

Review

# The roles of microRNA in redox metabolism and exercise-mediated adaptation

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## Abstract

MicroRNAs (miRs) are small regulatory RNA transcripts capable of post-transcriptional silencing of mRNA messages by entering a cellular bimolecular apparatus called RNA-induced silencing complex. miRs are involved in the regulation of cellular processes producing, eliminating or repairing the damage caused by reactive oxygen species, and they are active players in redox homeostasis. Increased mitochondrial biogenesis, function and hypertrophy of skeletal muscle are important adaptive responses to regular exercise. In the present review, we highlight some of the redox-sensitive regulatory roles of miRs.

**Keywords:** Adaptation; Exercise; MicroRNA; Oxidative damage; Reactive oxygen species; Redox regulation

## 1. Introduction

MicroRNAs (miRs) are a unique subset of noncoding RNA, whose primary function is to post-transcriptionally modulate gene expression. The first miRs were discovered in the model organism *Caenorhabditis elegans*, a popular organism in genetic studies.<sup>1</sup> The majority of miRs are transcribed from the nuclear DNA similarly to other mRNAs: by the polymerase II enzyme. After transcription, the so-called Pri-micro RNA undergoes a maturation process with multiple stages.<sup>2</sup> First, the characteristic “hairpin” structure forms: the transcript base pairs with itself, leaving single-stranded overhangs at the 5' and 3' end of the hairpin. miRs may be transcribed individually or in a cluster, and they may have their own promoter. There are examples that originate from an intronic sequence, like miR-499 of the *myosin heavy chain 14 (MYH14)* gene<sup>3</sup> and there are a few miRs even from protein coding exonic regions.<sup>4</sup> Subsequent enzymatic processes, executed by

Drosha and *DiGeorge syndrome chromosomal region 8 (DGCR8)*, cleave the overhanging parts, leaving only the hairpin (pre-miR), which is exported to the cytoplasm across the exportin-5 complex. In the cytoplasm, miRs are subjected to another enzymatic processing step where the Dicer ribonuclease (RNase) cleaves the stem-loop, leaving the 18–23 base pair (bp) long, double-stranded RNA, with 2-bp overhang at the 3' end.<sup>4</sup> In the following sections, principles of miR-dependent gene silencing will be discussed along with their implications in skeletal muscle adaptation and their role in redox biology.

## 2. Mechanism of action and tissue specificity

Mature miRs, as mentioned above, are processed in a consecutive manner to form double-stranded polynucleotides. However, during their silencing function (usually), only one strand is actively used. The active strand, which is termed “the guide strand”, is loaded to the RNA-induced silencing complex (RISC).<sup>5</sup> The other so-called passenger strand can be subject to enzymatic decay.<sup>6</sup> In some cases, the passenger and the guide strand have similar probability of getting accepted by the

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silencing apparatus.<sup>7</sup> In the RISC complex, the first 2–8 base, called the miR seed region, of the loaded miR 3' end can formulate base pairing with mRNA messages. Usually the complementary sequence is at the 3' untranslated region (3' UTR) of the mRNA, and the miR–mRNA association results in decreased mRNA levels or message translatability. It is important to note that base pairing is not necessarily limited to the seed region, as other “non-canonical” forms exist. For a more comprehensive review of seed matching and related mechanisms, the reader is directed to Bartel’s excellent publication.<sup>8</sup>

The accurate number of endogenous miRs in the human body is still a matter of debate. In mammals, it is believed that approximately 50% of the protein-coding RNAs are directly affected by miRs.<sup>9</sup> However, a recent study highlighted that the computational approaches are more likely to produce false positive results, and even results of more rigid algorithms can find connections with very little or no biological significance.<sup>10</sup> There are 1881 human miRs according to the latest GENCODE release (Version 33.0) (<https://www.genecodegenes.org/human/stats.html>); mirBase contains 1917 annotated hairpin precursors,<sup>11</sup> and the canonical human “bona fide” miR pool includes around 519 miRs.<sup>12</sup> Not surprisingly, the expression level of the individual RNA species is not homogeneous in different tissues.<sup>13–15</sup> Moreover, studies in some cases have demonstrated obligate tissue specificity, such as the heart-specific miR-208<sup>16</sup> and the skeletal muscle-specific miR-206.<sup>17</sup> In the following sections, described miRs refer to microRNAs in tissue unless they are claimed to be circulating or secreted.

