ISSN: 0976-3104
SPECIAL ISSUE: REDOX BIOLOGY IN CARDIOVASCULAR AND NEUROLOGICAL DISORDERS

REDOX REGULATION OF REGENERATION OF INFARCTED HEART WITH STEM CELLS: ROLE OF MICRO RNA

Partha Mukhopadhyay, Narasimman Gurusamy, and Dipak K. Das*

National Institute of Health (NIH), Bethesda, Maryland and Cardiovascular Research Center, University of Connecticut School of Medicine, Farmington, Connecticut, USA

Received on: 10th-Feb-2011; **Revised on:** 16th-Feb-2010; **Accepted on:** 18th-Feb-2010; **Published on:** 8th-Jul -2011
***Corresponding author:** Email: ddas@neuron.uchc.edu; Tel: (860) 679-3687, Fax: (860) 679-4606

ABSTRACT

Mobilization and homing of the hematopoietic stem cells appear to be regulated by mechanism involving redox cycling. Stem cells are localized inside bone marrow in a strictly hypoxic environment and must move to the injury site that is subjected to oxidative environment. Cytokines and adhesion molecules control stem cell mobilization through a redox-regulated process. The major hitch in stem cell therapy includes the life of the stem cells after the stem cell therapy; most cells do not survive beyond 24 to 72 hours. Sudden exposure of the stem cells from the hypoxic meleu into the oxidative environment likely to cause severe injury to the cells. FoxO-SirT network appears to be intimately involved in redox-regulated stem cell homeostasis while their differentiation process is regulated by redox factor protein-1 (Ref-1). Lack of oxygen [hypoxia], specifically controlled hypoxia can stimulate the growth of the stem cells in their niche and HIF-1a plays a significant role in their maintenance and homing mechanism. Recently, resveratrol, a polyphenolic phytoalexin prolonged the survival of the stem cells as evidenced by active proliferation and differention of the cells even after four months of cell therapy. The enhancement of stem cell survival was shown to be due to the ability of resveratrol to maintain a reduced tissue environment by over-expressing Nrf2 and Ref-1 in rat heart up to six months resulting in an enhancement of the regeneration of the adult cardiac stem cells as evidenced by increased cell survival and differentiation leading to improved cardiac function. Expression of SDF and myosin conclusively demonstrated homing of stem cells in the infracted myocardium, its regeneration leading to improvement of cardiac function.

Key words: *Cardiac stem cells; resveratrol; redox; nrf2; ref-1; NFkB; heart; ischemia*

[I] INTRODUCTION

The rapidly expanding fields of stem cell biology and its potential role in cardiac repair process have stimulated the investigators to explore the molecular mechanisms of stem cell mobilization and homing. Existing reports indicate cardiac chimerism resulting from the migration of primitive cells from the recipients to the grafted heart [1]. In this study, the authors showed that as compared with the ventricles of the control hearts, the ventricles of the transplanted hearts had higher number of cells, which were positive for C-kit and Sca-1. More recent studies suggest that cytokines and adhesion molecules might be involved in the stem cell homing process. The continuous presence of CD34+ cells in the peripheral blood during the steady state hematopoiesis support the role of adhesion molecules in the homing process [2]. Most of the adhesion molecules are believed to be members of b1 and b2 integrin, selectin and super immunoglobulin [3,4]. L-selectin that is responsible for the contact of leukocytes with endothelium, is highly expressed on CD34+ progenitor cells suggesting its role for homing [5]. Very late antigen-4 (VLA-4) is expressed on circulating CD34+ cells residing in the bone marrow suggesting a role of VLA-4 on the release and circulating the CD34+ cells [6,7]. Leukocyte function–associated molecule-1 (LFA-1) and CD18/CD11a also play a role in the interaction between CD34+ hematopoietic progenitor cells and bone marrow cells [5].

Similar to the adhesive molecules many cytokines are likely to be involved in stem cell mobilization. For example, a number of cytokines including IL-3, IL-8, IL-11, Flt-3 and stem cell factor (SCF) have been implicated for the mobilization of CD34+ cells [8, 9]. However, G-CSF and GM-CSF are best known for the mobilization of peripheral blood stem cell [10, 11]. These factors are involved in the differentiation of

THE IIOAB JOURNAL ISSN: 0976-3104

progenitor cells into granulocytes and monocytes, respectively.

There is no doubt that the success of stem cell therapy depends largely on the efficiency of the hematopoietic stem cells to home to bone marrow. Despite the role of cytokines and adhesive molecules in stem cell mobilization, exact mechanisms remain unclear. Interestingly, signal transduction pathways leading to cytokine expression and inflammatory response are redox regulated. For example, downregulation of intracellular glutathione level is associated with the enhancement of oxidative stress-mediated inflammation and is differentially involved in controlling redox-dependent cytokine regulation [12]. In another study, thioredoxin reduced cysteine residues of transcription factors in the nucleus to regulate their DNA binding and transactivation activities. Upon TNFα stimulation and subsequent generation of ROS, thioredoxin becomes oxidized and releases ASK-1 [13]. In another related study, an alteration of cytokine response was found to be related with activation of redox-dependent transcription factors [14].

From the above discussion, it should be clear that the principle factors of stem cell mobilization, cytokines and adhesion molecules, are redox regulated. Thus, it may be speculated that homing mechanisms of stem cell are also redox-regulated. In fact, a handful number of papers recently appeared that could substantiate this hypothesis. For example, a recent study showed impaired endothelial progenitor cell function in response to the oxidative stress [15]. Another study demonstrated a positive role of ROS in the regulation of normal and neoplastic hematopoiesis [16]. Another related study revealed that thioredoxin mediates redox regulation of the embryonic stem cell transcription factor Oct-4 [17]. More recently, it is found that the production of ROS is greatly stimulated by the inhibition of Ref-1, which ultimately results in induced differentiation of adult cardiac stem cells [18].

The purpose of this review is to discuss the potential redox regulation of stem cell biology and how the redox signaling can potentiate a homing mechanism leading to the repair of the injured cells.

[II] REDOX REGULATION OF STEM CELL IN REGENERATION OF INFARCTED MYOCARDIUM

2.1. ROS in stem cell biology

A growing body of evidence supports the notion that stems cells possess the ability to cope with oxygen overload through an unique adaptive mechanism by which they can upregulate their own antioxidant defense system [19]. Hematopoietic stem cells are located in a hypoxic environment inside the bone marrow where they remain quiescent. Upon mobilization, they are exposed to oxygenic environment, which potentiates proliferation and differentiation [20, 21]. Most cells and tissues exhibit alterations in their antioxidant reserve and capacity to undergo redox cycling during different stages of differentiation. Antioxidant protection abilities of the progenitor cells are highly amplified under stress enhancing their ability to exert resistance against oxidative stress [22]. ROS has been utilized by the embryonic stem cells as transducers of mechanical strain-induced cardiovascular differentiation [23]. A recent study showed that shear stress increased lysine acetylation of histone H3 at position 14, serine phosphorylation at position 10 and lysine methylation at position 79 [24]. Shear stress induced phosphorylation of Flk-1 is shown in Flk-positive embryonic stem cells in a recent study [25]. Interestingly enough, shear stress mediated angiogenic response is redox-regulated. Indeed, a recent study demonstrated redox regulation of the members of MAP kinase pathway including ERK1, 2, JNK and p38MAPK, which potentiate a signaling cascade for the initiation of cardiovascular differentiation of embryonic stem cells [23]. In another study, ROS was found to exert deleterious effects of oxidative stress on hematopoietic stem cells self-renewal and identifies p38MAPK as a key mediator of ROS-induced stem cell lifespan shortening [26].

