FoxO1–miRNA interacting networks as potential targets for mitochondrial diseases

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Mitochondrial homeostasis is important for the health and well-being of organ systems and organisms. Mitochondrial dysfunction is known to be the cause and consequence of metabolic diseases, including obesity, diabetes, cancer, neurodegeneration, cerebrovascular, and cardiovascular disease. For cardiovascular tissue, which relies mostly on oxidative phosphorylation, the role of mitochondria is inevitable. Rather than being biomarkers of mitochondrial health, miRNAs are now known as bioregulators of this important feature. Recent studies have shown a close interaction between Forkhead box other 1 (FoxO1) transcription factors and miRNAs in the cardiovascular system. These interactions have also been shown to regulate mitochondrial homeostasis. In this review, I highlight how understanding FoxO1 and miRNA interacting networks could enable us to limit mitochondrial dysfunction and associated pathologies.

Introduction

Mitochondria generate energy required for the cell in the form of ATP [1]. The cardiovascular system is a major organ system that relies on mitochondrial-derived ATP for its energy requirements [2,3]. Thus, there is a need to understand how mitochondrial homeostasis is maintained in the cardiovascular system following metabolic or infection-induced stress. Rather than at the mitochondrial level, metabolic complications start at the cellular level, where transporters of glucose, lipids, amino acids, or ketone bodies allow the unregulated entry of substrates [4–7]. The metabolic homeostasis of a cell is disrupted when the amount of substrate molecules entering the cell exceeds the capacity of that cell to process them. The unlimited or surplus supply of fatty acid substrates to cardiovascular tissue results from an excess of CD36 and fatty acid binding protein 4 (FABP4) in the sarcolemma [4–9]. This surplus fatty acid delivery also downregulates glucose oxidation by upregulating pyruvate dehydrogenase kinase 4 (PDK4), which is a crucial kinase that inhibits pyruvate dehydrogenase (PDH) activity, as well as being a target of FoxO1. This increased lipid utilization leads to various cellular compartmental stresses, resulting in free radical generation and, thus, influencing membrane, cytosolic, nuclear, endoplasmic reticulum (ER), and mitochondrial function [8]. FoxO1, a crucial transcription factor in cardiovascular tissue, is involved not only in development, survival, and apoptosis but also in regulating cardiovascular lipid and glucose metabolism by increasing lipid uptake and utilization along with downregulating glucose utilization, resulting in metabolic stress, followed by mitochondrial instability and cardiovascular damage [10]. A recent focus on the role of nonprotein-coding RNAs [i.e., short and long noncoding (lnc)RNAs] in metabolic health research has gained momentum, other than their use as biomarkers [11–17]. In this review, I discuss how a transcription factor, such as FoxO1, and a ncRNA, such as miRNA, could interact with each other and influence each other's function, ultimately impacting mitochondrial health in cardiovascular tissue following metabolic stress.

FoxO1 and cardiovascular metabolism

FoxO1 has a strong influence on cellular metabolic homeostasis because of its ability to target a range of proteins, from those localized on the cellular periphery or membrane to those residing in cytosol, nucleus, ER, and mitochondria [8,10,18]. One of the