### 3. miR involvement in myocellular pathways and the connection with oxidative stress

There are many different attributes for miRs, with distinct intracellular processes being involved, including hypoxamirs<sup>18</sup> or redoxomirs, and attributes that refer to tissue-specific myomiRs.<sup>19,20</sup> However, the fact is that miRs do not exclusively act on a single mRNA, but can down-regulate many mRNAs, thus making it difficult to characterize miR-related pathways without overlap. One mRNA may be affected by many miRs, and that is why—somehow unexpectedly in some cases—genetic ablation of an miR or miRs does not result in a significant phenotype. One possible explanation for this phenomenon is functional redundancy, since many miRs share a common seed sequence,<sup>21</sup> or the phenomenon can reflect the nature of some miR-related genes expression control where the suppression fine tunes the expression profile(s) rather than executes robust programs.<sup>22</sup> As an example, knocking-out the muscle-specific miR-206 and miR-133b<sup>23</sup> does not present with significant alteration in development or in muscle phenotype in unchallenged conditions. Similarly, the loss of the miR-23–27–24 cluster<sup>24</sup> does not affect muscle development or exercise tolerance significantly. Although miR-1<sup>25</sup> and miR-133a<sup>26</sup> have been shown to be substantial in cardiac development, miR-206, miR-133a, and miR-133b expression are believed to play a role in skeletal muscle differentiation and possibly in muscle growth.<sup>27</sup> With genetic ablation, some of these miRs produce abnormal phenotypes when the genetically engineered animals are exposed to

exercise.<sup>28</sup> In line with this, if miR-206 is overexpressed, as in a mouse model for Duchenne muscular dystrophy, the progression of the disease is substantially delayed.<sup>29</sup> Moreover, miR-206 helps to slow down denervation-induced muscle atrophy in rats.<sup>30</sup> Other studies have demonstrated the importance of miR in myoblast fate determination<sup>31,32</sup> and muscle regeneration.<sup>33</sup> *Paired-like homeodomain transcription factor 2* gene (*Pitx2*) and *Pitx3*, 2 transcription factors involved in paired box 7 (Pax7)-mediated muscle differentiation, have been shown to be associated with redox regulation during myogenesis, because double-conditional *Pitx2/3* mouse mutants accumulated abnormal levels of reactive oxygen species (ROS).<sup>34</sup> Furthermore, Lozano-Velasco et al.<sup>35</sup> found that *Pitx2c* overexpression increases myoblast proliferation with concomitant decreases in miR-targeting cyclins, namely, 15b (miR-15b), miR-23b, miR-106b, and miR-503.

The relationship between neuromuscular disorders and ROS signaling has been established by studies in amyotrophic lateral sclerosis (ALS) models. A common experimental setup for ALS is the *G93A super oxide dismutase 1 (SOD1)* transgenic mouse,<sup>36</sup> where multiple copies of the mutant DNA are inserted into the 12th chromosome.<sup>37</sup> Animals harboring the human *G93A SOD1* with a functional mutation die around the ~150th postnatal day.<sup>38</sup> During their shortened lifetime, accumulation of carbonylated SOD1, translationally controlled tumor protein (TCTP), ubiquitin carboxyl-terminal hydrolase-L1 (UCH-L1), and, possibly, alpha B-crystallin can be detected.<sup>39</sup> ALS mice gripping strength was 70% less than that of the B6SJL mice wild type, and there was a decrease in total sulfhydryl groups in the skeletal muscle of symptomatic ALS mice.<sup>40</sup> Indeed, during ALS progression, the loss of motoneurons leads to skeletal muscle atrophy and concurrent muscle weakness. Williams and colleagues<sup>41</sup> showed that miR-206 is up-regulated both in *G93A SOD1*-related ALS and in surgical denervation of skeletal muscle. According to their hypothesis, muscle innervation controls miR-206 expression with myoblast determination protein (MyoD) and fibroblast growth factors (FGF)-dependent mechanisms. During denervation, MyoD is upregulated and enhances miR 206 expression. Furthermore, miR-206 targets histone deacetylases 4 (HDAC4) mRNA, the product of which may down-regulate fibroblast growth factor-binding protein (FGFBP), a potential activator of skeletal muscle reinnervation. In the normal state, the feedback mechanism will eventually down-regulate itself (reinnervation blocks MyoD activity) and a dynamic balance occurs. But in pathological situations, runaway expression of miR-206 may take place. In accordance with this idea, another study showed that MyoD promoted myogenesis by down-regulating Twist-1 by miR-206 induction.<sup>42</sup> It is important to note that, similar to ALS, denervation is associated with increased muscle mitochondrial ROS production.<sup>43</sup> There is a possibility that miR-206, along with other myomiRs, indirectly modulate ROS defense in skeletal muscle by helping to tune the tissue-specific gene expression environment. In fact, if myomiR miR-133a-1 and miR-133a-2 are knocked out from the mouse genome, impaired exercise tolerance occurs, and the peroxisome proliferator-activated receptor  $\gamma$  coactivator-1 $\alpha$ /Mt transcription factor A (PGC-1 $\alpha$ -mtTFA) pathway activity decreases and decreased citrate synthase activity occurs.<sup>28</sup>

An interesting concept in miR biology is that miRs do not execute robust transcriptional programs. Instead, they change the expressional topology in respect to the tissue or other biochemical environment. The miR functional/physiological significance—meaning how severely the miR mutates or whether there is complete loss and how this affects the organism—is likely to depend on the process it modulates and on the target sensitivity or redundancy (Fig. 1).

