Review

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HypoxamiRs: regulators of cardiac hypoxia and energy metabolism

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Hypoxia and its intricate regulation are at the epicenter of cardiovascular research. Mediated by hypoxia-inducible factors as well as by several microRNAs, recently termed 'hypoxamiRs', hypoxia affects several cardiac pathophysiological processes. Hypoxia is the driving force behind the regulation of the characteristic metabolic switch from predominant fatty acid oxidation in the healthy heart to glucose utilization in the failing myocardium, but also instigates reactivation of the fetal gene program, induces the cardiac hypertrophy response, alters extracellular matrix composition, influences mitochondrial biogenesis, and impacts upon myocardial contractility. HypoxamiR regulation adds a new level of complexity to this multitude of hypoxia-mediated effects, rendering the understanding of the hypoxic response a fundamental piece in solving the cardiovascular disease puzzle.

Introduction

The demand for novel curative strategies to treat heart failure, still the number one cause of hospitalization and morbidity in the Western Hemisphere, remains urgent [1]. Heart failure is preceded by ventricular remodeling (also termed cardiac remodeling), a complex process that involves alterations in the size, shape, structure, and physiology of the heart that occur secondary to clinically prevalent forms of injury to the myocardium such as myocardial infarction, aortic stenosis, or chronic hypertension. Cardiac remodeling arises from a variety of biochemical and hemodynamic stressors that provoke hypertrophic growth of the cardiomyocyte, a form of growth of the heart muscle rather than hyperplasia [2]. Cardiac muscle characteristics in all of these settings include increased myocyte size, sarcomere formation, and reprogramming of cardiac gene expression [3]. In humans, cardiac hypertrophy is the principal risk factor for the development of heart failure and lethal arrhythmias. Despite the impressive metabolic flexibility and adaptation to nutritional status and exercise demand, the mammalian myocardium cannot produce sufficient energy under anaerobic conditions to sustain its contractile performance [4].

Under stress conditions, when oxygen or substrate supply is diminished, decreased efficiency of ATP synthesis can limit energy supply, leading to an overall failure to maintain vital processes, and contributing to cardiac remodeling. In fact, cardiac oxygen consumption exceeds that of any other organ in the body even at resting pulse rate, making the heart very susceptible to oxygen fluctuations [5]. As a potential trigger to cardiac disease, hypoxia has received increasing attention in recent years [6]. Although it is well established that hypoxia is a key factor in activating pro-angiogenic factors [7], the participation of hypoxia as contributor to heart failure remains controversial (Box 1).

The healthy adult myocardium relies primarily on mitochondrial fatty acid oxidation (FAO) for its energy production given the abundant supply of oxygen and fatty acids. Impaired mitochondrial fatty acid oxidation and a larger reliance on glucose utilization for energy production are consistent features of heart failure [8,9]. Owing to the strict aerobic nature of the heart and the inability to generate sufficient energy under anaerobic conditions, reliance on glycolysis has a major impact on available ATP levels [4]. As such, hypoxia is considered to be a characteristic of the failing heart and is intimately connected with cardiac substrate use for energy production [10–12].

Recently, new regulators of hypoxia have been identified, namely small non-coding regulatory RNA species or microRNAs (miRNAs, miRs; Box 2). miRNAs have a multitude of roles in different cellular processes, including myocardial remodeling, contractility, angiogenesis, and the cardiac metabolic switch from predominant reliance on fatty acid utilization in the healthy myocardium toward increased reliance on glucose metabolism in the diseased myocardium. We discuss here the complexity of myocardial hypoxia signaling with an emphasis on the contribution of miRNAs in hypoxia regulation of cardiac energy metabolism.