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major ground-breaking phases in FoxO1 research began when the role of the Forkhead transcription factors in regulating metabolism and their involvement in metabolic perturbations leading to cardiovascular complications were revealed [8,10,18,19]. An earlier study demonstrated the role of FoxO1 in upregulating inducible nitric oxide synthase (iNOS) followed by increased low-density lipoprotein (LDL) oxidation and endothelial NOS (eNOS) downregulation [20]. The authors showed that overexpression of FoxO1 mimics a hyperglycemic effect on vascular endothelium and also that hyperglycemia-induced endothelial dysfunction is mediated through FoxO1 [20]. This study was followed by another in which isolated cardiomyocytes incubated with excess glucocorticoids (1 μM) caused increased FoxO1 nuclear presence, accompanied by an increase in PDK4 induction, with increased PDH phosphorylation [10]. An increased PDH phosphorylation ultimately resulted in decreased enzyme activity of PDH and reduced glucose oxidation. This study was one of the early observations in isolated cardiac cells, in which the authors revealed the significance of FoxO1 in regulating cardiac glucose oxidation using glucocorticoid as a stressor agent [10]. FoxO1 was also shown to be involved in upregulating membrane CD36, a prominent fatty acid transporter in both palmitate-incubated cardiomyocytes (an *in vitro* model system) and intralipid infused rat hearts (an *in vivo* system) [8]. The study also revealed the role of FoxO1-induced iNOS in enhancing the presence of membrane-associated CD36. Enhanced CD36 at the membrane surface was followed by increased lipid uptake into cardiac cells. This ultimately led to the downregulation of mitochondrial oxidative capacity because of suppression of oxidative phosphorylation proteins (OXPHOS), an effect facilitated by CD36-delivered fatty acids. This was also accompanied by increased lipid storage in cardiac tissue [8]. These observations confirmed the role of FoxO1 in promoting excess lipid storage in cardiac tissue and also in disrupting mitochondrial homeostasis. In rodent models of streptozotocin (STZ) and diazoxide-induced hyperglycemia, FoxO1 caused a sequence of cardiac cellular events by mediating iNOS induction that led to cardiomyocyte death. iNOS induction was accompanied by glyceraldehyde 3-phosphate dehydrogenase (GAPDH) nitrosylation, poly (ADP-ribose) polymerase (PARP) activation, and apoptosis-inducing factor (AIF)-induced cell death, a death-signaling path that closely resembles parthanatos [21]. Given the relationship between AIF and oxidative phosphorylation proteins, it supports the possible role of FoxO1 in regulating mitochondrial oxidative phosphorylation proteins by two routes; (i) via a PARP–AIF-mediated path; or (ii) via a nutrient stress-mediated downregulation of OXPHOS proteins [21]. The above-mentioned studies also highlight the role of FoxO1 in regulating mitochondrial metabolism through both its transcriptional effect and FoxO1-induced iNOS-derived NO signaling [21]. Studies focused on the role of FoxO1 in endothelial cells demonstrated that FoxO1 ablation in endothelial cells has a protective phenotype in atherosclerosis mouse models [22]. Another study showed that, when the authors crossed constitutively active deacetylated FoxO1 with Ldl receptor $(Ldlr)^{-/-}$ mice, the offspring were prone to atherosclerosis characterized by increased intercellular adhesion molecule 1 (ICAM-1) and tumor necrosis factor (TNF)-α expression, with enhanced monocyte adhesion [23], demonstrating the significance of the post-translational modification of FoxO1.

The role of FoxO1 in regulating cardiovascular metabolism has been investigated in detail in not only cardiac or endothelial cell models, but also in whole-animal models and humans. In murine models of both genetic and high-fat diet-induced cardiomyopathy, FoxO1 depletion specifically in cardiac cells resulted in the preservation of cardiac function, insulin responsiveness, and decreased lipid accumulation [24]. Overexpression of constitutively active FoxO1 in endothelial cells resulted in increased arterial occlusion and resistance accompanied by cardiac failure and death [25]. Adipose tissue and endothelial cells from severely obese individuals had decreased FoxO1 phosphorylation because of insulin resistance. Administration of AS1842856, a FoxO1 inhibitor, in endothelial cells resulted in the restoration of insulin sensitivity [19]. In vascular endothelial cells, FoxO1–MYC signaling has a major role, in which FoxO1 acts as a negative regulator of MYC proteins, which have innate functions such as in glycolysis and maintaining the mitochondrial balance [26]. Restoration of MYC function in FoxO1-overexpressing endothelial cells stabilized glycolytic and mitochondrial functions accompanied by an endothelial proliferative function [26]. Together, these studies confirm the significance of FoxO1 in regulating metabolism in cardiac and endothelial cells in cardiovascular tissues and highlight the significance of maintaining FoxO1 homeostasis in the cardiovascular system.

Studies in vascular smooth muscle cells in the cardiovascular system suggest that FoxO1 has a unique function on individual cell types within the cardiovascular system. For example, FoxO1 acts as a mediator in thrombin-induced vascular smooth muscle cell proliferation. After FoxO1 knockdown using small interfering (si)RNA, the proliferation process was augmented, whereas the phosphoinositide 3-kinase (PI3K) inhibitor LY294002 prevented thrombin-mediated smooth muscle cell proliferation [27]. Another group suggested the unregulated proliferation of vascular smooth muscle cells as either a causative factor or a coexisting event in atherosclerosis [28]. Based on their observations, a crucial factor named 'Smooth Muscle and Endothelial Cell Enriched Migration/Differentiation-Associated Long non coding RNA' (SENCR) was highlighted as having a major role in this phenomenon, which the authors termed 'inappropriate proliferation'. Interestingly, SENCR was shown to regulate this proliferation process, and SENCR and FoxO1 had an inverse relationship as demonstrated by overexpression and knockdown studies [28]. Another study in a mouse model targeting smooth muscle cells with genetic ablation of phosphatase and tensin homolog (PTEN) resulted in arterial calcification, an event that was that was followed by Akt activation, FoxO1 inhibition, and Runx2 upregulation. This hypothesis was validated using *in vitro* and *in vivo* models. Thus these studies revealed a protective role for FoxO1 against metabolic diseases, such as atherosclerosis and stroke, specifically in vascular smooth muscle cells [29]. Thus, even though FoxO1 is an initiator of complications in cardiovascular tissues following metabolic stress, specifically in cardiac cells, it also has a protective function in vascular smooth muscle cells. This cell-specific role of FoxO1 makes it a unique regulator of cardiovascular function, which should be considered when developing drugs or molecules targeting FoxO1.