#### 4. Oxidative stress-related miRs

A number of miRs have been linked to processes associated with oxidative damage. In an intestinal ischemic-reperfusion model, Hu and colleagues<sup>44</sup> showed that miR-351-5p promotes oxidative injury, presumably by targeting *sirtuin 6 (SIRT6)* and *mitogen-activated protein kinase 13 (MAPK13)*. Findings from the same group strengthen the *SIRT6 MAPK13* interaction, because dioscin administration reduces the damage derived from hypoxia reoxygenation with co-occurring ROS reduction and miR-351-5p down-regulation.<sup>45</sup> In a similar ischemia/reperfusion mouse model, Wang and coworkers<sup>46</sup> found that significant miR-34a-5p and miR-495-3p increases aligned with ROS levels, but only with intestinal injury attenuated by miR-34a-5p inhibition. The authors suggest that this prophylactic effect is mediated by SIRT1-dependent ROS reduction, since wild-type *SIRT1* mRNA contains a miR-34a-5p target site at the 3' UTR, and mutation of that site decreases the luciferase reporter signal in Caco-2 cells. Sirtuin family member SIRT4 is also involved in ROS balance, since its translation is regulated by miR-15b. miR-15b inhibition leads to elevation of both *SIRT4* mRNA and protein, which eventually has a negative impact on ROS generation and mitochondrial membrane potential.<sup>47</sup>

Oxidative damage is a major player in the pathomechanism of cardiovascular events<sup>48,49</sup> and related inflammatory<sup>50</sup> signaling. In normoxic conditions, the endogenous free radical scavenging system can effectively neutralize the ROS. In the case of ischemia/reperfusion, there is an oxidative overflow with potential deleterious effects. miR-210 is known as a hypoxia-induced miR,<sup>51–53</sup> and it seems to have a tight connection with the hypoxic transcription factor-1 $\alpha$  (HIF-1 $\alpha$ ). miR-210 is induced by HIF-1 $\alpha$ . Under hypoxic conditions, HIF-1 $\alpha$  is stabilized and binds to the miR-210 promoter, thus inducing its expression.

Conversely, miR-210 can act as an HIF-1 $\alpha$  enhancer by targeting glycerol-3-phosphate dehydrogenase 1-like (GPD1L), an enzyme that promotes HIF-1 $\alpha$  proline hydroxylation<sup>54</sup>; thus, miR-210 indirectly limits the proteasomal degradation of HIF-1 $\alpha$ . In contrast, miR-429, another member of the hypoxamir family, acts through a negative feedback loop where its expression induced by HIF-1 $\alpha$  decreases HIF-1 $\alpha$  stability.<sup>55</sup>

*Iron-sulfur cluster scaffold homolog (ISCU)* and *cytochrome c oxidase assembly factor heme A (COX 10)* are merged as potential mRNA targets of miR-210.<sup>56</sup> The protein product of these genes can contribute to ROS accumulation by down-regulating mitochondrial respiration and up-regulating glycolytic processes. Also, miR-210 is known to target the succinate dehydrogenase complex, subunit D (SDHD), another member of the electron transport chain<sup>57</sup> in A549 cells. In miR-210-transfected A549 cells, the SDHD-fused luciferase reporter is decreased upon transfection and complex II subunit (SDHA) is also down-regulated, but without significant change of the complex I subunit (NDUFA9). Enlarged mitochondria exhibiting altered cristae organization are prevalent upon miR-210 induction.<sup>57,58</sup> In contrast, in the ischemic heart model, miR-210 overexpression is associated with better progression,<sup>59</sup> as indicated by reduced apoptosis and higher ventricular fractional shortening.