Hypoxia and small non-coding RNAs

Although initially considered transcriptional noise, transcriptome studies have revealed widespread transcription of a large number of non-coding RNAs (ncRNAs) classified into two groups according to their length [13]. Small

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Box 1. Heart failure and hypoxia

Heart failure, or the inability of the heart to meet hemodynamic demands, represents the end-stage of various forms of cardiac disease [1]. Hemodynamically challenged hearts display a characteristic return to the fetal metabolic pattern that is hallmarked by impaired mitochondrial fatty acid oxidation and a shift to further reliance on glucose metabolism [8]. Hypoxia is increasingly considered as a characteristic of heart failure. Altered gene expression as a response to oxygen availability in the heart is regulated by several mechanisms including the hypoxia-inducible factor (HIF) transcription factors [33]. Loss and gain of function studies support this contention, where targeted HIF1α deletion in mice leads to embryonic lethality at midgestation due to abnormal vascular development and hypertrophy of the embryonic heart [66]. Under normoxic conditions, the hydroxylation of HIF1α at two specific proline residues by the prolyl-4-hydroxylase domain-containing enzymes (PHD) targets HIFs for polyubiquitination and proteasomal degradation by the von Hippel–Lindau tumor-suppressor protein (VHL). However, at low oxygen levels, PHD activity is inhibited, preventing HIF1α from polyubiquitination and subsequent proteasomal degradation. In contrast to the HIF1α null mice, HIF2α-deficient mice display multiple organ pathology characterized by an impaired response to oxidative stress and vascular integrity [50,51].

Box 2. MicroRNAs and RNA therapeutics

MicroRNAs (miRNAs, miRs) belong to a class of small non-coding, regulatory, ~22 nt nucleic acids that play a pivotal role in post-transcriptional gene regulation. MicroRNAs are transcribed by RNA polymerase II as primary microRNA transcripts that can encode one or multiple microRNAs. Primary microRNAs create imperfectly base-paired hairpin structures that are cleaved by the RNase III endonuclease Drosha to generate multiple 60–100 bp hairpin-like structures called precursor microRNAs, which are transported to the cytoplasm in an exportin 5-dependent manner and cleaved by the cytoplasmic RNase III endonuclease Dicer to yield miR–miR* duplexes. The mature strand is loaded into the RNA-induced silencing complex (RISC), which guides the mature strand to cognate target mRNAs to induce post-transcriptional gene silencing by translational repression or mRNA cleavage [67]. MicroRNAs are thought to 'fine-tune' gene expression under homeostatic circumstances but, under conditions of stress, the regulatory functions of microRNAs become far more pronounced and seem to play more decisive roles in disease processes.

Therapeutic microRNA inhibition can be achieved by complementary oligonucleotides that are chemically modified for increased binding affinity and improved nuclease resistance, enabling *in vivo* targeting [68]. Owing to their small size and evolutionary conservation, pharmacological antisense oligonucleotide technology can be employed to efficiently silence microRNAs and/or create steric blockade, rendering mature microRNAs essentially inactive. Two major classes of so-called antisense 'antimirs' have been most abundantly used in the cardiovascular arena. The first class utilizes locked nucleic acid (LNA) modifications, where the 2′-hydroxyl group is linked to the 4′ carbon atom of the sugar ring, thereby forming a bicyclic sugar moiety. LNA-protected antisense antimirs exhibit high thermal stability when hybridized with their RNA target molecules. Indeed, LNA-protected antimirs possess the thermodynamically strongest duplex formation with complementary target RNA of all antimirs, show excellent mismatch discrimination, and show lack of toxicity in nonhuman primates [69,70]. Adverse outcomes from LNA-protected antimirs may result from tight double-strand binding which can lead to unintended binding to non-targeted microRNAs or precursor microRNAs [71]. The second class utilizes methylation of the hydroxyl group at the 2′ position of the ribose unit, phosphorothioate moieties in the linking backbone, and cholesterol conjugation to increase cellular uptake of the antisense oligonucleotide. This 'antagomir' approach exhibits broad biodistribution *in vivo*, has very high sequence specificity, and can efficiently degrade target microRNAs in all tissues tested without adverse toxicity even at relatively high doses [72].

ncRNAs are arbitrarily set at less than 200 nt in size and include several ncRNAs species including small interfering RNAs (siRNAs), Piwi-interacting RNAs (piRNAs), and microRNAs (miRNAs) [14,15]. miRNAs are transcribed by RNA polymerase II and fulfill key roles in a wide range of processes, including chromatin modifications, RNA processing, and transcriptional and post-transcriptional gene silencing [14–16].