miRNAs: from biomarkers to bioregulators of cardiovascular mitochondrial health

miRNAs are small ncRNAs of 22 nucleotides or less. Over the past few years, they have been shown to be not only a biomarker in

cancer and other metabolic diseases, but also a major target in regulating cardiovascular metabolism, fibrosis, hypertrophy, and other cardiovascular complications [30–34]. Here, I focus on miRNAs that are known to regulate cardiovascular metabolism by targeting mitochondrial function and homeostasis [30–34].

In the H9c2 rat myoblast cell line, miR-1 acts an initiator of mitochondrial defects characterized by cytochrome-c release and apoptosis following hyperglycemic stress. Another study in neonatal rat ventricular myocytes demonstrated that the miRs-15b, 16, 195, and 424 could downregulate ADP ribosylation factor-like 2 (Arfl2), a crucial regulator of mitochondrial ATP generation, by modulating the ADP/ATP exchanger, adenine nucleotide transporter 1. As a result of miR-15b overexpression, Arfl2 expression decreased with decreased mitochondrial function and reduced ATP levels. This was supported by another study that reaffirmed miR-15b as a major regulator of mitochondrial health [35]. However it is unknown whether miR-15b-mediated Arfl2 signaling has a role following nutrient stress conditions [36]. miR-15b was upregulated in Sprague–Dawley rat hearts subjected to ischemia-reperfusion and also in *in vitro* experiments in which cardiomyocytes were subjected to hypoxia-reoxygenation resulting in increased cytochrome-c release to cytosol, caspase-3 activation, and apoptosis [35]. Biopsies of hearts from patients with insulin resistance showed an increase in miR-223. By overexpressing miR-223 in rat neonatal ventricular cardiomyocytes via adenoviral-mediated delivery, the authors found an increase in GLUT4 expression, an effect that was independent of insulin signaling or other survival kinase pathways but had a transcriptional effect of the miRNA on GLUT4 [37]. This study highlighted not only the role of miRNA in regulating glucose uptake in cardiac tissue, but also the potential of miRNAs in regulating GLUT4 expression independent of insulin signaling.

Free radical generation and mitochondria-mediated cell death was reduced when miR-201 was overexpressed in mouse embryonic fibroblasts under hypoxic conditions. This protective role of miR-210 was mediated by both the p53 and Akt signaling pathways [38]. A study using a mouse model of ischemia-reperfusion showed that miR-214 has a protective role in the heart, especially in calcium handling by myocytes during ischemic-reperfusion injury, by downregulating sodium/calcium exchanger 1 [39]. This role was confirmed using a mouse model with genetic deletion of miR-214 [39]. Another study showed that miR-484 inhibited mitochondrial fission and apoptosis by downregulating the mitochondrial fission protein Fis1 [40]. Cardiomyocyte mitochondrial fission is also regulated by mitofusin 1, which prevents fission and mitochondria-mediated apoptosis [40]. Studies in both isolated cardiomyocytes and *in vivo* models showed that mitofusin 1 was downregulated following apoptosis stimuli and miR-140 was identified as a major contributor to the downregulation of mitofusin 1 and apoptosis-induced injuries in cardiac tissue [41]. miR-361 has been shown to downregulate prohibitin, accompanied by an increase in mitochondrial fission and apoptosis. These findings were validated using cardiac-specific prohibitin-overexpressing transgenic mice and knockdown of miR-361 in cardiomyocytes, which offered protection from excessive fission and apoptosis [42]. Using a primary culture of rat neonatal ventricular myocytes, it was shown that miR-181c overexpression downregulated mitochondrial cyclooxygenase 1 (mtCOX1) proteins without influencing mtCOX1 mRNA; these effects preceded mitochondrial reactive oxygen species (ROS) generation and negatively affected mitochondrial function [43]. Thus, this study revealed the transcription-independent regulation of mitochondrial proteins by miRNAs. This observation was strengthened by studies from the same group in which they showed that overexpression of miR-181c in the heart led to an altered mitochondrial complex IV gene along with altered matrix calcium that rendered the cardiac tissue more prone to dysfunction and failure [43–45]. Cardiomyocyte-specific overexpression of miR-30c caused dilated cardiomyopathy resulting from downregulation of the mitochondrial OXPHOS complex, specifically complex III and IV proteins. Thus, the above-mentioned studies reveal the role of miRNAs in directly regulating mitochondrial inner membrane or matrix-inhabiting proteins that are involved with oxidative phosphorylation and ATP synthesis [46].