There is great deal of interest in the role of miRs-mediated regulation of ROS and redox signaling in the brain. miR-210 increases microvascular density<sup>60</sup> in the stroke model, where, interestingly, the *brain-derived neurotrophic factor (BDNF)* mRNA is found to be a direct target of miR-210. Somewhat conversely, transfected animals have higher Hexaribonucleotide Binding Protein-3 doublecortin (NeuN+/DCX+) ratios, indicating promoted neurogenesis in the hippocampal and subventricular areas. The authors of the cited study suggest an explanation according to which the pro-BDNF/mature BDNF balance is the main regulator in focal angiogenesis and neurogenesis, since in their experimental conditions, the reduction mainly occurred in the pro-BDNF pool of the ipsilateral hemisphere. In a similar transfection-based study on lentiviral vector carrying miR-210,<sup>61</sup> it was found that overexpression of miR-210 results in up-regulation of vascular endothelial growth factor (VEGF) along with endothelial cell proliferation. HIF-1 is involved in VEGF up-regulation, and miR-210-mediated VEGF induction has recently been confirmed by novel *in vivo* and *in vitro* experiments using neural<sup>62,63</sup> and myocardial<sup>64</sup> cells. Ischemia/reperfusion brain injury resulted in decreased SOD activity in male C57BL/6J mice, but intracerebroventricular miR-93 antagomir injection increased SOD activity even above the control levels and reduced the infarct volumes.<sup>65</sup> Moreover, miR-93 antagomir inhibited primary cortical neuron death after H<sub>2</sub>O<sub>2</sub> treatment.

Nuclear factor erythroid-derived 2-like 2 (NFE2L2) has a pinnacle importance in governing endogenous antioxidant defense. Triggered by oxidative stress, NFE2L2 protein (complex) is stabilized and translocated into the nucleus where it binds to antioxidant response element (ARE) cis acting elements.<sup>66</sup> NFE2L2 is a member of the cap “n” collar (CNC) transcription factor family, and includes NFE2L1, NFE2L3, Bach1, and Bach2<sup>67</sup> proteins. *NFE2L1* knockout mice

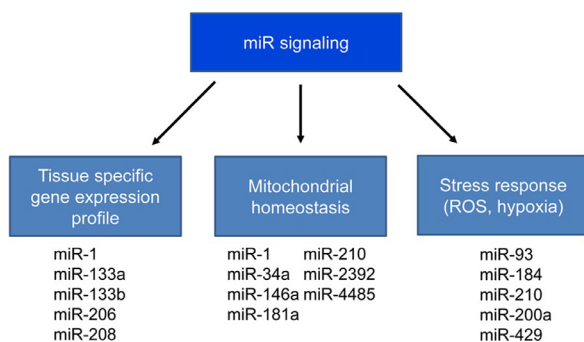


Fig. 1. The tissue specificity of miR production. MicroRNAs have complex regulatory roles but show tissue specificity as well. miR = microRNA; ROS = reactive oxygen species.

embryos die at a late stage of the embryonic development;<sup>68,69</sup> loss of *NFE2L2* seems to be more tolerable, but the *NFE2L2*-null mice are sensitive to oxidative stress<sup>70,71</sup> and develop autoimmune diseases and die prematurely.<sup>72,73</sup> Data suggest that miR-155 can directly influence the expression of *NFE2L2*.<sup>74</sup> *NFE2L2* can form hetero dimers with small musculoaponeurotic fibrosarcoma (Maf) proteins and activate gene transcription related to oxidative stress.<sup>75,76</sup> Maf family member v-Maf avian Maf oncogene homolog G (MAFG) is repressed by miR-128 and impaired *NFE2L2* activity.<sup>77</sup> MiR-128 also promotes ROS increase<sup>78</sup> by polycomb complex protein (Bmi-1) inhibition; and during hypoxia, miR-128 levels decrease, with an increase in MAFG and heme oxygenase 1 (HMOX-1)<sup>77</sup> protein levels in C2C12 cells and mouse hind-limb adductor muscles.

Guanine is the most susceptible to oxidative damage among the 4 DNA bases. In contact with ROS, guanine may convert to 8-oxoguanine or 8-hydroxyguanine, which can lead to DNA mutation if left uncorrected. There is a significant body of literature on ROS-related DNA modification. Importantly, a recent study showed that RNA can be also a target of ROS. One miR-related example is the oxidative modification of miR-184 and its role in apoptosis.<sup>79</sup> In H9c2 cells, oxidative stress increased the abundance of 8-oxo-7, 8-dihydroguanosine in miR-184 and, strikingly, the oxidized form was able to reduce Bcl-w and Bcl-xl protein levels and subsequently promote apoptosis. This mechanism may serve as an emergency switch: if the cellular environment is critical in terms of redox balance, the induction of apoptosis can tone down the detrimental effect at the organismal level by hijacking the more deleterious necrosis or excessive DNA mutation.