A continuous increase in ROS activity was demonstrated during the time course of differentiation of embryonic stem cells [27]. A NADPH oxidase like enzyme was identified as source of ROS in embryonic stem cell-derived embryoid bodies, which appeared to interfere with diverse signaling cascades thereby affecting stem cell differentiation. A subsequent study showed that upregulation of HIF at the gene and protein levels that led to an increase in VEGF activity, which is critical for vasculogenesis in embryonic stem cells [23, 28].

Manipulation of subcellular p53 localization in response to endogenous ROS is efficiently done by Sirt1 for the regulation of apoptosis and Nanog expression in mouse embryonic stem cells [29]. The authors showed that SirT1 blocks nuclear translocation of cytoplasmic p53 in response to endogenous ROS and triggers mitochondrial-dependent apoptosis in mouse embryonic stem cells. Nanog expression of SirT1-/- embryonic stem cells clearly revealed an accelerated sensitivity to ROS and a simultaneous p53-mediated repression of Nanog expression suggesting that ROS is important for stem cell maintenance in culture. Interestingly, it is FoxO-deficient hematopoietic stem cells that have a significant increase in ROS suggesting that there might be a link between ROS and cell cycle activities [30]. In this study, FoxO-deficient mice exhibited marked reduction in the lineage-negative Sca1+ and c-Kit+ compartment that contains hematopoietic stem cells. In concert, there was a significant increase in ROS in FoxO-deficient stem cells compared to wild type cells correlating with changes in gene expression that regulate ROS. N-acetyl cysteine, a cell-permeable antioxidant, reduced FoxO-deficient stem cell phenotype and corrected the deficiencies in cell cycle regulation.

Several recent studies have indicated that oxidative stress can regulate FoxOs through a Ral/Jnk-dependent mechanism [31].

THE IIOAB JOURNAL ISSN: 0976-3104

Conditional deletion of some members of FoxO reduced hematopoietic stem cell lineages simultaneously reducing resistance to oxidative stress [30]. Another recent study showed that FoxO3 is specifically required for induction of proteins, which regulate redox signaling in murine erythrocytes [32]. Accordingly, the animals lacking FoxO3 in hematopoietic cells undergo sudden death when exposed to ROS, which also reduces the amount of ROS scavenging enzymes [32]. FoxO-deficient hematopoietic stem cells are subjected to increased amount of oxidative stress and undergo apoptosis. Several studies suggest crucial roles of FoxOs and ROS as signaling network partners in hematopoietic stem cell homoeostasis [33].

2.2. Redox Regulation of Stem cell proliferation and differentiation

Stem cells are usually sheltered in a stable microenvironment called niches, which preserves the survival and replication potential of stem cells in an organ [34, 35]. ROS are known to play a major role in induction of the exit of hematopoietic stem cells from the niche in bone marrow. Redox effector protein-1 (Ref-1) plays an essential role in DNA repair and redox regulation of several transcription factors. In a recent study, we examined the role of Ref-1 in maintaining the redox status and survivability of adult cardiac stem cells challenged with sub-toxic level of H_2O_2 under inhibition of Ref-1 by RNA interference. Treatment with low concentration of hydrogen peroxide in mouse embryonic stem cells is shown to induce the components of NADPH oxidase, and vital cardiac transcriptional regulators such as Nkx2.5, MEF2C and GATA4 [18, 36]. When adult cardiac stem cells are treated with low concentration of H_2O_2 (10 µM) under the inhibition of Ref-1, the amount of ROS production was tremendously increased via activation of components of NAPDH oxidase such as p22 phox, p47 phox and Nox4, leading to the differentiation (increased expression of Nkx2.5, MEF2C, GATA4 and α-sarcomeric actinin) and cell death by apoptosis. In this study, the involvement of ROS in the induction of cardiac differentiation was confirmed by pre-treating cardiac stem cells with N-acetyl-L-cysteine, a scavenger of ROS, which abolished the Ref-1 inhibition-mediated induction of NADPH oxidase components, and cardiac differentiation transcription factors. Moreover, a role for phosphatidylinositol-3-kinase has been identified in ROS-mediated cardiac differentiation of embryonic stem cells [37]. These results indicate that Ref-1 plays an important role in maintaining the redox status of cardiac stem cells and protects from oxidative injury-mediated cell death and differentiation [18]. **[Figure–1]**

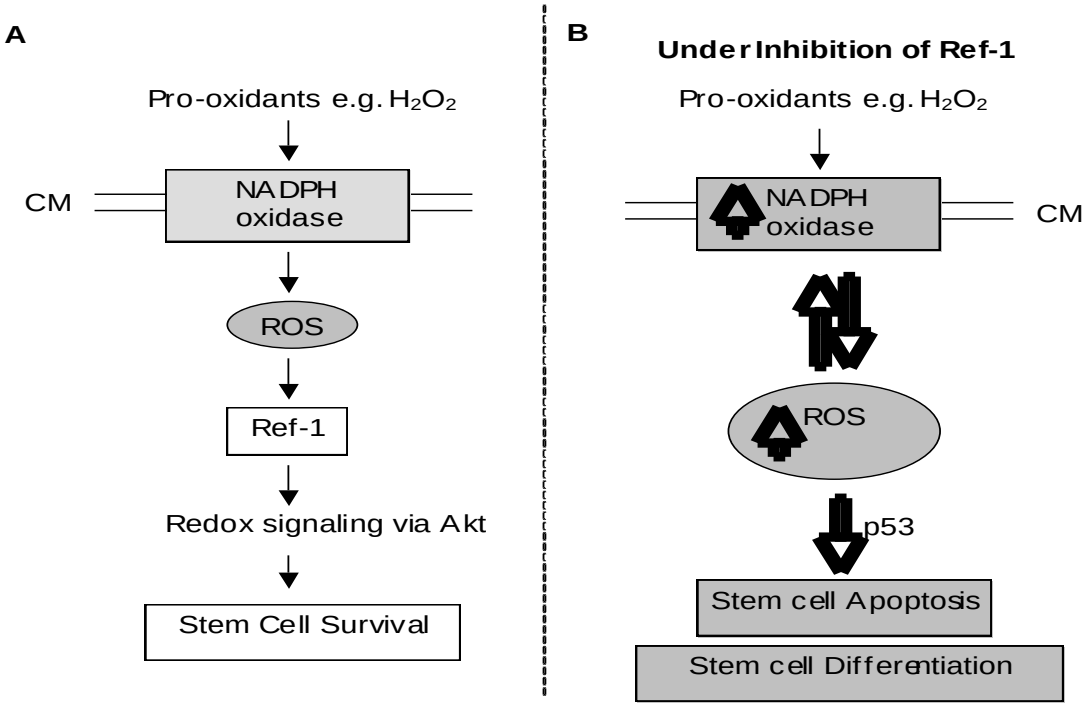

Fig: 1. Ref-1 mediated redox signaling protect stem cells. (A)-Addition of pro-oxidants like hydrogen peroxide at low concentration induces redox signaling mediated through Ref-1 and Akt leading to the survival of stem cells. (B)-Treatment with low concentration of hydrogen peroxide under inhibition of Ref-1 induced the level of reactive oxygen species, and the level of NADPH leading to p53-mediated apoptosis and differentiation in adult cardiac stem cells.

