Macrophages play a critical role throughout atherogenesis, from the initiating events leading to plaque formation to the development of plaque instability and thrombosis. Uptake of modified forms of low-density lipoprotein by macrophages within the arterial wall leads to their transition into arterial foam cells and the development of atherosclerotic plaques. In addition, as plaques mature and macrophage-derived foam cells continue to accumulate lipids, many undergo apoptosis, and the inability of other macrophages to efficiently remove apoptotic cells within atherosclerotic plaques leads to the formation of the necrotic core, a hallmark of plaque instability. Finally, macrophages produce and secrete several matrix metalloproteinases that can promote degradation of the fibrous cap and increase likelihood of plaque rupture.

Inhibition of miR-33 Increases Mitochondrial Biogenesis and Improves Mitochondrial Function

In this issue of Circulation Research, Karunakaran et al. confirm that pharmacological inhibition of mitochondrial function in macrophages reduces CE capacity, and further demonstrate that macrophages from mice lacking PGC1α, the master regulator of mitochondrial biogenesis, also have an impaired ability to efflux cholesterol. This work further demonstrates that treatment of macrophages with inhibitors of the small noncoding RNA microRNA-33 (miR-33) leads to increased mitochondrial respiration and ATP production. These findings are consistent with previous work establishing miR-33 as an important regulator of the availability of fuel sources used in mitochondrial respiration (CROT, HADHB, CPT1), as well as expression of PGC1α and AMP-activated protein kinase (AMPK), a kinase responsive to changes in cellular energy status and required for activation of PGC1α. miR-33 has previously been found to play an important role in many aspects of cellular metabolism in macrophages, and inhibition of miR-33 has been shown to promote RCT by derepression of ABCA1, and delay the development of atherosclerotic plaques in mice and nonhuman primates.

Enhanced CE After AntimiR-33 Therapy Requires Mitochondrial Respiration

In addition to confirming the regulation of PGC1α by miR-33, Karunakaran et al identify several other genes involved in mitochondrial function whose 3′ untranslated region is targeted by miR-33. The authors further demonstrate that the ability of antimiR-33 treatment to promote CE is dependent on mitochondrial respiration, as these effects are lost in cells with impaired mitochondrial function. These findings suggest that in addition to the induction of ABCA1, improvement in mitochondrial function may be involved in the beneficial effects of antimiR-33 therapy because of derepression of PGC1α and other mitochondrial genes. In addition to known and newly identified miR-33 targets involved in mitochondrial function, several other mitochondrial factors, including NRF1, one of the key nuclear transcription factors involved in induction of mitochondrial biogenesis, and TFAM, the primary mitochondrial transcription factor, were found to be induced on inhibition of miR-33. This suggests an overall induction of mitochondrial biogenesis, and consistent with this, the expression of mitochondrial ECT components, as well as mitochondrial DNA copy number, was found to be elevated in macrophages treated with antimir-33. Increasing PGC1α activity by treatment with activators of SIRT1 or AMPK is known to induce mitochondrial biogenesis in macrophages and enhance CE. These new findings suggest that inhibition
of miR-33 may promote RCT in a similar manner by increasing the energy available to carry out this process as well as the previously known derepression of ABC transport proteins.

Questions Remaining to Be Answered

Although the work of Karunakaran et al serves as a needed reminder of the important role that mitochondrial function plays in RCT and the development of atherosclerosis, and identifies miR-33 as a novel regulator of mitochondrial respiration in macrophages, several questions remain concerning the mechanisms by which miR-33 regulates mitochondrial function and the means by which this mediates RCT.

Although the work of Karunakaran et al demonstrates that inhibition of miR-33 in macrophages improves mitochondrial function and increases cellular ATP levels, it does not address the potential impact of other mitochondrial functions on RCT under these conditions. This question is important, as alterations in mitochondrial reactive oxygen species production and cholesterol transport have previously been shown to regulate RCT.6,7 Moreover, prior work has demonstrated that treatment of macrophages with oligomycin, as reported in this article, impairs cholesterol efflux. However, in this earlier work, experiments were performed at doses that do not deplete cellular ATP levels, and the effects of oligomycin on cholesterol efflux were proposed to have been due to alterations in mitochondrial cholesterol transport and cellular cholesterol homeostasis.4 Therefore, further exploration into how inhibition of miR-33 affects other mitochondrial functions, including the apoptotic signaling cascade, reactive oxygen species production, and cholesterol transport, will be essential for truly understanding the mechanisms by which antimiR-33–induced mitochondrial biogenesis affects RCT and cardiovascular disease (CVD).

Previous work has established that PGC1α is regulated by miR-33,9 and these findings are confirmed by the work of Karunakaran et al, who also identified additional, novel, mitochondrial targets of miR-33. However, this work fails to demonstrate how targeting of these novel mitochondrial genes affects RCT. The authors do show that loss of PDK4 partially abrogates the effects of antimiR-33 therapy on RCT, while responsiveness was not altered in SLC25a25−/− mice. However, although these findings indicate that PDK4 is involved in the effects of miR-33 on mitochondrial function and RCT, this does not indicate a direct role of miR-33 in targeting PDK4 under these conditions because PGC1α is a known regulator of PDK4. Indeed the complete repression of the ability of anti-miR-33 treatment to induce RCT in mice lacking PGC1α indicates its critical role in these effects.