Caloric restriction (CR) is a potent modulator of health in laboratory model organisms. In fact, CR has the capacity to increase non-selective metabolic stress resistance, including oxidative challenges. Caloric or dietary restriction can positively change the redox balance and ROS-associated molecular damage,<sup>80</sup> but CR seems to follow a hormetic dose trajectory, since severe CR increases oxidative damage and decreases antioxidant capacity of hepatocytes.<sup>81</sup> Interestingly, aging increases 45 known miRs species levels in the mice circulation, and CR has the potential to reduce the rising trajectory.<sup>82</sup> There are a number of rodent studies demonstrating the CR effect on miR expression profile in different tissues, including mouse breast tissue,<sup>83</sup> mouse liver,<sup>84,85</sup> mouse heart,<sup>86</sup> intrauterine rat pancreas,<sup>87</sup> rat cerebro-microvascular endothelia,<sup>88</sup> and rat cerebral cortex.<sup>89</sup> In a primate model, CR has reduced the age-related s-t of miR-181b, miR-451, and miR-144<sup>90</sup> in skeletal muscle. These studies mainly demonstrated the positive effects of CR in mitochondrial related pathways.<sup>91</sup> In a recent study, 12 weeks of CR (first week at 20% and then 11 weeks at 40%) significantly induced mitochondrial miRs in mouse liver.<sup>92</sup> In that experimental setup, miR-122 increase was the highest during CR and miR induction was concomitant with enhanced production of mtDNA-encoded proteins. There is a connection between CR and nuclear respiratory factor 1 (NRF-1) activity, since NRF-1, PGC-1 $\alpha$ , and cytochrome c oxidase subunit 4 (COXIV) protein levels keep their middle-age levels during long-term CR in rats.<sup>93</sup> Moreover,

NRFs are involved in mitochondrial transport because it regulates the gene expression of TOM genes.<sup>94,95</sup> With respect to redox balance, mitochondrial-enriched SIRT3 helped to reduce ROS levels by activating SOD2 through deacetylation<sup>96</sup> in CR mice and, consequently, the age-associated accumulation of cellular oxidative damage may be mitigated by CR interventions. The miRs that have been associated with the above-mentioned molecular pathways are listed in Table 1.

## 5. Can miR regulate mitochondrial function and ROS production?

Mitochondria are organelles that play a fundamental role in oxidative metabolism and cellular redox balance. Therefore, it is not surprising that any perturbation of the organellar gene expression—whether it is of nuclear or mitochondrial origin—has a major impact on cellular homeostasis. In skeletal or cardiac muscle, the mitochondrion can be a significant source of ROS production. After extracting high-energy electrons from the upstream metabolic processes, the harvested electrons progressively enter lower energy states, and this process drives the maintenance of mitochondrial membrane potential. But during metabolic stress (exercise, ischemic-reperfusion event) or in disease-related conditions, elevated numbers of electrons can escape from the system and form ROS. High-intensity interval training results in impaired oxygen supply during exercise bouts, while at the resting periods the blood supply is normalized; hence, this type of exercise training can create a situation similar to ischemia/reperfusion in the skeletal muscle.

It has been well demonstrated that endurance capacity is strongly related to mitochondrial biogenesis.<sup>97</sup> Overexpression of PGC-1 $\alpha$  in mice leads to switch to slow type of muscle fibers and greater resistance to fatigue.<sup>98</sup> PGC-1 $\alpha$  is often referred to as the master regulator of mitochondrial biogenesis. Indeed, there is a great deal of literature about the involvement of PGC-1 $\alpha$ <sup>99</sup> in the induction of mitochondrial respiratory genes along with mitochondrial transcriptional factors like mtTFA and mtTFB, the 2 proteins controlling mtDNA replication. This mechanism depends on NRFs (NRF1 and NRF2) and downstream targets of PGC-1 $\alpha$ . PGC-1 $\alpha$  can be post-translationally modified by 5' AMP-activated protein kinase (AMPK)<sup>100,101</sup> and SIRT1.<sup>102,103</sup> Furthermore, PGC-1 $\alpha$ -mediated pathways are activated during physical exercise and CR in AMPK<sup>104</sup> and SIRT1-dependent<sup>105</sup> pathways. However, SIRT1 involvement in CR cell physiology is not equivocal in all publications.<sup>106</sup> Both *SIRT1* and *PGC-1 $\alpha$*  mRNA can be miR targets (Fig. 2).

It has been shown that miR-696 increased by 4 weeks of exercise training and decreased by 5 days of immobilization, and this was paralleled by the changes of PGC-1 $\alpha$  content.<sup>107</sup> The related cell culture studies confirmed that miR-696 is one of the regulators of PGC-1 $\alpha$ .<sup>107</sup>

Because mitochondria play a major role in energy production during skeletal muscle contraction, it is plausible that during exercise adaptation, miRs may affect nuclear and even mitochondrial encoded transcripts. A major obstacle of the regulation of mt-transcripts by miRs is that the RNA-induced



Table 1  
MicroRNAs target various cellular signaling pathways.