Upregulating miR-145 had beneficial effects on mitochondrial health. Its ability to prevent cell death via the mitochondrial pathway was demonstrated in ischemia-reperfusion models of mouse hearts and also in neonatal rat ventricular myocytes exposed to H_2O_2 stress [47]. The study also revealed that this protective role in mitochondrial resulted from inhibition of the proapoptotic protein, Bnip3 [47]. An inner mitochondrial membrane phosphate transporter protein, solute carrier family 25 member 3 (Slc25a3), which is a major contributor of phosphate ions and facilitates ATP generation at the mitochondrial level, was downregulated in hyperglycemic mice following 5 weeks of multiple STZ injections [48]. The downregulation of Slc25a3 expression and decrease in ATP generation following hyperglycemic stress were identified to be downstream effects of miR-141-mediated 3′-untranslated region (3′-UTR) binding of the *Slc25a3* gene [48]. This study also demonstrated the regulatory effect of miRNAs on mitochondrial interfibrillar proteins. In mouse primary cardiomyocytes, miR-24 offers protection against the ER-mediated intrinsic apoptosis pathway, a process that includes interaction of miR-24 with CCAAT-enhancer-binding protein homologous protein (CHOP) proteins and suppression of a CHOP-mediated induction of the pathway [49]. In a mouse model of ischemia–reperfusion, miR-2861 bound directly to the promoter and repressed adenine nucleotide translocase 1 mRNA and protein expression, thus rendering the cardiac tissue more prone to necrotic injury [50]. These studies demonstrate a direct effect of miRNAs on targets in the mitochondrial membrane or targets that could directly regulate membrane stability, impacting mitochondrial function and health in cardiovascular tissue [50]. Whether these are direct effects of the above-mentioned miRNAs or result from their interaction with transcription factors is yet to be investigated.

In terms of the role of miRNAs in regulating mitochondrial functions, a study in hepatic and muscle tissues showed miR-378 and miR-378* to be regulators of mitochondrial function. Studies based on mitochondrial fractions of liver and slow muscle fibers of wild-type and miR-378 and miR-378* knockout mice showed that following miR- 378 and miR-378* knockout, mitochondrial carbon dioxide and acid soluble metabolite production increased [51]. This effect was suggested to result from upregulation of carnitine-*O*-acetyltransferase (*CRAT*) and *MED13* (a component of the mediator of mitochondrial fatty acid metabolism) genes following miR- 378 and miR-378* knockout [51]. However, detail investigations are required to determine whether the same rela-

tionship between miR-378 and miR-378* and mitochondrial health is present in cardiovascular tissue following metabolic stress.

Independent of metabolic stress, the relevance of miR-378 and miR-378* was demonstrated from a developmental biology perspective in cardiovascular tissue. Cardiac tissue of aged mice (10-months old) exhibited increased miR-378 expression compared with tissue from younger mice (1-month old). This increase in miR-378 following aging was accompanied by decrease in insulin-like growth factor 1 (IGF-1) expression and signaling, as observed by Akt phosphorylation at Thr-308, an effect that was also validated in an *in vitro* model using rat cardiomyocytes and miR-378 mimics [12]. Reduced Akt phosphorylation also resulted in decreased phosphorylation of FoxO3a, ultimately leading to FoxO3a activation [12]. This effect was also perceived in a H_2O_2-induced oxidative stress model in rat cardiomyocytes. Use of anti-miRs for miR-378 resulted in nuclear exclusion of FoxO3a, revealing the role of miR-378 in promoting nuclear entry of FoxO3a [12]. Although it is unknown whether the same relationship exists between miR-378 and FoxO1, if it does exist, then further studies are required to determine whether, following metabolic stress, this positive regulation observed between miR-378 and FoxO3a is also maintained between miR-378 and FoxO1. Studies to identify the relationship between miR-378, FoxO1, and mitochondria health will reveal the role of the miR-378–FoxO1 interacting network in regulating cardiovascular mitochondrial health.