Simultaneous occurrence of apoptosis and differentiation has been observed during embryonic stem cell differentiation [38,

THE IIOAB JOURNAL ISSN: 0976-3104

39]. Tumor suppressor protein p53 regulates cell cycle checkpoint, differentiation and induces cell apoptosis. p53 was shown to be involved in the simultaneous induction of apoptosis and differentiation [40, 41]. Induction of endogenous p53 is found to be associated with differentiation in mouse cultured keratinocytes, mouse embryonic stem cells, hematopoietic and muscle cells [41,42]. High level of p53 was found in undifferentiated embryonic stem cells, and it was decreased as differentiation proceeds [43, 44]. The addition of retinoic acid, a physiological regulator of embryonic development, onto murine embryonic stem cells caused an increase the level of p53 followed by accelerated neural differentiation and apoptosis [45]. Redox-dependent and redox-independent mechanisms have been shown to regulate p53 [46]. Ref-1 is a potent activator of p53 [47]. The activation of survival signaling kinase Akt inhibits p53, whereas pro-apoptotic stimuli-induced p53 inhibits Akt [48]. When the cardiac stem cells are treated with low concentration of H_2O_2, the level of p53 is decreased than normal cellular levels. H_2O_2 treatment under Ref-1 inhibition almost completely abolished the activation of survival signaling molecule Akt; and at the same time the level of p53 was significantly higher than normal levels leading to an enhanced level of ROS production and ROS-mediated cell death and differentiation. The above findings thus indicate that p53 play an important role in determining the fine balance between growth, differentiation and cell death [18].

2.3. Hypoxic regulation of Stem cell differentiation.

Hypoxic preconditioning has been found to extrapolate the potency of mesenchymal stem cells to repair infarcted myocardium, which was attributed to reduced cell death and apoptosis of implanted cells and increased angiogenesis/ revascularization [49]. In vivo and in vitro studies have showed an enhancement in the expression of pro-survival and pro-angiogenic factors including hypoxia-inducible factor 1, angiopoietin-1, vascular endothelial growth factor and its receptor, Flk-1, erythropoietin, Bcl-2, and Bcl-xL with a simultaneous decrement in caspase-3 activation in these cells in response to hypoxic preconditioning compared to their normoxic counterpart. Transplantation of normoxic versus hypoxic mesenchymal stem cells after myocardial infarction resulted in comparable increment in angiogenesis, as well as enhanced morphologic and functional benefits of stem cell therapy in the latter group. Another study demonstrated that the quiescent stem cells survive in hypoxic niches of hematopoietic tissue with the corresponding increase in the mitochondrial number [50, 51]. The authors were able to demonstrate that the activated stem cells move to less hypoxic areas close to the niches, and in better oxygenated areas, they would undergo proliferation and differentiation. In another related study, culture of mesenchymal stem cells in conditions of low oxygen increased expression of c-Met and migration rate in response to chemoattractant gradients [52]. To demonstrate in vivo efficacy the scientists administered control mesenchymal stem cells, and mesenchymal stem cells that have been preconditioned for 24 hours by hypoxia to mice having undergone femoral artery ligation. The mesenchymal stem cells were administered intra-arterially. While both groups had positive response, increased vascularity and reduced limb loss was observed in the groups that received mesenchymal stem cells that were preconditioned with hypoxia.

Recently, specific signaling pathways such as Notch and the expression of transcription factors such as Oct4 that control stem cell self renewal and multipotency, are shown to be activated by HIFs [53]. In another recent study, exposing embryoid bodies derived from embryonic stem cells to ambient oxygen at or below 5% resulted in stabilization as well as an increased transcription of hypoxic responsive genes such as HIF-1α [54]. Interestingly enough, HIF-1a expression peaked to the highest level after 48 hours of hypoxia and then declined to undetectable levels in spite of continued hypoxic exposure.

Consistent with this report, a study demonstrated that prolonged hypoxia in conjunction with serum deprivation caused massive human mesenchymal stem cell death [55]. Indeed, transplantation of mesenchymal stem cells into ischemic heart causes over 99% cell death within 96 hours [55]. In contrast, neonatal cardiomyocytes grafted into a vascular bed survived better than cells transplanted into ischemic tissues [56]. To resolve this problem, a study was undertaken to modify mesenchymal stem cells with a hypoxia-regulated HO-1 plasmid to enhance the survival of stem cells in acute myocardial infarction (MI) heart. In this study, mesenchymal stem cells collected from bone marrow were transfected with either HO-1 or LacZ plasmids. The MSCHO-1 group had higher expression of HO-1 and a 2-fold reduction in the number of terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate in situ nick end labeling-positive cells compared to that of the MSCLacZ group, in the ischemic myocardium. At seven days after implantation, not only that the survival in MSCHO-1 was five-fold greater than that of the MSCLacZ group; but MSCHO-1 also attenuated post-ischemic left ventricular remodeling with an enhancement in the functional recovery of infarcted hearts two weeks after MI [57].

2.4. Redox regulation of stem cell mobilization

The importance of redox regulation of stem cells is increasingly realized as ROS have been implicated in pathological, biological and physiological control of stem cell maintenance and mobilization. ROS are of particular importance for maintaining a critical balance between preservation of the stem cells in an undifferentiated state and mobilization of the cells to the site [homing] where they can undergo differentiation process [58]. It is now believed that

REVIEW

THE IIOAB JOURNAL ISSN: 0976-3104

homeostatic regulation of hematopoietic stem cells is fine tuned by redox signaling, which include their maintenance, proliferation, differentiation, mobilization and finally homing [59].

As mentioned earlier, stem cells can survive better under lower oxygen atmosphere. For example, the self-renewal potential of the hematopoietic stem cells is higher in the low-oxygenic osteoblastic niche [60]. The hematopietic stem cells present in the low-oxygenic niche express higher level of Notch1, N-cadherin, calcium receptor, telomerase, Bcrp and p21, and expresses a lower level of p38 MAPK, p53 and mTOR [60]. On the other hand, hematopietic stem cells in the high ROS population express higher level of p38 MAPK and mTOR, where treatment with an antioxidant, a p38 MAPK inhibitor or rapamycin, an inhibitor of mTOR restore the function of hematopietic stem cells in the high ROS population [60]. These results indicate that ROS-related signaling plays an important role in the preservation of stem cells' self renewal potential, and relatively enhanced proliferation of the stem cells at lower oxygen may be due to their adaptation to hypoxic condition in original niche bone marrow, where oxygen concentration is relatively low [60]. Interestingly, antioxidants can enhance the self-renewal of hematopoietic stem cells through ataxia telangiectasia mutated (ATM) gene, which maintains maintains [delete] genomic stability by activating a key cell-cycle checkpoint in response to DNA damage, telomeric instability or oxidative stress [61]. Ito et al [61] have also shown that ATM-mediated inhibition of oxidative stress potentiates the self-renewal capacity of hematopoietic stem cells. Yalcin et al [62] have shown that Forkhead transcription factor Foxo3 represses ROS via regulation of ATM and thus maintains the stem cell pool.

Both mobilization and homing of the stem cells appear to be redox regulated. The important growth factors VEGF and erythropoietin that can mobilize stem cells are certainly under the control of redox regulation [58, 63]. The homing of stem cells to bone marrow is mediated by the binding of chemokine stromal cell-derived factor-1 (SDF-1) to CXCR4 receptor present on the circulating cells [64]. Ceradini et al [65] have shown that reduced oxygen tension mediated expression of HIF-1 regulates and induces the expression of SDF-1 in the regenerating ischemic tissues. A recent study showed that uncoupling of endothelial nitric oxide synthase (eNOS) resulting in superoxide anion formation caused diabetic endothelial dysfunction while eNOS regulated mobilization and vascular repair of endothelial progenitor cells [66]. Urao et al [67] have shown that hindlimb ischemia increased the production of ROS and Nox2 in bone marrow mononuclear cells, where Nox-2-derived ROS play an important role in the mobilization, homing and angiogenic capacity of stem or progenitor cells leading to the revascularization of ischemic tissue. Piccoli et al [68] have shown that bone marrow derived hematopoietic stem or progenitor cells express multiple isoforms of NADPH oxidase such as NOX1, NOX2 and NOX4 and its regulatory subunits such as p22, p40, p47, p67, rac1, rac2, NOXO1 and NOXA1. The activation of NOX isoforms facilitate the fine tuning of the ROS level, which balances the self renewal and differentiation in stem cells [68].