Since the first descriptions of their regulatory functions, miRNAs have displayed unique functions in a broad range of cellular adaptive processes including cellular differentiation, proliferation, apoptosis, and metabolism [16,17]. The endoRNAse DICER is a key component in the processing of pre-miRNAs into mature miRNAs. Furthermore, DICER facilitates incorporation of miRNAs into the RNA-induced silencing complex (RISC), enabling post-transcriptional gene silencing via changes in target mRNA stability and/or translation. The importance of DICER and proper miRNA biogenesis is exemplified by studies demonstrating that mice embryos lacking DICER die at embryonic day 7.5 as a result of arrested development during gastrulation [18]. In addition, conditional heart muscle-restricted DICER deletion results in severe cardiac phenotypes, including excessive myocardial growth, fibrosis, and dramatic loss of cardiac contraction [19]. Intriguingly, despite cardiomyocyte-restricted depletion of miRNA biogenesis, myocardial vascularization was severely dysregulated, indicating a specific role for miRNAs expressed in cardiac muscle in maintaining cardiac vascular homeostasis (Figure 1). Likely, the increase in oxygen diffusion distance as a result of the hypertrophied cardiomyocyte, and the decreased contractile function in the DICER null hearts, provides an ideal combination for the creation of a hypoxic milieu in cardiomyocytes.

A major advance in our understanding how miRNAs participate in cellular hypoxia was provided by a study where it was demonstrated that global miRNA biogenesis was disrupted during chronic hypoxia. In this study, chronic hypoxia resulted in a general decrease in expression of mature miRNAs and the accumulation of precursor miRNAs, indicative of disrupted miRNA biogenesis precisely at the step of DICER activity. The reduction in DICER activity in this study depended crucially on the Von Hippau–Lindau tumor suppressor (VHL), and concurred with decreased protein stability of both the hypoxia-inducible factor (HIF)1α and HIF2α isoforms as well as dysregulation of vascular endothelial growth factor receptor 2 (VEGFR2) [20]. Taken together, these data exemplify how hypoxia is capable of driving global changes in cardiac mature miRNA expression profiles by altering miRNA biogenesis. Future studies should reveal the mechanisms how hypoxia can lead to alterations in DICER activity or additional components of the miRNA biogenesis machinery.

Hypoxia-associated miRNAs (hypoxamiRs)

Apart from hypoxia-mediated global changes in mature miRNA expression, cellular adaptations to hypoxia are also heavily influenced by single miRNAs, as indicated by a steadily rising list of hypoxamiRs [21,22]. The list