miR	mRNA target	Tissue/cell line	Seed location in 3' UTR (hsa)	PMID	Human reference target mRNA	miR sequence
miR-28-5p	NFE2L2	MCF-12A, MCF-7 and HEK293T cells	55	21638050	NM_001145413	AAGGAGCUCACAGUCUAUUGAG
miR-93-5p	NFE2L2	mouse N2A cells	186	27300700	NM_001145412	CAAAGUGCUGUUCGUGCAGGUAG
miR-93-5p	NFE2L2	MCF-10A and T47D human breast cancer cell line	186	23492819	NM_001145412	CAAAGUGCUGUUCGUGCAGGUAG
miR-153-3p	NFE2L2	SH-SY5Y neuronal cells	98	23236440	NM_001145412	UUGCAUAGUCACAAAAGUGAUC
miR-27a-3p	NFE2L2	SH-SY5Y neuronal cells	62	23236440	NM_001145412	UUCACAGUGGCUAAGUUCGCG
miR-142-5p	NFE2L2	SH-SY5Y neuronal cells	83	23236440	NM_006164	CAUAAAAGUAGAAAGCACUACU
miR-144-3p	NFE2L2	SH-SY5Y neuronal cells	265, 370	23236440	NM_006164	UACAGUAUAGAUGAUGUACU
miR-200a-3p	KEAP-1	Rat hepatic stellate cell	131	25049078	NM_203500	UAACACUGUCUGGUAACGAUGU
miR-200a-3p	KEAP-1	MDA-MB-231 and Hs578T cells	131	21926171	NM_012289	UAACACUGUCUGGUAACGAUGU
miR-200a-3p	KEAP-1	Mahlavu and HuH7 cells	131	23857252	NM_203500	UAACACUGUCUGGUAACGAUGU
miR-196a-5p	Bach1	mouse embryonic fibroblast cells	2161, 2280	27343195	NM_001186	UAGGUAGUUUCAUGUUGUUGGG
miR-196a-5p	Bach1	9-13 human hepatoma cells	2161, 2280	20127796	NM_001186	UAGGUAGUUUCAUGUUGUUGGG
miR-155-5p	C/EBP $\beta$	human and mouse mesenchymal stem cells	554	28967703	NM_001285878	UUA AUGCUAAUCGUGAUAGGGGUU
miR-128-3p	MAFG	HEK293 and C2C12 cells	1564	29138682	NM_032711.4	UCACAGUGAACCGGUCUCUUU
miR-195-3p	HIF-1 $\alpha$	ATDC 5 and HEK293T cells	803	25753868	NM_001243084	CCAAUUAUUGGUCUGUCGUCUCC
miR-199a-5p	HIF-1 $\alpha$	A2780 cells	177	24706848	NM_181054	CCCAGUGUUCAGACUACCGUUC
miR-217-5p	PGC-1 $\alpha$	MCF-7, MDA-MB-231 and HEK-293T cells	3746	27916422	NM_013261	UACUGCAUCAGGAACUGAUUGGA
miR-494-3p	PGC-1 $\alpha$	3T3-L1 white and beige adipocytes	3788	30305668	NM_013261	UGAAACAUAACACGGGAAACCUC
miR-34a-5p	SIRT1	primary rat hepatocytes	891, 1434	24421392	NM_001142498	UGGCAGUGUCUUAGCUGGUUGU
miR-34a-5p	SIRT1	HCT116 cells	891, 1435	18755897	NM_001142498	UGGCAGUGUCUUAGCUGGUUGU
miR-133a-3p	SIRT1	H9c2 cells	403	29487709	NM_001142498	UUUGGUCCCCUUAACACAGCUG
miR-504-5p	NRF-1	CNE2 and HEK 293 cells	506	26201446	NM_001040110	AGACCCUGGUCUGCACUCUAUC
miR-494-3p	SIRT3	SH-SY5Y neuronal cells	1618	29567426	NM_012239.6	UGAAACAUAACACGGGAAACCUC

Abbreviations: Bach1 = transcription regulator protein BACH1; C/EBP $\beta$  = CCAAT/enhancer-binding protein  $\beta$ ; HEK = human embryonic kidney; HIF-1 $\alpha$  = hypoxia-inducible factor 1- $\alpha$ ; KEAP-1 = Kelch-like ECH-associated protein 1; Maf = musculoaponeurotic fibrosarcoma; MAFG = v-Maf avian Maf oncogene homolog G; miR = microRNA; NFE2L2 = nuclear factor erythroid 2-related factor 2; NRF-1 = nuclear respiratory factor 1; p = point mutation; PGC-1 $\alpha$  = peroxisome proliferator-activated receptor gamma coactivator 1- $\alpha$ ; PMID = PubMed Unique Identifier; SIRT = sirtuin; UTR = untranslated region.

silencing complex (RISK) complex processes mRNA in the cytoplasm, and in order to implement an intra-mitochondrial effect, RISK elements, along with effector miRs, need to be transported to the mitochondrial compartment. There are some miRs that have been found to be spatially associated with mitochondria.<sup>108</sup>