[III] MICRO RNA AS REGULATOR OF RESVERATROL MEDIATED CARDIOPROTECTION

3.1. Emerging role of microRNA

The rapid pace of outstanding findings in the RNA interference research followed by the completion of human genome project leads to the development of critical tools to understand the basic processes of life and disease. One of the key discoveries is MicroRNA (miRNA), which includes over thousands from many species and these were identified by bioinformatics, genetics and molecular biology approach. Genes for miRNAs are an essential component of the genetic program of all species, most of them also being evolutionarily conserved [69]. The first report of RNA silencing was found to be in plant system [70], but the fundamental study is carried out in C. elegans where a gene loci lin-4 is found to be regulator of developmental gene expression [71]. Molecules like Resveratrol regulate expression of microRNA genes in heart by direct or indirect mechanisms. MicroRNAs are the mature form of processed pre-miRNA. Pre-miRNAs are processed by Drosha from bigger poly-adenylated transcripts, known as pri-miRNA, in the nucleus and export to cytoplasm by Exportin 5 [72]. Further maturation of pre-miRNA to miRNA occurs in both nucleus and cytoplasm through Dicer and other protein complexes **[Figure–1]**. miRNAs target their mRNA by base pairing complimentary sequence located mainly at 3'UTR (un-translated region). miRNAs also target 5'UTR or coding regions of mRNA [73,74]. In addition to sequence specific targeting of mRNA, miRNA function as a ribonucleoprotein complex (miRNPs), also known as miRISCs (miRNA-induced silencing complex). Key components of miRISCs include AGO (Argonaute) and GW182 (glycine-tryptophan repeat-containing protein family. Although mature miRNAs are generally thought to be stable due its small size, however they are prone to degradation by both 5` to 3` and 3` to 5` exoribonucleases present in cells [75, 76]. miRNA stability also determined by its sequence complexity [77]. miRNAs are well known for its role as inhibitor of protein synthesis and thus interfering with target protein molecules. Recently miRNAs were also shown to activate protein synthesis [74, 78, 79].

3.2. Redox regulation of MicroRNA in cardiovascular health.

Cardiovascular diseases are complex process involving

REVIEW

THE IIOAB JOURNAL ISSN: 0976-3104

different cells type including cardiomyocytes, fibroblasts, endothelial cells, smooth muscle cells, neurons and various blood cells. The signatures of miRNA are different in those cell types and thus can be explained based on specific disease models. Cardiac fibrosis, where cardiac fibroblasts take the lead role in the development of many diseases like cardiomyopathy, hypertension, myocardial infarction (MI), chronic cardiomyopathy and regulate the cardiac extracellular matrix components [80-82]. The dysfunction of miRNA metabolism leads to hypertrophy and ventricular fibrosis [83]. Cardiac-specific overexpression of miR-208 resulted in cardiac hypertrophy, whereas genetic deletion of miR-208 blunted the hypertrophic response and decreased interstitial fibrosis following aortic banding [84, 85]. Dysregulation of miRNA (miR-29) family were observed in acute myocardial infarction model and knocking down of the miRNA resulted reduced collagen expression in fibroblasts [86]. Increased expression of miR-21, miR-214 and miR-224 and reduced expression of miR-29b and miR-149 are also found in myocardial infarction 86. Similar studies with microarray and northern blot analyses leads to the discovery of miR-21 over-expression in failing heart and miR-21 observed to regulate ERK-MAP kinase pathways [87]. CTGF, a key players in fibrosis is regulated at post-translational level by miR-133 and miR-30 [88]. In Ischemic heart disease, miR-1 has been shown to upregulated in human studies and overexpression studies in rat correlate miR-1 expression with arrhythmogenesis, cardiac conduction disturbance and membrane potential abnormality [89]. Another miRNA (miR-133), encoded by the same loci of miR-1,induced myoblast proliferation in vitro and shown to proliferate skeletal as well as cardiac muscle after overexpression in Xenopus embryos [90]. Hypoxia-inducible factor (Hif1α) is the transcription factor involved in cardiac hypoxia and beneficial to treatment of ischemic injury [91]. Hypoxia induced HIF1α up-regulation is partly regulated by a microRNA miR-199 [92]. Bcl2, a key regulator of apoptosis by mitochondrial pathway, is regulated by miR-1 and miR-15 family [93]. miR-92a is present in endothelial cells and is up-regulated upon induction of ischemia and knockdown of miR-92a resulted in improved recovery after MI due to accelerated vessel growth [94]. miR-320 regulate heat shock proteins (Hsp) and HSP mediate cardio protection against ischemic condition in heart [95]. Ischemic preconditioning of bone marrow-derived mesenchymal stem cells improved by their survival following engraftment in the infarcted heart and miR-210 has crucial role in the process [96].

3.3. Role of microRNA in resveratrol mediated cardioprotection

Recent Real-time PCR based array studies with resveratrol demonstrate unique expression pattern for resveratrol pretreated hearts. Differential expression is observed in ex vivo ischemia reperfused (IR) heart over 50 miRNAs, some of them are previously implicated [97]. Based on computational analyses, the target genes for the differentially expressed miRNA include genes of various molecular functions such as metal ion binding, transcription factors, cytoskeleton remodeling which may play key role in reducing IR injury. IR samples pretreated with resveratrol or its commercial formulation reverse the up or down regulation in IR samples in the opposite direction in more than 50% of differentially expressed miRNAs and either resveratrol or its commercial formulation, but not both, reverse the up or down regulation compared to IR control in 20% of miRNA. There is a significant upregulation of miR-21 expression with resveratrol. miR-21 is shown to regulates the ERK-MAP kinase signaling pathway in cardiac fibroblasts, which has impacts on global cardiac structure and function [87]. It has been shown earlier that resveratrol triggers MAPK signaling pathway as a preconditioning mechanism [98]. FOXO1 is regulated by miR-27a in cancer cells whereas VEGF is modulated by miR-20b through HIF1α [99, 100]. SIRT1 is observed to be regulated by miR-9 in stem cells and miR-199 in cardiomyocyte [92, 101]. Both microRNAs are modulated in resveratrol treated rat heart. Complex statistical analyses such as principal component analyses reveal that the IR samples pretreated with resveratrol are remarkably similar to vehicle sample in terms of miRNA gene expression [97]. These results are indeed of utmost importance, as they document that resveratrol can protect the ischemic heart by restoring the IR-induced up-regulation or down-regulation of gene expression. Future studies will be based on the mechanistic action and stability of miRNA. Further detailed in vivo and in vitro studies like targeting those miRNA followed by loss/gain of function will able to explore the complex mechanism underlying the cardioprotection by resveratrol **[Figure–2]**.