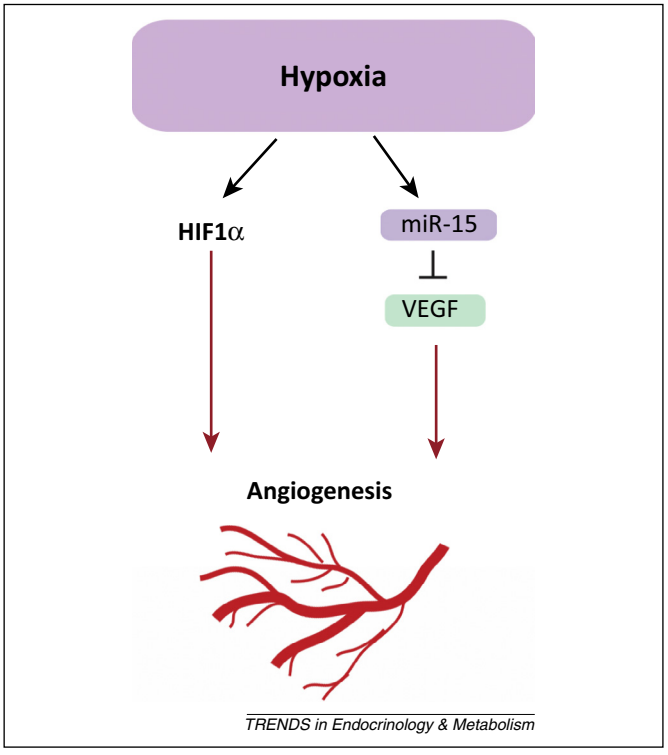

Figure 1. Functional roles for hypoxamirs in regulating angiogenesis. Understanding the relation between hypoxia and several key processes to changes in cardiac tissue oxygenation remain highly complex. Apart from the cellular response tools to hypoxia such as metabolic adaptation, declining oxygen tension in the heart is sufficient for dramatic structural and functional changes. An example for this complexity is the fact that chronic hypoxia results in a general decrease in production of mature miRNAs. Disruption of the DICER activity results in destabilization of HIF1α and dysregulation of VEGFR2, thereby negatively influencing cardiac angiogenesis. The miR-15 hypoxamir family shows differential expression patterns in models of cardiac disease by targeting VEGF, a key requirement for cardiac angiogenesis.

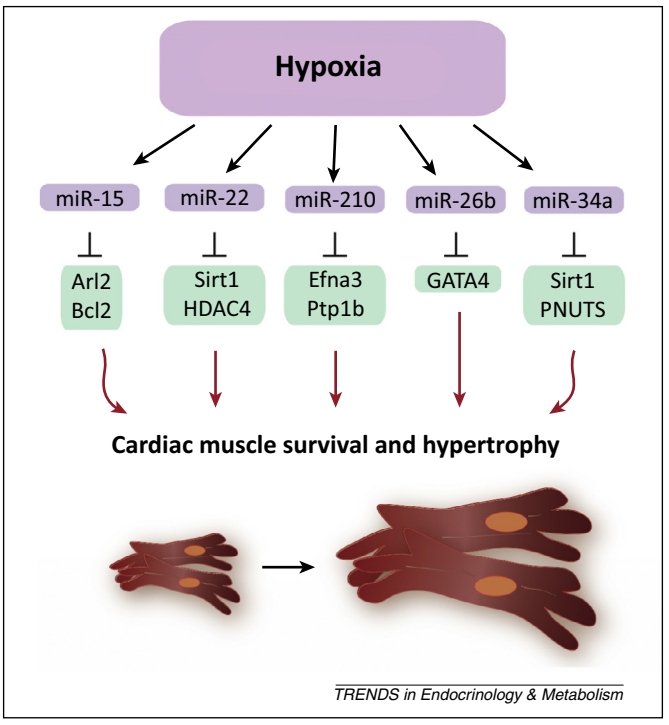

Figure 2. Functional roles for hypoxamirs in regulating cardiac remodeling. The stressed adult heart is characterized by myocyte hypertrophic growth and the reactivation of the fetal gene program. The processes driving cardiac remodeling and the reactivation of the fetal gene program by chronic cardiac hypoxia remain poorly understood. Among the transcription factors that also have fundamental roles in cardiac development is GATA4. During cardiac hypertrophy, GATA4 is upregulated and its overexpression in the heart suffices for inducing cardiac hypertrophy. A role for hypoxamirs in regulating hypertrophic growth and the reactivation of the fetal gene program is exemplified by miR-26 and miR-22. miR-26 is suppressed in hypertrophied hearts resulting in the derepression of GATA4. Sirtuin1 (Sirt1) has a key role in cell survival and is protective against cardiac hypertrophy. Sirt1 is targeted by the hypoxamir miR-22. While Sirt1 is protective in the setting of cardiac hypertrophy, another target of miR-22, HDAC4, is involved in rapid epigenetic modifications in response to variations in cardiac load.

now includes over 90 miRNAs, each acting on specific organs and providing mechanistic insight into the specific involvement of miRNAs and their target genes in several hypoxia-related disease phenotypes [23,24]. Several hypoxamiRs are implicated in cardiac development and cardiac disease, and the best-characterized examples are discussed briefly below [25].

