation of a stem-loop sequence, called mir-125b-1 to miR-125b-5p and to the partially complementary, passenger strand, miR-125b*. Complementary miR-star sequences were previously thought to be degraded, however, according to recent findings depending on the biological context (cell type, pathological condition, developmental stage) they often have biological functions, e.g. acting as paracrine regulators, as shown in case of the cardiac fibroblast-derived miR-21* in a mouse model of angiotensin II-induced cardiac hypertrophy [7, 8]. miR-125 family members are mostly expressed

in clusters with other miRNAs, such as miR-99 and let-7 family members, suggesting that their expression is regulated by promoters and transcription factors common with other miRs [9, 10].

miR-125b is cardioprotective against acute ischemia/reperfusion injury

miR-125b has been previously found as a potential player in cardioprotection against ischemia/reperfusion injury by two independent groups. We have previously assessed early changes in miRNA expression profiles with a miRNA microarray in acute cardioprotection by ischemic pre- and postconditioning and compared the expression changes to ischemia/reperfusion injury and non-ischemic conditions in rat hearts [11] and found that ischemia/reperfusion injury markedly downregulated miR-125b* which was inhibited by both ischemic pre- and postconditioning, i.e. both pre- and postconditioning induced miR-125b* as compared to ischemia/reperfusion. Furthermore, transfection of primary cardiomyocytes with miR-125b* showed cardiocytoprotection against simulated ischemia/reperfusion. We have termed mimics or antagomiRs of certain miRNAs that elicit cardioprotection “protectomiRs” [11]. In an elegant set of experiments, Wang et al. examined miR-125b expression levels in H9C2 cardiomyoblasts and adult primary cardiomyocytes exposed to 2 hours of hypoxia followed by 24 hours of reoxygenation. They found a similar decrease in the level of miR-125b that has been reversed by antioxidant treatment (PDTC or *N*-acetyl cysteine), implicating that miR-125b is a mediator of pharmacologically-induced cardiocytoprotection. In further *in vivo* studies, they also confirmed that overexpression of miR-125b confers cardioprotection against myocardial ischemia/reperfusion injury, potentially by decreasing myocyte apoptosis via down-regulating pro-apoptotic p53 and Bak1 expression levels [6].

The recent study by Bayoumi *et al.* in *J Mol Cell Cardiol* is a further confirmation of the cardioprotective properties of miR-125b [12]. The authors used first a knock-down approach *in vivo* to decrease miR-125b levels in mice. Upon permanent coronary artery ligation, mice treated with the antagomiR of miR-125b developed larger infarcts, had poor contractile function, and more fibrosis. They found increased cardiomyocytes apoptosis, when miR-125b levels were reduced *in vitro*. They also tested if the cardioprotective drug carvedilol [13, 14] affects expression levels of miR-125b and found an induction of miR-125b both in HL-1 atrial

cardiomyocytes line, in H9C2 cardiomyoblasts, and in primary neonatal cardiomyocytes upon cardiocytoprotection by carvedilol.

miR-125b in heart failure and cardiac fibrosis

There are conflicting results in relation to miR125b expression level in heart failure models. In an early study Busk *et al.* reported upregulation of miR-125b in a rat model of pressure overload-induced heart failure [15]. There are two studies available in human patients when miR-125b levels have been measured. Marquez *et al.* assessed miRNA release to the coronary sinus in patients suffering from advanced heart failure (9 patients – non-specified etiology) and compared the changes to healthy individuals (8 subjects), thereby assuming miRNA expression changes occurring in the myocardium due to advanced heart failure. They found a significant decrease in the release of miR-125b-5p to the coronary sinus in this advanced heart failure population, awaiting cardiac transplantation [16]. In another human study, miRNA expression levels have been assessed in peripheral blood mononuclear cells in patients suffering from heart failure of different etiologies. MiR-125b has been found also to be decreased in monocytes due to heart failure of ischemic origin [17], however, the relevance of transcript data from circulating monocytes are questionable (see for a recent position paper: [3]). In contrast Nagpal *et al.* described recently upregulation of miR-125b in explanted failing human hearts, and investigated the role of miR-125b in myocardial fibrosis [18]. The authors have demonstrated with various in vitro and in vivo approaches that upregulation of miR-125b is dependent on angiotensin-II-TGF β signaling and that miR-125b-related downstream effects involve downregulation of apelin and p53 in fibroblasts thereby promoting fibroblast-to-myofibroblast transition. They concluded that miR-125b inhibition might be a promising therapeutic approach to inhibit myocardial fibrosis. Confirming these fibroblast-specific effects, Bie *et al.* has transfected miR-125b mimics into cardiac fibroblasts that resulted in fibroblast activation as assessed by increased expression of the myofibroblast markers (alpha-smooth muscle actin and vinculin) [19]. Though, cardiac fibrosis is generally considered to be a maladaptive event in structural and functional remodeling, the effects of TGF β is particularly interesting, since beside being a major mediator in fibrotic processes, it is a positive regulator of cardiomyocyte survival as well in response to cardiac injuries. Exogenous administration of TGF β during reperfusion has been shown to decrease myocardial infarct size in rats [20]. Furthermore, TGF β may also induce paracrine, intercellular

miRNA-dependent signaling as described by Climent et al. showing that TGF β triggers miR-143/145 transfer from smooth muscle cells to endothelial cells [21], a mechanism that is highly plausible between cardiomyocytes and fibroblasts, either via nanotubes, exosomes, or direct gap junctional miRNA transfer. Thus, miRNAs might have a complex multicellular involvement in promoting fibrosis, hypertrophy, and at the same time cardiomyocyte survival. Indeed, known pro-fibrotic miRNAs (miR-21, miR-208) may act as pro-survival microRNAs (protectomiRs) in cardiomyocytes subjected to ischemia/reperfusion injury. In the same study, it was shown that one of the most robust cardiocytoprotective effect is observed by transfection of the cardiac myocytes with the mimic of miR-125b* (also known as miR-125b-1-3p) [11].

Although, the cardiocytoprotective function of miR-125b and its induction by cardioprotective interventions (pharmacological as well as ischemic conditioning) has been confirmed by three independent groups, further explorations of the anti-fibrotic and cardiocytoprotective mechanisms are needed to confirm the effectiveness miR-125b therapy in cardioprotection against acute myocardial infarction and in post-infarction heart failure and remodeling.

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Conflict of interest disclosures

ZVV and PF are inventors of an international patent application, describing the use of miRNA compounds including miR-125b* in ischemia/reperfusion injury (WO 2013/057527). PF is an owner of a pharmaceutical/biotechnological company.

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