FoxO1–miRNA interacting networks in cardiovascular tissue

As a metabolic bifunctional switch, FoxO1 has the ability to bind to many co-transcription factors as well as other transcription factors leading to the activation or suppression of its target [8,10,18,19,21,52,53] through Forkhead responsive elements. There are numerous studies showing the direct and indirect regulation of FoxO1 by miRNAs, although there is less research highlighting the effect of miRNAs on FoxO1 and vice versa with respect to mitochondrial metabolism and health. There are only a few studies providing evidence that helps us understand the FoxO1–miR interacting network in cardiovascular tissue. Most of the emphasis herein has been on the mutual regulation of FoxO1 and miRNAs specifically in cardiovascular tissue (Fig. 1). Using a dual-luciferase reporter assay in human umbilical vein endothelial cells, FoxO1 was identified as the major target of miR-370, following miR-370 mimic transfection [54]. When overexpressed, miR-370 downregulated FoxO1 luciferase activity and enhanced angiogenesis, an effect that was normalized when a point mutation of the miR-370-binding 3′-UTR at the FoxO1 promoter was used [54]. When FoxO1 was overexpressed without the 3′-UTR to rescue miR-370-mediated binding and repression, FoxO1 was able to prevent angiogenesis [54]. miR-181b, which is normally downregulated under obese conditions, was upregulated in endothelial tissue surrounding fat when rescued using systemic intravenous delivery, and was able to normalize glucose homeostasis and reverse insulin resistance mediated by upregulated Akt and FoxO1 phosphorylation and downregulated FoxO1 activity [55]. However, this study questions how miRNAs could negatively regulate the post-translational modification of FoxO1 as seen in FoxO1 phosphorylation other than miRNAs regulating FoxO1 at 3′-UTR, thus affecting transcription? It is still unclear whether miR-181-b has any role in post-translational modifications, such as in increasing Akt and FoxO1 phosphorylation and/or influencing FoxO1 degradation by ubiquitination. A similar study in endothe-

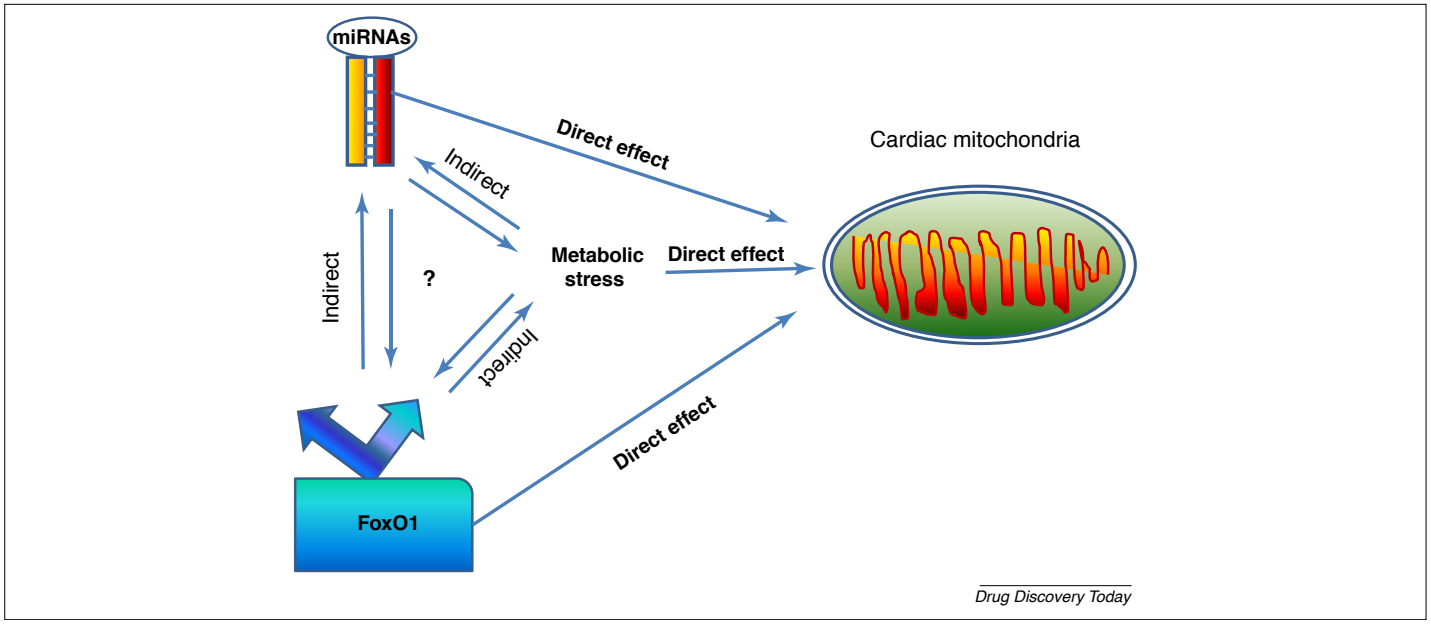

FIGURE 1

Forkhead box other 1 (FoxO1), miRNAs, and metabolic stress can not only act as both independent and direct regulators of mitochondrial health, but also regulate mitochondrial function by influencing each other. FoxO1 can influence miRNA expression and activity and vice versa. In addition, FoxO1 and miRNAs could directly regulate mitochondrial function by influencing certain gene and/or protein targets in mitochondria or indirectly by initiating metabolic stress and then metabolic stress-induced mitochondrial dysfunction. The reverse is also true, whereby metabolic stress could influence FoxO1 or miRNA expression first, which would then influence mitochondrial function.