The hypoxamiR miR-26 was implicated in the balance between cardiac hypertrophy and cardiomyocyte survival by dual targeting of the GATA-motif transcription factor GATA4 and the kinase phospholipase C-β1 (Figure 2) [26]. The zinc finger-containing DNA-binding factor GATA4 is essential for proper cardiogenesis in the developing embryo [27] and is essential for proper neo-angiogenesis in the stressed heart [28] because both gain- and loss-of-function studies for GATA4 in the mouse produce severe cardiac remodeling [29]. In addition, the prototype signaling enzyme phospholipase C-β1 is activated by mechanical stretch to generate the Ca^{2+}-releasing messenger inositol trisphosphate (IP_3) and sn-1,2-diacylglycerol (DAG), an activator of protein kinase C subtypes. Activation of phospholipase C-β1 directly contributes to cardiac remodeling [30]. As such, the hypoxia-induced reduction in miR-26 in myocardium therefore directly contributes to maladaptive remodeling by dual repression of both GATA4 and phospholipase C- β1 (Table 1).

The cardiac- and skeletal muscle-enriched hypoxamiR miR-22 is more abundantly expressed in conditions of early cardiac remodeling. Using gain- and loss-of-function mouse models for miR-22, it was demonstrated that overexpression of miR-22 was sufficient to induce cardiac remodeling, while miR-22 gene deletion sensitized mice to the development of dilated cardiomyopathy under stress conditions. Both NAD-dependent deacetylase sirtuin 1 (Sirt1) and histone deacetylase 4 (Hdac4) were confirmed as intracellular miR-22 targets in the heart that mechanistically explain the pathological functions of this hypoxamiR (Figure 2). Reduction in the expression of deacetylase Sirt1 results in morphological and functional mitochondrial abnormalities, because mitochondrial genes were the most affected in Sirt1-deficient mice [31], as well as an altered acetylation pattern of the genes encoding myocyte enhancer factor 2 (Mef2) transcription factors, which are crucial for normal heart development and mitochondrial integrity [32,33]. An additional target of miR-22, Hdac4, is part of the machinery that regulates the accessibility of regulatory DNA sequences to transcriptional activators and repressors by reversible posttranslational modifications of histones. Acetylation of histones by histone acetyltransferases stimulates gene expression by relaxing chromatin structure,

Table 1. Cardiac hypoxamiR regulation and function[a]

Name	Genes they target		Genes that regulate them		Tissue/cell expression profile	Response to hypoxia	Mechanism of action in the heart	Refs
	Induce	Repress	Induced by	Repressed by				
miR-26		GATA4, PLC1b			Heart muscle	Increased	Cardiac remodeling	[26]
miR-22		Sirt1, Hdac4			Heart muscle	Increased	Mitochondrial abnormalities, pathological gene expression	[32]
miR-34a		Sirt1, PNUTS			Heart muscle Renal tubular cells	Increased	Telomere shortening, DNA damage response, cardiomyocyte apoptosis	[36,37,40]
miR-210		Efna3, Ptp1b, HIF3α	HIF1α		Heart muscle	Decreased	Impairs neoangiogenesis, cardiomyocyte apoptosis	[40,63,64]
miR-15		VEGF, Arl2, Bcl-2		c-Myc	Heart muscle	Increased	Impairs neoangiogenesis, cardiomyocyte apoptosis	[41–46]
miR-27b		PPARγ	TGF-β		Heart muscle	Increased	Cardiac remodeling	[53–55]
miR-214		PPARδ	HIF1α		Heart muscle	Increased	Reduced mitochondrial FAO	[56]
miR-199a		HIF1α, Sirt1	HIF1α		Heart muscle	Increased	Hypoxia	[57]
miR-696		PGC1α			Heart and skeletal muscle	Increased	Impaired mitochondrial biogenesis	[58]
miR-181c	Mt-COX2	Mt-COX1			Heart muscle	Decreased	Higher mitochondrial respiration, ROS production	[63]
miR-484		Fis1			Heart muscle	?	Regulates mitochondrial fission and apoptosis	[64]
miR-223	GLUT4				Heart muscle	?	Increased glucose uptake in diabetic heart	[65]