It has been suggested that miR-4485 regulates mitochondrial ribosomal RNA processing, and its abundance increases after H<sub>2</sub>O<sub>2</sub> treatment.<sup>109</sup> Transfection with miR-4485 mimetic decreased the activity of mitochondrial respiratory complex I. However, ROS levels and membrane potential are decreased in breast cancer-derived mitochondria. Conversely, miR-4485 inhibitor also increases ROS levels, along with complex I activity and membrane potential. In fact, miR-4485 negatively affected most of the 13 protein-coding transcripts in human embryonic kidney 293 (HEK293) cells, which suggests transcriptional inhibition.<sup>109</sup>

Drawing general conclusions from studies dealing with miR subcellular location should be done with caution. Sometimes the discrepancies between the published mitomiR pathways and the reported spatial ambiguities may not always have

robust biological meaning. Rather, this finding highlights the technical difficulties inherent in isolating pure cellular compartments. Contamination in miR studies can be an issue, but using cytoplasmic-enriched controls like miR-145<sup>110</sup> aids in filtering out false detections. Subjecting the isolated mitochondria to RNase A<sup>109</sup> digestion may help to avoid cytoplasmic or outer-membrane-bound RNA contamination.

## 6. miR and exercise

During physical activity the metabolic state of the working skeletal muscle changes significantly: ATP production and demand increases,<sup>111</sup> and the tissue is subjected to greater mechanical<sup>112</sup> and oxidative stress.<sup>113,114</sup> High-volume or high-intensity exercise may perturb the intramuscular homeostasis at such a level that the metabolic processes can be shifted to mainly anaerobic energy production. This phenomenon results in excessive lactate accumulation,<sup>115,116</sup> and decreased oxygen tension leads to the activation of the hypoxia-sensitive genes, like the already mentioned HIF-1 $\alpha$ .<sup>117,118</sup> The acute and chronic metabolic changes can turn on and off miR expression processes (like

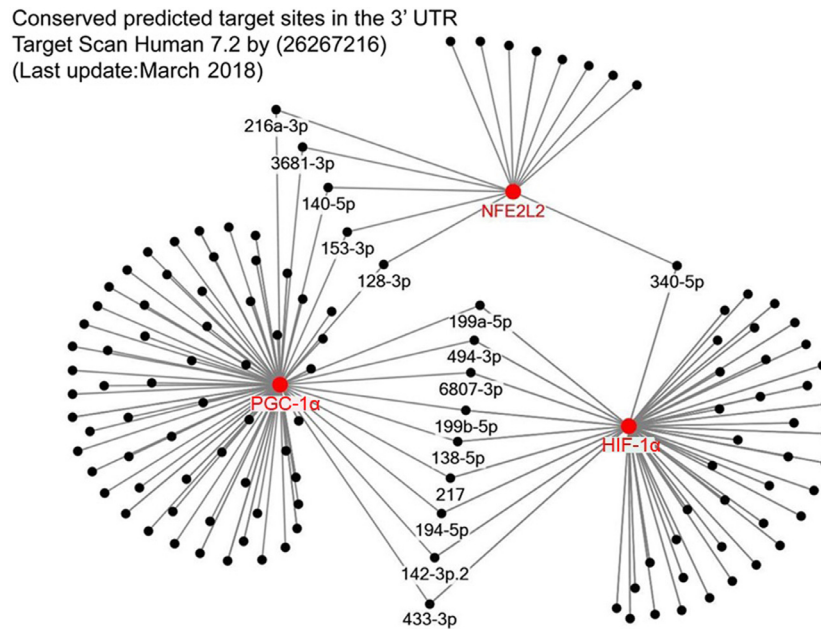


Fig. 2. MicroRNA-mediated regulation of NFE2L2, PGC-1 $\alpha$ , and HIF-1 $\alpha$ . The suggested microRNA-associated regulation of 3 important signaling proteins in the cell. HIF-1 $\alpha$  = hypoxia-inducible factor 1- $\alpha$ ; NFE2L2 = nuclear factor erythroid 2-related factor 2; PGC-1 $\alpha$  = peroxisome proliferator-activated receptor gamma coactivator 1- $\alpha$ ; UTR = untranslated region; p = point mutation.

the HIF-1 $\alpha$ –miR-210 pathway), which is likely to contribute to exercise-induced skeletal muscle adaptation.

There are numerous studies published on how various exercise stimuli affect the miR levels in the circulation,<sup>119–121</sup> but almost always the connection is correlative, without providing information about the nature of causality. Indeed, miR-1, miR-133a, and miR-206 circulatory levels have been shown to be increased after a half-marathon run,<sup>122</sup> but it has not been shown whether this is always a controlled secretion process with distinct physiological function. A vast number of miRs have been shown to increase in blood due to different types of exercise stimuli.<sup>119</sup> However, the appearance of miR in the plasma can be an outcome of temporal loss of sarcolemma integrity during exercise and is not necessarily accountable to increased miR expression and/or secretion.