lial cells demonstrated that, following high glucose stress, endothelial cell FoxO1 expression was upregulated and that of miR-181c was downregulated. These authors also showed that overexpressing miR-181c in the presence of high glucose stress suppressed FoxO1 expression because of its direct promoter-binding ability, which ultimately resulted in alleviation of nitration stress in endothelial cells [56]. These observations suggest either that high glucose-mediated stress is able to prevent the miR-181-c-mediated repression of FoxO1 by interfering with 3′-UTR or that high glucose stress directly influences this process by: (i) preventing the pre-miRNA from maturing into miR-181-c; or (ii) promoting miR-181c degradation. It is well known that nitration stress interferes with mitochondrial VDAC channels and destabilizes the mitochondrial membrane, with resultant ROS generation and the promotion of apoptosis. Thus, miR-181c could be a therapeutic target to regulate FoxO1-dependent and -independent mitochondrial membrane destabilization resulting from nitration stress. miR-331-5p was shown to influence glucose metabolism, activate mitochondrial function using the transforming growth factor m, (TGF)β1–Stat3–FoxO1 signaling network [57]. These effects were reversed by peroxisome proliferator-activated receptor (PPARγ) activation, resulting in the inhibition of TGFβ1–Stat3–FoxO1 signaling. Here, the role of FoxO1 is more of an intermediate regulator of miR-331-5p action but requires further investigation [57].

In rat models of ischemia–reperfusion, administration of urocortin 1 and 2 during the early stages of reperfusion restored cardiac function by differentially regulating miRNAs [58]. Urocortin 1 and 2 offer cardiac protection following ischemia–reperfusion by downregulating miR-139-3p. However, when cardiomyocytes were transfected with miR-139-3p by a lentivirus-mediated mechanism, FoxO1 mRNA expression was downregulated in these cardiac cells [58]. In contrast to the other studies mentioned here, this study questions whether there is any protective role for FoxO1 in ischemia–reperfusion injury. However, further research is required to determine whether there is a protective role for FoxO1 offers following ischemia–reperfusion and whether it involves mitochondrial regulation.

FoxO1 is inevitably involved in the development of cardiovascular tissue [18,53]. Recent studies highlight a possible strong interacting molecular or signaling network between FoxO proteins and miRNAs. Cardiac muscle-specific overexpression of miR-486 downregulated FoxO1 and PTEN [59]. A decrease in PTEN was associated with overactivation of PI3Kinase/Akt signaling [59]. Thus, it could also be possible that enhanced Akt phosphorylation results in Akt-mediated FoxO1 phosphorylation at sites including Thr 24, Ser 253, and Ser 316, which are specific to Akt, leading to FoxO1 expulsion from nucleus to cytosol and ubiquitination-mediated degradation. Thus, even though miR-486 could have a direct impact on FoxO1 by regulating its expression, it could also regulate FoxO1 post translationally through the PTEN–Akt pathway.

Work has also demonstrated that, following cancer in muscle cells, inflammatory cytokines could have a negative impact on miR-486 expression, specifically in the muscle tissue [60]. This reduced miR-486 expression was accompanied by an increase in the expression of FoxO1 and PTEN, an effect similar to that observed by other research groups under different pathological states. This study also replicated and confirmed the mechanism using an *in vitro* model system, where cultured media from tumor cells were used to trigger C2C12 myoblast cells, which caused the downregulation of miR-486 and Akt signaling, and an upregulation of PTEN and FoxO1 [60]. Thus, even though cancer is accompanied by an increased growth signaling activation, it could be that, unlike cancer or developing tumor tissue, the muscle tissue could have reduced growth or survival kinase activation. This would also explain the cachexia or muscle atrophy accompanying most cancers. Once FoxO1 is upregulated, it could promote the transcription of atrophy-related genes. Another study in vascular smooth muscles of diabetic db/db mice reported that miR-135a expression was increased. In this study, miR-135a was shown to have a proinflammatory effect that co-occurred with FoxO1 downregulation [61]. Unlike cardiac cells, FoxO1 in vascular smooth muscle cells tends to show anti-inflammatory effects [61]. However, further work is required to determine why FoxO1 shows differential effects in cardiac cells, endothelial cells, and smooth muscle cells.