[a]Abbreviations: Arl2, ADP-ribosylation factor-like 2; Blc-2, B cell leukemia/lymphoma 2; c-Myc, myelocytomatosis oncogene; Efna3, ephrin A3; Fis1, mitochondrial fission protein; GLUT4, glucose transporter type 4; HIF1α/3α, hypoxia-inducible factors 1α/3α; Mt-COX1/2, mitochondrial cytochrome *c* oxidase subunit1/2; PGC1α, PPARγ co-activator 1α; PLC1, phospholipase C1; PNUTS, protein phosphatase 1, regulatory subunit 10; PPARδ/γ, peroxisome proliferator activated receptors δ/γ; Ptp1b, protein tyrosine phosphatase, non-receptor type 1; TGF-β, transforming growth factor β, VEGF, vascular endothelial growth factor.

whereas deacetylation of histones by histone deacetylases promotes chromatin condensation and transcriptional repression [34]. Hdac4 represses the expression of Mef2 transcription factors, which control pathological cardiac growth and gene expression in response to acute and chronic stress stimuli [35]. Taken together, the increased abundance of hypoxia-sensitive miR-22 affects cardiac mitochondrial integrity, growth and gene expression by the repression of its downstream targets Sirt1 and Hdac4 (Table 1).

The hypoxamiR miR-34 family members (miR-34a/b/c) are upregulated in the heart in response to stress and share Sirt1 as a direct target [36]. In addition, miR-34a is induced in the aging heart, and genetic deletion of miR-34a reduced age-associated cardiomyocyte cell death and improved functional recovery after acute myocardial infarction [37]. Protein phosphatase 1 regulatory subunit 10 (PPP1r10 or PNUTS), which interacts with the telomere regulator telomeric repeat binding factor (TRF2) and is involved in DNA repair [38,39], was discovered as novel mechanistic target of miR-34a, linking telomere shortening, DNA damage responses, and cardiomyocyte apoptosis to age-induced expression of miR-34a (Figure 2). In two separate studies, locked nucleic acid (LNA)-modified 'antimirs' (Box 1) were employed to therapeutically silence miR-34a in the mouse, and in both cases this new therapeutic approach improved systolic function and attenuated pathological cardiac remodeling following sustained pressure overload or acute myocardial infarction [37,40].

The miR-15 family members (miR-15a, miR-15b, miR-16-1, miR-16-2, miR-195, and miR-497) are regulated by hypoxia and are consistently upregulated in mouse and pig models of heart disease [41,42]. Nucleotides 2–8 at the 5′ end of a miRNA, referred to as the 'seed sequence', are important for miRNA:target interaction. Interestingly, the miR-15 family members share homologous seed sequences and differ in their 3′ portions, and consequently miRNA family members can presumably regulate overlapping target genes. The miR-15 family is predicted to influence cardiomyocyte cell survival by regulating the expression of several prosurvival proteins, including ADP-ribosylation factor-like 2 (Arl2) and B cell leukemia/lymphoma 2 (Bcl2) [43,44]. In line, miR-15 family inhibition by (LNA)-antimiRs enhances cardiac function and reduces infarct size in response to ischemic damage in mice by increasing cardiomyocyte survivability and by derepression of its additional target VEGF, suggesting a new mechanism where miR-15 reduces the angiogenic response despite the hypoxic milieu in failing hearts [45,46].

miR-210, sometimes referred to as the 'master hypoxamiR' [24], exhibits cardioprotective effects after intramyocardial injections in mouse hearts, leading to preserved cardiac function, improved neovascularization, and inhibition of apoptosis [47], effects which were mechanistically explained by suppression of two targets of miR-210, ephrin A3 (Efna3) and protein tyrosine phosphatase, non-receptor type 1 (Ptp1b). Interestingly, although miR-210 was also shown to target HiF3α, a negative regulator of HIF1α, no differences in HIF1α activity were observed under normoxic or hypoxic conditions when miR-210 expression is altered [48], indicating that miR-210 has non-HIF1α-dependent downstream effects. Taken together, the aforementioned hypoxamiRs provide mechanistic insight how dysregulation of individual miRNAs affect diverse processes including myocyte survivability, gene expression, and cardiac vascularization downstream of cardiac hypoxic conditions. The above examples also exemplify

how new classes of RNA therapeutics based upon antisense RNA technology can open new avenues to intervene at the level of hypoxia and cardiac remodeling.