EDITED BY – PROF. S.K. MAULIK; MD, PHD

REVIEW

THE IIOAB JOURNAL
ISSN: 0976-3104

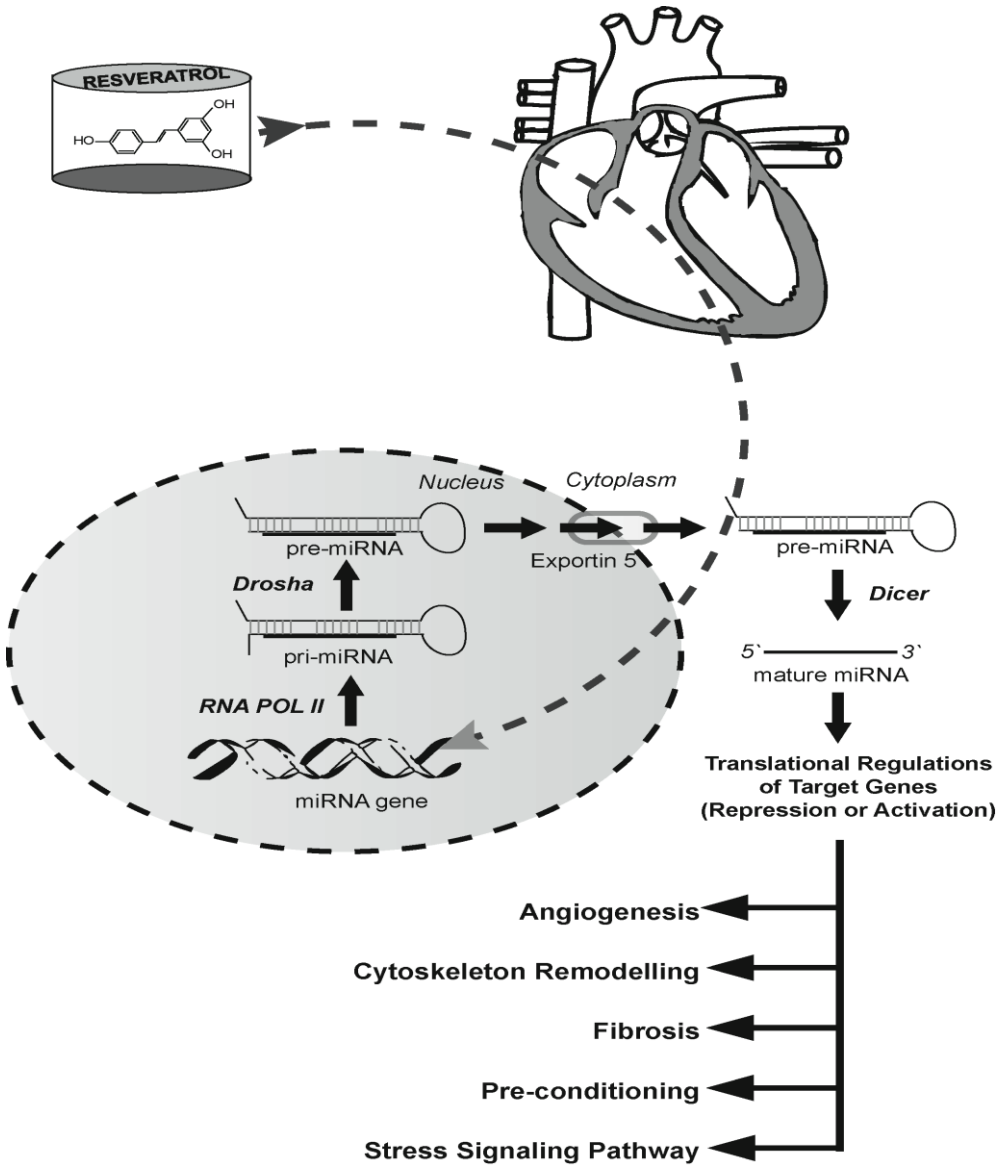

Fig:2. Mechanism of MicroRNA-mediated cardioprotetion by resveratrol. Resveratrol target miRNA gene and after synthesis by RNA Polymerase II (RNA POL II), primary transcript of miRNA (pri miRNA) are recognized by Drosha and Pasha which excise the hairpin precursor generating precursor miRNA (pre miRNA). These are transported to cytoplasm by Exportin 5 and further processed by Dicer to mature ~23nt miRNA. Mature miRNA associated with Argonaute and other factors leads to the targeted translational regulation. Release from Argonaute or absence of protection machinery leaves miRNA prone to degradation by exoribonuclease. miRNA modulate translation either by repression or activation although the mechanism is different. The target genes of miRNA include various cardiac molecular function as described.

[IV] CONCLUSION

In conclusion, the process of stem cells maintenance and growth in their niche appears to be regulated by controlled hypoxia and HIF-1α while the mobilization and homing is controlled by cytokines and/or adhesive molecules, which are driven by redox signaling that in turn appears to be regulated by redox-controlled FoxO-SirT network.

In essence, microRNA regulate target gene mostly by translational repression and sometimes through translational activation. Resveratrol regulates miRNA expression in healthy heart and ischemic-repurfused heart. Future detailed studies based on this approach and analyses will pave the way for

EDITED BY – PROF. S.K. MAULIK; MD, PHD

THE IIOAB JOURNAL ISSN: 0976-3104

development of novel therapeutic intervention for cardioprotection in acute IR injury. As more studies of the importance of miRNA appear in publication database, a tremendous impulse is generated for the feasibility of its therapeutic potential. There are some limitations to this process. First of all, the mechanism of action for miRNA is still unfolding and more information is required such as how it is transported, metabolized and targets the specific as well as non-specific genes. Other limitation is related to the stability of miRNA and administration at high dose based on animal studies and related toxic effect (if any) in targeted or non-targeted tissue delivery. Currently several modified version of antisense oligonucleotides, commonly known as antagomirs or anti-miRs, are available. These includes 2′-O-methoxy ethyl/phosphorothioate (2`-MOE), Locked Nucleic Acid (LNA) and hairpin inhibitors [102-104] Another important aspect of miRNA in cardiovascular research is its potential use as biomarker of cardiovascular disease [105-107]. More insights of microRNA are required before it actually implemented in clinical use.

FINANCIAL DISCLOSURE AND ACKNOWLEDGEMENT

This study was supported in part by NIH HL 34360, HL 33889, and HL 22559.

REFERENCES

[1] Quaini F, Urbanek K, Beltrami AP, et al. [2002] Chimerism of the transplanted heart. *N Engl J Med* 346:5–15.

[2] Kronenwett R, Martin S, Haas R. [2000] The role of cytokines and adhesion molecules for mobilization of peripheral blood stem cells. *Stem Cells* 18:320–330.

[3] Kinashi T, Springer TA. [1994] Adhesion molecules in hematopoietic cells. *Blood Cells* 20:25–44.

[4] Carlos TM, Harlan JM. [1994] Leukocyte-endothelial adhesion molecules. *Blood* 84:2068–2101.

[5] Mohle R, Murea S, Kirsch M, Haas R. [1995] Differential expression of L-selectin, VLA-4, and LFA-1 on CD34+ progenitor cells from bone marrow and peripheral blood during G-CSF-enhanced recovery. *Exp Hematol* 23:1535–1542.

[6] Lichterfeld M, Martin S, Burkly L, Haas R, Kronenwett R. [2000] Mobilization of CD34+ haematopoietic stem cells is associated with a functional inactivation of the integrin very late antigen 4. *Br J Haematol* 110:71–81.

[7] Yamaguchi M, Ikebuchi K, Hirayama F, et al. [1998] Different adhesive characteristics and VLA-4 expression of CD34(+) progenitors in G0/G1 versus S+G2/M phases of the cell cycle. *Blood* 92:842–848.

[8] Laterveer L, Lindley IJ, Heemskerk DP, et al. [1996] Rapid mobilization of hematopoietic progenitor cells in rhesus monkeys by a single intravenous injection of interleukin-8. *Blood* 87:781–788.