Potential biphasic role of a FoxO1–miRNA interacting network: mitohormesis versus mitochondrial damage

Based earlier studies on cardiovascular tissue, mitohormesis, a phenomenon in which low amounts of ROS induction helps mitochondria to adapt to future stress, was thought to have a role in cardiac protection [62]. Low amounts of mitochondrial ROS resulting from statin administration were suggested to help counteract the assaults or to prepare cardiac mitochondria for future assaults, thus protecting cardiac tissue [62]. However, this protective effect was not evident in skeletal muscle mitochondria, which produce enormous amounts of ROS following statin administration, ultimately resulting in muscle damage [62]. Although the hypothesis that mitohormesis has a protective role in heart is justifiable, what makes the same drug (in this case a statin) act in a different manner in different tissues remains unknown.

Another study reported that the dephosphorylated state of ATPase inhibitory factor (IF1) is important for inhibiting the hydrolase activity of H+-ATP synthase, an effect that was reversed by protein kinase A-mediated inhibition [63]. This study advocated the role of IF1 in cardiac mitohormesis [63]. A recent study in muscle and liver tissues showed that low-level exposure to irradiation also triggered mitohormesis in these tissues, offering them protection from future radiation-mediated stress and damage [64]. Based this evidence, it might be that, in certain tissues, FoxO1 has either a positive or protective role, whereas, in other tissues, it has a detrimental role.

Thus, the questions remain: does a FoxO1–miRNA interacting network have a role in cardiovascular mitohormesis? Do FoxO1 and miRNA also act in a differential manner in different cell types. From the data reviewed here, FoxO1 appears to have unique, but different roles in endothelial cells, vascular smooth muscle cells, and cardiac cells, which are also influenced by metabolic stress. Whether a FoxO1–miRNA interacting network determines mitohormesis in cardiovascular tissue requires further clarification. Understanding tissue and/or cell-specific regulation of a FoxO1–miRNA interacting network under normal and metabolic stress-induced conditions will help us determine whether this network

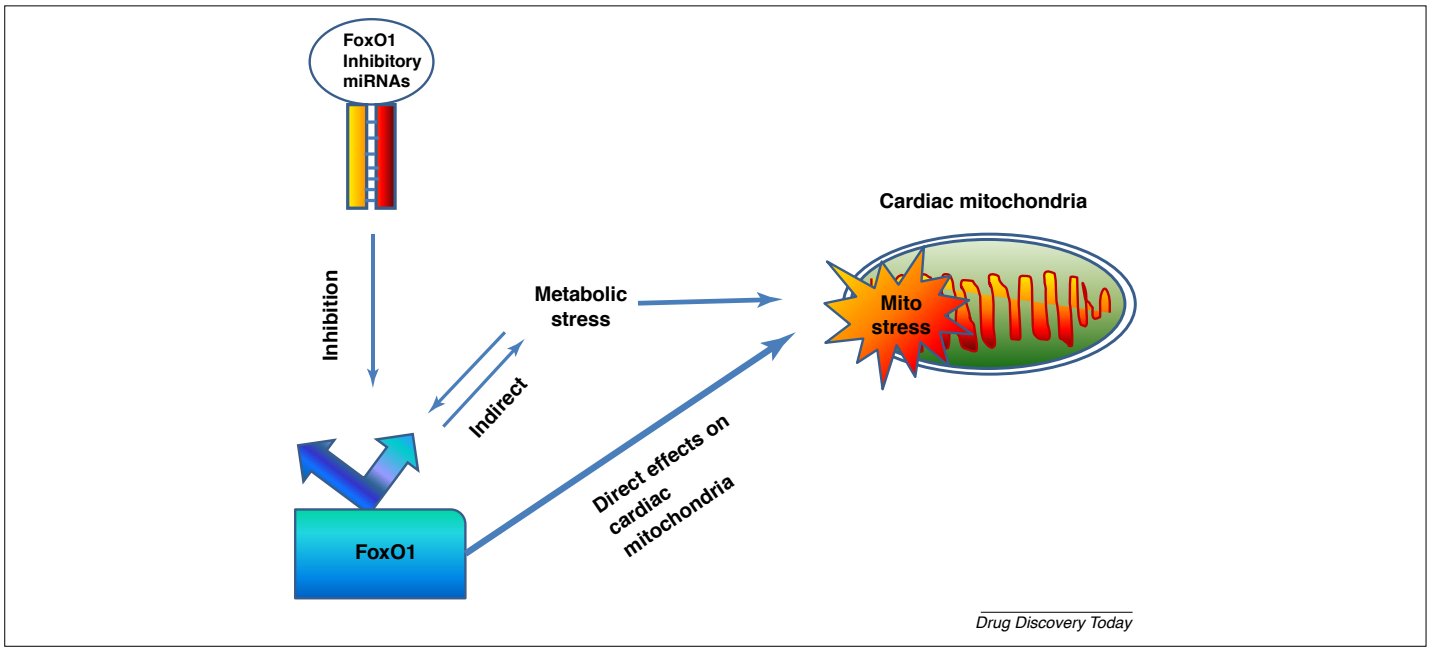

FIGURE 2

Conditions in which Forkhead box other 1 (FoxO1) has been shown to regulate mitochondrial function directly or indirectly through metabolic stress. Thus, it might be possible to normalize mitochondrial function in cardiovascular tissue via the targeted delivery of miRNAs capable of influencing FoxO1 activity or expression.

has a role in mitohormesis or triggers damage in cardiovascular tissue.