HypoxamiRs in cardiac mitochondrial function

Depending on nutritional status and cardiac demand, the heart is able to generate a continuous ATP supply by using fatty acids, glucose, ketone bodies, lactate, and amino acids as substrates for oxygen-dependent mitochondrial oxidative phosphorylation, which accounts for the vast majority (90%) of ATP production in the heart [49]. Whereas fatty acid oxidation is the primary source of energy in the adult myocardium, the end-stage failing heart demonstrates a striking impairment of mitochondrial fatty acid oxidation and a shift to larger reliance on glucose metabolism [50]. Increased fatty acids levels, as a consequence of impaired mitochondrial fatty acid oxidation, promote the synthesis of uncoupling proteins and the deterioration of the electrochemical gradient across the inner mitochondrial membrane [51], eventually hampering mitochondrial activity, leading to reduced ATP production and diminished cardiac function [52].

miR-27b, elevated in conditions of cardiac remodeling, directly targets peroxisome proliferator-activated receptor-γ (PPAR-γ) in cardiomyocytes (Figure 3). Consistently, miR-27b transgenic mice displayed significantly lower levels of PPAR-γ, while *in vivo* silencing of miR-27b using a specific antagomir in a pressure-overload-induced mouse model of heart failure increased cardiac PPAR-γ expression and attenuated cardiac hypertrophy and dysfunction [53]. This interaction between miR-27b and PPARγ has also been confirmed in neuroblastoma cells [54], and in adipocytes where it affects adipogenesis (Table 1) [55].

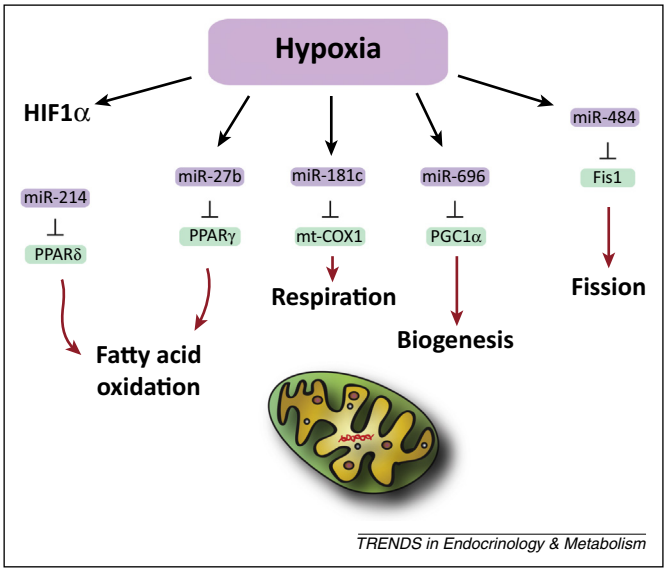

Figure 3. Functional roles for hypoxamiRs in regulating cardiac metabolism. MicroRNAs are essential for the adaptation of cardiac metabolism to changes in tissue oxygenation. A well-established cellular adaptation to hypoxia consists of repressed mitochondrial respiration and decreased mitochondrial biogenesis. Canonical hypoxia signaling via the HIF1α pathway induces the expression of the microRNA cluster miR-199a~214 to target peroxisome proliferator activated receptor δ (PPARδ), thereby suppressing fatty acid oxidation. Another PPAR isoform, PPARγ, is targeted by the hypoxamiR miR-27b, thereby regulating the metabolic shift in heart failure through suppression of fatty acid fueling of mitochondria. Another way of repressing mitochondrial respiration is mediated by the hypoxamiR miR-181c which targets the mitochondrial cytochrome *c* oxidase subunit 1 (mt-COX1), causing electron transport chain complex IV remodeling. The pathophysiology of heart failure is also hallmarked by a decrease in cardiac mitochondrial content and mitochondrial DNA. In healthy hearts mitochondrial biogenesis and fission events participate in regulating mitochondrial homeostasis. An important regulator of mitochondrial biogenesis is PGC1α, targeted by miR-696. In addition, miR-484 targets mitochondrial fission protein (Fis1), which is responsible for regulating mitochondrial fission and apoptosis in the heart, thereby disrupting the .maintenance of normal mitochondrial function.