In addition to transcriptional regulation, PGC1α is known to be regulated by post-transcriptional mechanisms including acetylation and phosphorylation. Indeed phosphorylation of PGC1α by AMPK is critical for its ability to induce mitochondrial biogenesis.10 As the activity of AMPK is regulated in response to changes in cellular AMP/ATP ratio, its function is inexorably linked to the cellular energetic state. In response to ATP depletion, AMPK mediates several critical changes in signaling pathways to maintain and restore cellular energy pools including regulation of lipid and glucose metabolism, as well as induction of mitochondrial biogenesis, through activation of PGC1α.11 In addition, AMPK has been shown to be involved in regulation of RCT and compounds capable of activating AMPK, including AICAR, metformin, and resveratrol, have been shown to improve RCT and reduce the development of atherosclerotic plaques.12,13,14 Despite previous work identifying AMPK as a target of miR-33,6 the work of Karunakaran et al fails to address the impact of miR-33 inhibition on the expression and activity of AMPK or its capacity to activate PGC1α. This could have important implications considering the central role of AMPK in the monitoring and maintenance of cellular energy demands.

Central Role of miR-33 in Regulation of Cellular Metabolic Function

Recently, miRNAs have garnered a great deal of interest as potential therapeutic targets, in part because of their ability to regulate many different targets including multiple genes within the same or related pathways, leading to mRNA degradation or impaired translation. An excellent example of this is miR-33, which has previously been demonstrated to target critical genes involved in glucose (IRS2, PGC1α, G6PC) and cholesterol metabolism (ABCA1, ABCG1), fatty acid oxidation (CROT, CPT1α, HADHB), and bile acid synthesis (CYP7A1).9,11,12,22 In addition to this, the work of Karunakaran et al demonstrates an important role of miR-33 in the regulation of mitochondrial biogenesis and aerobic respiration in macrophages.

Efflux of cholesterol through ABCA1 and ABCG1 requires ATP and depletion of cellular ATP pools can impair cholesterol efflux capacity. In response to ATP depletion, AMPK is activated leading to several cellular changes, including activation of PGC1α. Once activated, PGC1α promotes the transcription of key genes involved in mitochondrial biogenesis, including transcription factors responsible for the induction of nuclear-encoded mitochondrial genes, NRF1 and NRF2, and TFAM, the factor responsible for mediating mitochondrial DNA synthesis and transcription of mitochondrial genes. This in turn leads to increased mitochondrial mass, enhanced mitochondrial respiration, and increased ATP production capacity.

In this issue of Circulation Research, Karunakaran et al demonstrate an important role of miR-33 in the regulation of mitochondrial biogenesis and respiration and argue that this may be involved in the ability of miR-33 to impair RCT. Consistent with this, miR-33 has previously been shown to regulate PGC1α, the master regulator of mitochondrial biogenesis, and the cellular energy sensing kinase, AMPK. In addition, miR-33 is known to influence mitochondrial fuel source availability by targeting genes involved in fatty acid metabolism. The current work identifies additional mitochondrial genes that may be targeted by miR-33 and shows that the ability of antimiR-33 treatment to improve cholesterol efflux requires functional mitochondria indicating that induction of mitochondrial biogenesis, in addition to the direct stimulation of ABCA1 and ABCG1, may be involved in the ability of antimiR-33 to improve RCT and reduce atherosclerotic plaque burden (Figure).

Regulation of Mitochondrial Function by miR-33: Conclusions and Broader Implications

In addition to the known role of mitochondrial dysfunction in promoting CVD, impaired mitochondrial function has also...
Figure. Proposed model of how microRNA-33 (miR-33) regulates mitochondrial energy production and cholesterol efflux. High rates of cholesterol efflux through cholesterol transporters ABCA1 and ABCG1, such as those observed in macrophage-derived foam cells can deplete cellular ATP stores. In response to ATP depletion, AMP-activated protein kinase (AMPK) becomes activated initiating several cellular changes necessary to restore cellular energetic balance. One of these changes involves the activation of PGC1α and stimulation of mitochondrial biogenesis. This results in numerous improvements in mitochondrial function, including increased respiration capacity and ATP production, thus restoring cellular energy stores necessary for continued removal of intracellular cholesterol. In this study, Karunakaran et al demonstrate that miR-33 plays an important role in regulation of mitochondrial biogenesis and function through targeting of PGC1α and other mitochondrial genes. Together with earlier work these findings demonstrate the central role of miR-33 in regulating this energetic pathway by direct repression of ABCA1 and ABCG1 (only in rodents), as well as targeting critical genes involved in cellular energy sensing (AMPK), mitochondrial biogenesis, and mitochondrial fatty acid oxidation (CROT, CPT1a, HADHB). This figure was prepared using the Servier Medical Art resources (http://servier.com). HDL indicates high-density lipoprotein; miR-33, microRNA-33; and ROS, reactive oxygen species.

been implicated in numerous other disease states associated with aging and metabolic function. These include muscle insulin resistance and atrophy, multiple neurodegenerative disorders, and type II diabetes mellitus. Interestingly, one of the most commonly prescribed drugs for treatment of diabetes mellitus, metformin, is thought to function primarily through activation of AMPK. This suggests that derepression of AMPK and PGC1α by inhibition of miR-33 may work in concert with metformin treatment to improve mitochondrial function and delay the progression of diabetes mellitus and CVD. However, reports of increased liver TG accumulation, elevated body weight and impaired glucose homeostasis in mice lacking miR-3331 indicate the potentially complicated role of miR-33 in metabolic dysfunction and demonstrate the need to more fully understand the role of miR-33 in different metabolic tissues. Overall, the findings of Karunakaran et al demonstrate an important role of miR-33 in the regulation of mitochondrial biogenesis and mitochondrial ATP production. They further explore the role of this in the regulation of macrophage cholesterol efflux, demonstrating that this may play an important role in the ability of antimiR-33 therapy to prevent CVD and further suggesting that inhibition of miR-33 may provide beneficial effects on other diseases associated with mitochondrial dysfunction.

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Disclosures
None.

References


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