[9] Maurer AM, Liu Y, Caen JP, Han ZC. [2007] Ex vivo expansion of megakaryocytic cells. *Int J Hematol* 71:203–210.

[10] Duhrsen U, Villeval JL, Boyd J, Kannourakis G, Morstyn G, Metcalf D.[1988] Effects of recombinant human granulocyte colony-stimulating factor on hematopoietic progenitor cells in cancer patients. *Blood* 72:2074–2081.

[11] Gazitt Y. [2001] Recent developments in the regulation of peripheral blood stem cell mobilization and engraftment by cytokines, chemokines, and adhesion molecules. *J Hematother Stem Cell Res* 10:229–236.

[12] Haddad JJ. [2002] Redox regulation of pro-inflammatory cytokines and IkappaB-alpha/NF-kappaB nuclear translocation and activation. *Biochem Biophys Res Commun* 296:847–856.

[13] Liu H, Nishitoh H, Ichijo H, Kyriakis JM. [2000] Activation of apoptosis signal-regulating kinase 1 (ASK1) by tumor necrosis factor receptor-associated factor 2 requires prior dissociation of the ASK1 inhibitor thioredoxin. *Mol Cell Biol* 20:2198–2208.

[14] Wilmanski J, Siddiqi M, Deitch EA, Spolarics Z. [2005] Augmented IL-10 production and redox-dependent signaling pathways in glucose-6-phosphate dehydrogenase-deficient mouse peritoneal macrophages. *J Leukoc Biol* 78:85–94.

[15] Case J, Ingram DA, Haneline LS. [2008] Oxidative stress impairs endothelial progenitor cell function. *Antioxid Redox Signal* 10:1895–1907.

[16] Ghaffari S. [2008] Oxidative stress in the regulation of normal and neoplastic hematopoiesis. *Antioxid Redox Signal* 10:1923–1940.

[17] Guo Y, Einhorn L, Kelley M, et al. [2004] Redox regulation of the embryonic stem cell transcription factor oct-4 by thioredoxin. *Stem Cells* 22:259–264.

[18] Gurusamy N, Mukherjee S, Lekli I, Bearzi C, Bardelli S, Das D. [2008] Inhibition of Ref-1 Stimulates the Production of Reactive Oxygen Species and Induces Differentiation in Adult Cardiac Stem Cells. *Antioxid Redox Signal*

[19] Li Z, Li L. [2006] Understanding hematopoietic stem-cell microenvironments. *Trends Biochem Sci* 31:589–595.

[20] Kopp HG, Avecilla ST, Hooper AT, Rafii S. [2005] The bone marrow vascular niche: home of HSC differentiation and mobilization. *Physiology (Bethesda)* 20:349–356.

[21] Haneline LS. [2008] Redox regulation of stem and progenitor cells. *Antioxid Redox Signal* 10:1849–1852.

[22] Dernbach E, Urbich C, Brandes RP, Hofmann WK, Zeiher AM, Dimmeler S. [2004] Antioxidative stress-associated genes in circulating progenitor cells: evidence for enhanced resistance against oxidative stress. *Blood* 104:3591–3597.

[23] Schmelter M, Ateghang B, Helmig S, Wartenberg M, Sauer H. [2006] Embryonic stem cells utilize reactive oxygen species as transducers of mechanical strain-induced cardiovascular differentiation. *FASEB J* 20:1182–1184.

[24] Illi B, Scopece A, Nanni S, et al. [2005] Epigenetic histone modification and cardiovascular lineage programming in mouse embryonic stem cells exposed to laminar shear stress. *Circ Res* 96:501–508.

[25] Yamamoto K, Sokabe T, Watabe T, et al. [2005] Fluid shear stress induces differentiation of Flk-1-positive embryonic stem cells into vascular endothelial cells in vitro. *Am J Physiol Heart Circ Physiol* 288:H1915–1924.

[26] Ito K, Hirao A, Arai F, et al. [2006] Reactive oxygen species act through p38 MAPK to limit the lifespan of hematopoietic stem cells. *Nat Med.* 12:446–451.

[27] Sauer H, Rahimi G, Hescheler J, Wartenberg M.[2000] Role of reactive oxygen species and phosphatidylinositol 3-kinase in cardiomyocyte differentiation of embryonic stem cells. *FEBS Lett* 476:218–223.

[28] Wartenberg M, Donmez F, Ling FC, Acker H, Hescheler J, Sauer H. [2001] Tumor-induced angiogenesis studied in

THE IIOAB JOURNAL ISSN: 0976-3104

confrontation cultures of multicellular tumor spheroids and embryoid bodies grown from pluripotent embryonic stem cells. *FASEB J* 15:995–1005.

[29] Han MK, Song EK, Guo Y, Ou X, Mantel C, Broxmeyer HE. [2008] SIRT1 regulates apoptosis and Nanog expression in mouse embryonic stem cells by controlling p53 subcellular localization. *Cell Stem Cell* 2:241–251.

[30] Tothova Z, Kollipara R, Huntly BJ, et al. [2007] FoxOs are critical mediators of hematopoietic stem cell resistance to physiologic oxidative stress. *Cell* 128:325–339.

[31] Essers MA, Weijzen S, de Vries-Smits AM, et al. [2004] FOXO transcription factor activation by oxidative stress mediated by the small GTPase Ral and JNK. *EMBO J* 23:4802–4812.

[32] Marinkovic D, Zhang X, Yalcin S, et al. [2007] Foxo3 is required for the regulation of oxidative stress in erythropoiesis. *J Clin* 2133-2144.

[33] Invest. 117Tothova Z, Gilliland DG. [2007] FoxO transcription factors and stem cell homeostasis: insights from the hematopoietic system. *Cell Stem Cell* 1:140–152.

[34] Hosokawa K, Arai F, Yoshihara H, et al. [2007] Function of oxidative stress in the regulation of hematopoietic stem cell-niche interaction. *Biochem Biophys Res Commun* 363:578–583.

[35] Spradling A, Drummond-Barbosa D, Kai T. [2001] Stem cells find their niche. *Nature* 414(6859):98–104.

[36] Angkeow P, Deshpande SS, Qi B, et al. [2002] Redox factor-1: an extra-nuclear role in the regulation of endothelial oxidative stress and apoptosis. *Cell Death Differ* 9:717–725.

[37] Allen RG, Venkatraj VS. [1992] Oxidants and antioxidants in development and differentiation. *J Nutr* 122(3 Suppl):631–635.

[38] Beltrami AP, Barlucchi L, Torella D, et al. [2003] Adult cardiac stem cells are multipotent and support myocardial regeneration. *Cell.* 114:763–776.

[39] Beckman BS, Balin AK, Allen RG. [1989] Superoxide dismutase induces differentiation of Friend erythroleukemia cells. *J Cell Physio* 139:370–376.

[40] Almog N, Rotter V. [1997] Involvement of p53 in cell differentiation and development. *Biochim Biophys Acta* 1333:F1–27.

[41] Bachelder RE, Ribick MJ, Marchetti A, et al. [1999] p53 inhibits alpha 6 beta 4 integrin survival signaling by promoting the caspase 3-dependent cleavage of AKT/PKB. *J Cell Biol* 147:1063–1072.

[42] Gottlieb TM, Leal JF, Seger R, Taya Y, Oren M. [2002] Cross-talk between Akt, p53 and Mdm2: possible implications for the regulation of apoptosis. *Oncogene* 21:1299–1303.

[43] Keren-Tal I, Suh BS, Dantes A, Lindner S, Oren M, Amsterdam A. [1995] Involvement of p53 expression in cAMP-mediated apoptosis in immortalized granulosa cells. *Exp Cell Res* 218:283–295.