It has been reported that acute high-intensity resistance training changed the levels of miR-23a, miR-133a, miR-146a, miR-206, miR-378b, and miR-486 in the biopsy samples of skeletal muscle, and a relationship was not found between muscular and circulating miR levels.<sup>123</sup> Yin and co-workers<sup>124</sup> examined the effects of uphill and downhill running on myo-miRs (miR-1, miR-133a, miR-133b, miR-206, miR-208a, and miR-499) in quadriceps, gastrocnemius, soleus, and cardiac muscle and in the blood (exosomes and freely circulating). Results revealed that the exercise modality is one of the key factors that affects myo-miR levels. Moreover, the changes of the same miRs in the skeletal muscles, cardiac muscles, exosomes, and circulation are not significantly related.<sup>124</sup>

Some studies, on the other hand, have shown that miRs were released from microvascular compartments into the circulation due to exercise training.<sup>125,126</sup>

When the muscle biopsy samples of master athletes and age-matched control subjects were studied, it was demonstrated that aged, untrained skeletal muscle contains elevated

levels of miR-7.<sup>127</sup> Because miR-7 has been linked to impaired cellular repair and inflammation, this result suggests that 4–5 decades of training by master athletes has provided protection against sarcopenia.<sup>127</sup> Five myo-miRs—miR-1, miR-34a, miR-133a, miR-133b, and miR-206—were measured from the biopsy samples that were taken 2 h after the acute training, with and without blood flow restriction, during the rest periods of high-intensity resistance training.<sup>128</sup> Only the level of miR-206 was altered (i.e., significantly decreased in the leg), which was subjected to blood flow restriction. Because inhibition of miR-206 can readily enhance satellite cell proliferation and increase Pax7 protein levels, it was suggested that down-regulation of miR-206 could be important to cope with blood-flow restriction and training-caused damage and might be a necessary part of the adaptive response. One of the consequences of adaptation induced by high-intensity resistance training is muscle hypertrophy, although it is not easy to induce muscle hypertrophy in animal models using weight or anaerobic training. Therefore, we selected the very reliable compensatory hypertrophy model to study the possible role of miR in hypertrophy. Our data revealed that 2 weeks of compensatory hypertrophy resulted in a 40% increase in muscle mass and a significant increase in SIRT1 content and activity.<sup>129</sup> It is well documented that sirtuins are redox-sensitive proteins.<sup>130</sup> Compensatory hypertrophy was associated with decreased levels of miR-133a, which has binding sites for the *SIRT1* mRNA 3' UTR region; hence, it can down-regulate SIRT1 through its mRNA.<sup>131</sup> Thus, it is suggested that decreased level of miR-133a was important to the upregulation of SIRT1 in the muscle hypertrophy model. Not only was miR-133a changed with compensatory hypertrophy, but the levels of miR-1 also decreased. Previously it has been demonstrated that miR-1 levels increased during muscle atrophy, so the down-regulation of

miR-1 during hypertrophy is in accordance with earlier findings. We found in the hypertrophy model that the miR-214 levels increased nearly 10-fold. It has been reported that miR-214 can decrease superoxide production via nicotinamide adenine dinucleotide phosphate (NADPH) oxidase 4 (NOX4).<sup>132</sup> This fits nicely with our results in that we found a negative correlation between miR-214 and 2',7'-dichlorodihydrofluorescein diacetate (H<sub>2</sub>DCFDA) fluorescence ( $r^2 = -0.816$ ,  $p < 0.05$ ) and NADH levels ( $r^2 = -0.837$ ,  $p < 0.05$ ), suggesting that miR-214 can modulate the generation of various oxidants during muscle hypertrophy.<sup>129</sup>

## 7. Conclusion

Our knowledge of miR-regulated redox metabolism signaling is expanding day by day. Despite the complex overlapping effects of various microRNAs, it is clear that they play an important role in adaptive responses to oxidative challenge. miRs are involved in ROS production and in the regulation of antioxidant systems and repair as well. The miR-associated control of mitochondrial function, including ROS production, is also well established. Exercise-associated redox-sensitive miR regulation is an important element for exercise-mediated adaptation on redox homeostasis.

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## Authors' contributions

FT, ZG, MJ, IB, MT, TM, and FG contributed by searching for finding and discussing the importance of all relevant literature and contributed to the manuscript writing; ZR contributed by searching for finding and discussing the importance of all relevant literature, drafted the final version of the paper, and contributed to the manuscript writing. All authors have read and approved the final version of the manuscript, and agree with the order of presentation of the authors.

## Competing interests

The authors declare that they have no competing interests.

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