As a part of repressed mitochondrial respiration, decreased mitochondrial biogenesis is a well-established cellular adaptation to hypoxia [58], but the underlying molecular mechanisms are still incompletely understood. Studies in both rodent and human hearts confirmed a decrease in cardiac mitochondrial content and mitochondrial DNA (mtDNA) as a hallmark in the pathophysiology of heart failure. Mitochondrial biogenesis is regulated by several pathways, most of which rely on the coregulator of PPARδ, peroxisome proliferator-activated receptor γ coactivator 1α (PGC-1α) [59]. PGC-1α is regulated by miR-696 in skeletal muscle in response to physical activity, and mitochondria biogenesis and fatty acid oxidation were decreased following miR-696 overexpression (Figure 3) [60]. Cardiac-specific PGC-1α transgenic mice exhibit uncontrolled mitochondrial proliferation and biogenesis [61] through binding of PGC-1α to, and co-activation of, the nuclear respiratory factors (NRFs) 1 and 2, promoting the expression of mitochondrial proteins of the β-oxidation complexes (Table 1) [62].

The nucleus-encoded miR-181c causes electron transport chain complex IV remodeling through regulation of the mitochondrial cytochrome *c* oxidase subunit 1 (mt-COX1) at the translational level [63]. Moreover, overexpressing miR-181c in neonatal rat ventricular myocytes indirectly induced increased the mitochondrial cytochrome *c* oxidase subunit 2 (mt-COX2) mRNA and protein levels, resulting in higher mitochondrial respiration and reactive oxygen species generation (Figure 3, Table 1) [63]. In addition, miR-484 regulates mitochondrial fission and apoptosis in the heart via targeting of mitochondrial fission protein (Fis1), thereby disrupting maintenance of normal mitochondrial function (Figure 3) [64]. Finally,

insulin-regulated glucose transporter type 4 (GLUT4), an important player in glucose metabolism and uptake, was also shown to be indirectly increased upon miR-223 overexpression in cardiomyocytes, resulting in an increase in glucose uptake as an adaptive/homeostatic response to restore normal glucose uptake in the diabetic heart [65] (Table 1).

The above examples indicate a strong link between the hypoxic response and alterations in cardiac mitochondrial integrity and energy metabolism. It becomes clear that although hypoxia facilitates the metabolic shift from fatty acid utilization in the healthy myocardium towards increased reliance on glucose utilization in heart failure, it is accompanied by direct or indirect miRNA regulation, which serves to fine-tune the balance between fatty acid oxidation versus glucose oxidation and glycolysis.

Concluding remarks

Changes in cardiac tissue oxygenation remain a highly complex and controversial field of research into cardiovascular disease. A better understanding of the relationship between hypoxia and several processes key in the response of the heart to pathological stimuli can provide important insights towards targeted therapeutic approaches. Effects of hypoxia range from reactivation of the fetal gene program to regulation of the metabolic switch from predominant reliance on fatty acid utilization in the healthy myocardium toward increased reliance on glucose metabolism in heart failure, mitochondrial biogenesis, myocardial contractility, hypertrophic response, and extracellular matrix composition. Hypoxia on its own is able to induce signaling cascades that promote cardiac remodeling, and unraveling the underlying mechanisms is imperative in understanding what drives heart failure.

In this review we have discussed the role of hypoxia in cardiac miRNA biogenesis via the regulation of DICER, the central role of individual miRNAs in the regulation of hypoxia-related pathways and the direct involvement of miRNAs in mitochondrial homeostasis. Deciphering hypoxamiR function and regulation is central in our effort to understand cardiac physiology, and their ease of therapeutic targeting renders them attractive targets for the development of new interventions against cardiovascular disease.

Acknowledgments

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