Is targeted delivery of miRs a cure for cardiac mitochondrial dysfunction?

Among the various FoxO1–miRNA interactions discussed above, some miRNAs in cardiovascular tissue or cell types are able to downregulate FoxO1 expression. In addition, they also tend to have a protective effect against increases in ROS generation, mitochondrial membrane destabilization, and apoptosis in cardiovascular tissue, effects that are FoxO1 regulated (Fig. 2 and Table 1) [10,18,21]. Based on these observations, an ideal solution for the metabolic stress induced mitochondrial dysfunction and associated cardiovascular complications would be to develop targeted drug delivery of protective miRNAs that are capable of inhibiting FoxO1. Novel technologies, such as nanospheres, nanovesicles, and or exosomes, could be potential vehicles for the delivery of protective miRNAs into cardiovascular tissue, which could then regulate FoxO1 activity following metabolic stress.

Concluding remarks and limitations

Following a pathological state in cardiovascular tissue, multiple miRNAs get turned either on or off. Protein–RNA interaction-determining tools and techniques could be helpful in understanding how FoxO1 and miRNAs behave following metabolic or any other inflammatory stress. Understanding the nature and consequences of FoxO1–miRNA interactions, and how they regulate mitochondrial health in cardiovascular tissue, will be useful. One limitation of research so far is that the focus has been on FoxO1–miRNA interactions only in cardiovascular tissue or cardiovascular cell types following metabolic stress rather than in any other cell types. In addition, how exactly FoxO1 and miRNAs interact is not clear. Many questions remain, including: (i) do they mutually repress each other's transcription? (ii) Are they involved in stabi-

TABLE 1

Influence of the differential expression of miRNA–FoxO1 interacting networks on cardiovascular health

miRNA	Regulation	Cell type	Effect on FoxO1	Consequence	Refs
miR-34a	Upregulation	Endothelial progenitor cells	Increased acetylation	↑ Angiogenesis	[65]
miR-182	Upregulation	Zebrafish lymphatic vasculature	Decreased expression	↑ Angiogenesis	[66]
miR-200c	Upregulation	Murine femoral arteries	Increased acetylation	↓ Endothelial function	[67]
miR-181c	Upregulation	Aortic endothelial cells	Decreased expression	↓ Endothelial nitrosative stress	[56]
miR-135a	Upregulation	Vascular smooth muscle cells	Decreased expression	↑ Vascular inflammation	[61]
miR-217	Upregulation	Coronary artery endothelial cells	Increased acetylation	↑ Endothelial senescence	[68]
miR-370	Upregulation	Human umbilical vein endothelial cells	Decreased expression	↑ Angiogenesis	[54]
miR-486	Downregulation	Cardiac muscle/myoblast cells	Increased expression	↓ Downregulation of growth signals	[59]
miR-378	Upregulation	Cardiac muscle/rat cardiomyocytes	Increased FoxO3a expression. No known effect on FoxO1	↓ Downregulation of growth signals	[12]
miR-143-3p	Downregulation	Cardiac tissue/H9C2 cells	Increased activation	↓ Downregulation of cardiac hypertrophy	[69]

lizing or destabilizing the transcriptional machinery? And (iii) do they bring about any epigenetic changes in their respective DNA-binding sites? Thus, more research is required to address these and other questions. It might be that, apart from FoxO1, miRNAs could also be forming an interacting network with other metabolically active transcription factors, such as PPARα, γ, and β, and also estrogen and androgen receptors. Finally, FoxO1 could also have a role in regulating the transcription function of other small ncRNAs (e.g., small nuclear RNA or piwi-interacting RNA) and lncRNAs, or could be involved in an interacting network with them at the transcription level. However, further research is required to investigate these hypotheses. Although it is unfortunate that the literature to date focuses on the significance of FoxO1 and miRNAs in regulating cardiovascular metabolism and mitochondrial health, understanding this FoxO1–miRNA interaction in detail could help preserve mitochondrial health, thus limiting cardiovascular complications resulting from metabolic stress.

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