[44] Eizenberg O, Faber-Elman A, Gottlieb E, Oren M, Rotter V, Schwartz M. [1996] p53 plays a regulatory role in differentiation and apoptosis of central nervous system-associated cells. *Mol Cell Biol* 16:5178–5185.

[45] Sabapathy K, Klemm M, Jaenisch R, Wagner EF. [1997] Regulation of ES cell differentiation by functional and conformational modulation of p53. *EMBO J* 16:6217–6229.

[46] Ostrakhovitch EA, Cherian MG. [2005] Role of p53 and reactive oxygen species in apoptotic response to copper and zinc in epithelial breast cancer cells. *Apoptosis* 10:111–121.

[47] Jayaraman L, Murthy KG, Zhu C, Curran T, Xanthoudakis S, Prives C. [1997] Identification of redox/repair protein Ref-1 as a potent activator of p53. *Genes Dev* 11(5):558–570.

[48] Liu B, Chen Y, St Clair DK. [2008] ROS and p53: a versatile partnership. *Free Radic Biol Med* 44:1529–1535.

[49] Hu X, Yu SP, Fraser JL, et al. [2008] Transplantation of hypoxia-preconditioned mesenchymal stem cells improves infarcted heart function via enhanced survival of implanted cells and angiogenesis. *J Thorac Cardiovasc Surg* 135:799–808.

[50] Cipolleschi MG, Dello Sbarba P, Olivotto M. [1993] The role of hypoxia in the maintenance of hematopoietic stem cells. *Blood* 82:2031–2037.

[51] Vemuri VKR. [2009] Stem Cells, Hypoxia and Hypoxia-Inducible Factors. In: Vemuri VKRaMC, ed. Regulatory Networks in Stem Cells. New York: *Humana Press* 211–231.

[52] Rosova I, Dao M, Capoccia B, Link D, Nolta JA. [2008] Hypoxic preconditioning results in increased motility and improved therapeutic potential of human mesenchymal stem cells. *Stem Cells* 26:2173–2182.

[53] Keith B, Simon MC. [2007] Hypoxia-inducible factors, stem cells, and cancer. *Cell* 129:465–472.

[54] Cameron CM, Harding F, Hu WS, Kaufman DS. [2008] Activation of hypoxic response in human embryonic stem cell-derived embryoid bodies. *Exp Biol Med (Maywood)* 233:1044–1057.

[55] Potier E, Ferreira E, Meunier A, Sedel L, Logeart-Avramoglou D, Petite H. [2007] Prolonged hypoxia concomitant with serum deprivation induces massive human mesenchymal stem cell death. *Tissue Eng* 13:1325–1331.

[56] Ivanovic Z, Dello Sbarba P, Trimoreau F, Faucher JL, Praloran V. [2000] Primitive human HPCs are better maintained and expanded in vitro at 1 percent oxygen than at 20 percent. *Transfusion* 40:1482–1488.

[57] Tang YL, Tang Y, Zhang YC, Qian K, Shen L, Phillips MI. [2005] Improved graft mesenchymal stem cell survival in ischemic heart with a hypoxia-regulated heme oxygenase-1 vector. *J Am Coll Cardiol* 46:1339–1350.

[58] Csete M. [2005] Oxygen in the cultivation of stem cells. *Ann N Y Acad Sci*1049:1–8.

[59] Saretzki G, Armstrong L, Leake A, Lako M, von Zglinicki T. [2004] Stress defense in murine embryonic stem cells is superior to that of various differentiated murine cells. *Stem Cells* 22:962–971.

[60] Jang YY, Sharkis SJ. [2007] A low level of reactive oxygen species selects for primitive hematopoietic stem cells that may reside in the low-oxygenic niche. *Blood* 110:3056–3063.

[61] Ito K, Hirao A, Arai F, et al. [2004] Regulation of oxidative stress by ATM is required for self-renewal of haematopoietic stem cells. *Nature* 431(7011):997–1002.

[62] Yalcin S, Zhang X, Luciano JP, et al. [2008] Foxo3 is essential for the regulation of ataxia telangiectasia mutated and oxidative stress-mediated homeostasis of hematopoietic stem cells. *J Biol Chem* 283:25692–25705.

[63] Rabbany SY, Heissig B, Hattori K, Rafii S. [2003] Molecular pathways regulating mobilization of marrow-derived stem cells for tissue revascularization. *Trends Mol Med* 9:109–117.

[64] Peled A, Grabovsky V, Habler L, et al. [1999] The chemokine SDF-1 stimulates integrin-mediated arrest of CD34(+) cells on vascular endothelium under shear flow. *J Clin Invest* 104:1199–1211.

THE IIOAB JOURNAL ISSN: 0976-3104

[65] Ceradini DJ, Kulkarni AR, Callaghan MJ, et al. [2004] Progenitor cell trafficking is regulated by hypoxic gradients through HIF-1 induction of SDF-1. *Nat Med* 10:858–864.

[66] Thum T, Fraccarollco D, Schultheiss M, et al. [2007] Endothelial nitric oxide synthase uncoupling impairs endothelial progenitor cell mobilization and function in diabetes. *Diabetes* 56:666–674.

[67] Urao N, Inomata H, Razvi M, et al. [2008] Role of nox2-based NADPH oxidase in bone marrow and progenitor cell function involved in neovascularization induced by hindlimb ischemia. *Circ Res* 103:212–220.

[68] Piccoli C, D'Aprile A, Ripoli M, et al. [2007] Bone-marrow derived hematopoietic stem/ progenitor cells express multiple isoforms of NADPH oxidase and produce constitutively reactive oxygen species. *Biochem Biophys Res Commun* 353:965–972.

[69] Ambros V. [2004] The functions of animal microRNAs. *Nature* 431(7006):350–355.

[70] Hamilton AJ, Baulcombe DC. [1999] A species of small antisense RNA in posttranscriptional gene silencing in plants. *Science* 286(5441):950–952.

[71] Olsen PH, Ambros V. [1999] The lin-4 regulatory RNA controls developmental timing in Caenorhabditis elegans by blocking LIN-14 protein synthesis after the initiation of translation. *Dev Biol* 216:671–680.

[72] Hammond SM. [2005] Dicing and slicing: the core machinery of the RNA interference pathway. *FEBS Lett* 579:5822–5829.

[73] Rigoutsos I. [2009] New tricks for animal microRNAS: targeting of amino acid coding regions at conserved and nonconserved sites. *Cancer Res* 69:3245–3248.

[74] Orom UA, Nielsen FC, Lund AH. [2008] MicroRNA-10a binds the 5'UTR of ribosomal protein mRNAs and enhances their translation. *Mol Cell* 30:460–471.

[75] Kai ZS, Pasquinelli AE. [2010] MicroRNA assassins: factors that regulate the disappearance of miRNAs. *Nat Struct Mol Biol* 17:5–10.

[76] Krol J, Loedige I, Filipowicz W. [2010] The widespread regulation of microRNA biogenesis, function and decay. *Nat Rev Genet* 11:597–610.

[77] Bail S, Swerdel M, Liu H, et al. [2010] Differential regulation of microRNA stability. *RNA* 16:1032–1039.

[78] Buchan JR, Parker R. [2007] Molecular biology. The two faces of miRNA. *Science* 318(5858):1877–1878.

[79] Vasudevan S, Steitz JA. [2007] AU-rich-element-mediated upregulation of translation by FXR1 and Argonaute 2. *Cell* 128:1105–1118.

[80] Mukhopadhyay P, Rajesh M, Batkai S, et al. [2010] CB1 cannabinoid receptors promote oxidative stress and cell death in murine models of doxorubicin-induced cardiomyopathy and in human cardiomyocytes. *Cardiovasc Res* 85:773–784.

[81] Raman SV. [2010] The hypertensive heart. An integrated understanding informed by imaging. *J Am Coll Cardiol* 55:91–96.

[82] Jellis C, Martin J, Narula J, Marwick TH. [2010] Assessment of nonischemic myocardial fibrosis. *J Am Coll Cardiol* 56:89–97.

[83] Da Costa Martins PA, Bourajjaj M, Gladka M, et al. [2008] Conditional dicer gene deletion in the postnatal myocardium provokes spontaneous cardiac remodeling. *Circulation* 118:1567–1576.

[84] van Rooij E, Sutherland LB, Qi X, Richardson JA, Hill J, Olson EN. [2007] Control of stress-dependent cardiac growth and gene expression by a microRNA. *Science* 316(5824):575–579.

[85] Callis TE, Pandya K, Seok HY, et al. [2009] MicroRNA-208a is a regulator of cardiac hypertrophy and conduction in mice. *J Clin Invest* 119:2772–2786.

[86] van Rooij E, Sutherland LB, Thatcher JE, et al. [2008]. Dysregulation of microRNAs after myocardial infarction reveals a role of miR-29 in cardiac fibrosis. *Proc Natl Acad Sci U S A.* 105:13027–13032.

[87] Thum T, Gross C, Fiedler J, et al. [2008] MicroRNA-21 contributes to myocardial disease by stimulating MAP kinase signalling in fibroblasts. *Nature* 456(7224):980–984.

[88] Duisters RF, Tijsen AJ, Schroen B, et al. [2009] miR-133 and miR-30 regulate connective tissue growth factor: implications for a role of microRNAs in myocardial matrix remodeling. *Circ Res* 104:170–178.

[89] Yang B, Lin H, Xiao J, et al. [2007] The muscle-specific microRNA miR-1 regulates cardiac arrhythmogenic potential by targeting GJA1 and KCNJ2. *Nat Med* 13:486–491.

[90] Chen JF, Mandel EM, Thomson JM, et al. [2006] The role of microRNA-1 and microRNA-133 in skeletal muscle proliferation and differentiation. *Nat Genet* 38:228–233.

[91] Loor G, Schumacker PT. [2008] Role of hypoxia-inducible factor in cell survival during myocardial ischemia-reperfusion. *Cell Death Differ* 15:686–690.

[92] Rane S, He M, Sayed D, et al. [2009] Downregulation of miR-199a derepresses hypoxia-inducible factor-1alpha and Sirtuin 1 and recapitulates hypoxia preconditioning in cardiac myocytes. *Circ Res* 104:879–886.

[93] Tang Y, Zheng J, Sun Y, Wu Z, Liu Z, Huang G. [2009] MicroRNA-1 regulates cardiomyocyte apoptosis by targeting Bcl-2. *Int Heart J* 50:377–387.

[94] Bonauer A, Dimmeler S. [2009] The microRNA-17-92 cluster: still a miRacle? *Cell Cycle* 8:3866–3873.

[95] Ren XP, Wu J, Wang X, et al. [2009] MicroRNA-320 is involved in the regulation of cardiac ischemia/reperfusion injury by targeting heat-shock protein 20. *Circulation* 119:2357–2366.

[96] Kim HW, Haider HK, Jiang S, Ashraf M. [2009] Ischemic preconditioning augments survival of stem cells via miR-210 expression by targeting caspase-8-associated protein 2. *J Biol Chem* 284:33161–33168.

[97] Mukhopadhyay P, Mukherjee S, Ahsan K, Bagchi A, Pacher P, DasDK. [2010] Restoration of Altered MicroRNA Expression in the ischemic heart with Resveratrol. PLosone.5(12):e15705.

[98] Das S, Tosaki A, Bagchi D, Maulik N, Das DK. [2006] Potentiation of a survival signal in the ischemic heart by resveratrol through p38 mitogen-activated protein kinase/mitogen- and stress-activated protein kinase 1/cAMP response element-binding protein signaling. *J Pharmacol Exp Ther* 317:980–988.

[99] Cascio S, D'Andrea A, Ferla R, et al. [2010] miR-20b modulates VEGF expression by targeting HIF-1 alpha and STAT3 in MCF-7 breast cancer cells. *J Cell Physiol* 224:242–249.

[100] Guttilla IK, White BA. [2009] Coordinate regulation of FOXO1 by miR-27a, miR-96, and miR-182 in breast cancer cells. *J Biol Chem* 284:23204–23216.

[101] Saunders LR, Sharma AD, Tawney J, et al. [2010] miRNAs regulate SIRT1 expression during mouse embryonic stem cell differentiation and in adult mouse tissues. Aging (Albany NY)

[102] Huynh C, Segura MF, Gaziel-Sovran A, et al. [2010] Efficient in vivo microRNA targeting of liver metastasis. *Oncogene*

THE IIOAB JOURNAL
ISSN: 0976-3104

[103] Elmen J, Lindow M, Schutz S, et al. [2008] LNA-mediated microRNA silencing in non-human primates. *Nature* 452(7189):896–899.

[104] Vermeulen A, Robertson B, Dalby AB, et al. [2007] Double-stranded regions are essential design components of potent inhibitors of RISC function. *RNA* 13:723–730.

[105] Fichtlscherer S, De Rosa S, Fox H, et al. [2010] Circulating MicroRNAs in Patients With Coronary Artery Disease. *Circ Res*

[106] Kumarswamy R, Anker SD, Thum T. [2010] MicroRNAs as circulating biomarkers for heart failure: questions about MiR-423-5p. *Circ Res* 106:e8; author reply e9.

[107] Wang GK, Zhu JQ, Zhang JT, et al. [2010] Circulating microRNA: a novel potential biomarker for early diagnosis of acute myocardial infarction in humans. *Eur Heart J Mar* 31(6):659–666.

ABOUT AUTHORS

Partha Mukhopadhyay *completed Ph.D. (1998) in Biotechnology from Jawaharlal Nehru University, New Delhi, India on protein engineering of a redox sensitive enzyme. Dr. Mukhopadhyay completed his postdoctoral studies on oxidative stress at Cell Biology and Metabolism Branch, NICHD, National Institutes of Health, Bethesda, Maryland, USA. His current research interests focus on the molecular mechanism of redox biology in cardiovascular diseases and other tissue injury models. He has published over 50 articles in peer reviewed journals including PNAS, Cell, Nature Structural and Molecular Biology, The Journal of American College of Cardiology, JBC, FRBM etc. Dr. Mukhopadhyay is currently Staff Scientist at National Institutes of Health, Bethesda, Maryland, USA.*

Mr. Dipak K Das, *Ph.D., Sc.D., M.D. (hon), FAHA, FACN, FICA, FACS, FIACS is a professor and Director of Cardiovascular Research Center, University of Connecticut School of Medicine. Dr. Das also received the title of Dr. Honores Scouza from the University of Debrecen. Originally trained in biochemistry and molecular biology, Dr. Das is considered as a recognized leader in redox signaling and cardiovascular research. He has approximately 600 publications, 150 review articles and book chapters and 15 books. He also served as guest editors of special issues of several journal including Annals of the New York Academy of Sciences. Dr. Das is the Editor-in-Chief and the founding editor of Antioxidant and Redox Signaling [impact factor 7.6]. He also served as Associate Editor of American Journal of Physiology and Consulting Editor of Molecular and Cellular Biochemistry. Dr. Das serves in the editorial board of many cardiology and redox-related journals. Dr. Das is considered as renowned investigator in wine and resveratrol research for which he holds several patents.*