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Review

MicroRNAs as regulators of mitochondrial function: Role in cancer suppression☆

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ABSTRACT

Background: Mitochondria, essential to the cell homeostasis maintenance, are central to the intrinsic apoptotic pathway and their dysfunction is associated with multiple diseases. Recent research documents that microRNAs (miRNAs) regulate important signalling pathways in mitochondria, and many of these miRNAs are deregulated in various diseases including cancers.
Scope of review: In this review, we summarise the role of miRNAs in the regulation of the mitochondrial bioenergetics/function, and discuss the role of miRNAs modulating the various metabolic pathways resulting in tumour suppression and their possible therapeutic applications.
Major conclusions: MiRNAs have recently emerged as key regulators of metabolism and can affect mitochondria by modulating mitochondrial proteins coded by nuclear genes. They were also found in mitochondria. Reprogramming of the energy metabolism has been postulated as a major feature of cancer. Modulation of miRNAs levels may provide a new therapeutic approach for the treatment of mitochondria-related pathologies, including neoplastic diseases.
General significance: The elucidation of the role of miRNAs in the regulation of mitochondrial activity/bioenergetics will deepen our understanding of the molecular aspects of various aspects of cell biology associated with the genesis and progression of neoplastic diseases. Eventually, this knowledge may promote the development of innovative pharmacological interventions. This article is part of a Special Issue entitled Frontiers of Mitochondrial Research.

1. Introduction

MicroRNAs (miRNAs, MiRs) are endogenous 20–25 nucleotide-long non-coding RNAs that participate in numerous physiological and pathological processes. They are genome-encoded and negatively regulate gene expression at a post-transcriptional level. Single miRNAs can have multiple target sites in the 3′ untranslated regions (UTRs) of particular mRNAs, therefore causing their repression. Furthermore, mRNAs are predicted to be targets of many distinct miRNAs, suggesting that different miRNAs might act in a concerted manner to regulate mRNA translation and turnover [1]. Certain miRNAs have also been shown to affect multiple targets in linear pathways or interconnected nodes in regulatory networks, thereby exerting a larger cumulative effect [2]. MiRNAs have been found to substantially contribute to several type of regulatory circuits [3,4]: miRNAs can mediate or modulate signals, as well as suppress or amplify signals by participating in negative or positive feedback loops, respectively. MiRNAs' functions under normal physiological conditions might be integrated into multi-layered control circuits ensuring proper development and homeostasis; dysregulation of miRNA expression or function in response to intrinsic factors (genetic or epigenetic) or extrinsic factors (environmental cues or

Abbreviations: ACL, ATP citrate lyase; AGO, Argonaut; ARL2, ADP-ribosylation factor-like 2; BH3, Bcl-2 homology-3; CAT, catalase; COX IV, cytochrome c oxidase IV; CI, complex I; CII, complex II; CIV, complex IV; CPT, camptothecin; DGCR8, DiGeorge syndrome critical region 8; Drp-1, dynamin-related protein-1; FOXJ3, Forkhead box J3; FOXO1, Forhead box-O class 1; GLS, glutaminase; GPD, glycerol-3-phosphate dehydrogenase; HIF, hypoxia-inducible factor; IRS1, insulin receptor substrate-1; KSRP, KH-type splicing regulatory protein; LDHA, lactate dehydrogenase A; MiR/miRNA, microRNA; MM, malignant mesothelioma; MOM, mitochondrial outer membrane; mtDNA, mitochondrial DNA; NOX, NADPH oxidase; OXPHOS, oxidative phosphorylation; PCK1, phophoenolpyruvate carboxykinase; PDH, pyruvate dehydrogenase; PGC-1β, peroxisome proliferator-activated receptor γ co-activator-1; PHD, prolyl 4-hydroxylase; PI3K, phosphoinositol-3 kinase; PTP, permeability transition pore; RC, reductive carboxylation; RISC, RNA-induced silencing complex; RNAi, RNA interference; ROS, reactive oxygen species; snRNP, small nuclear ribonucleic particle; SOD2, superoxide dismutase-2; TCA, tricarboxylic acid; TFAM, mitochondrial transcriptional factor A; Txnrd2, thioredoxin reductase-2; usnRNA, uridylate-rich small nuclear RNAs; UTR, 3′untranslated region; $\Delta\Psi_{m,i}$, mitochondrial inner trans-membrane potential

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stress) may contribute to aberrant gene expression patterns underlying abnormal developmental patterning.

MiRNAs have recently emerged as key regulators of metabolism [1] and can affect mitochondria by modulating mitochondrial proteins coded by nuclear genes. MiRNAs have been found in mitochondria [5,6], and may contribute to the mitochondrial (dys)function [7]. Mitochondrial function is fundamental to metabolic homeostasis. In addition to converting the incoming nutrients into energy in the form of ATP, mitochondria generate intermediates for biosynthesis and reactive oxygen species (ROS) that serve as a secondary messenger to mediate signal transduction and metabolism. Alterations of mitochondrial function, dynamics, and biogenesis have been observed in various metabolic disorders, including aging, obesity, diabetes, and cancer.

Cancer is a disease where cells have lost their normal checks of cell proliferation. Intrinsic and extrinsic molecular mechanisms converge to alter cellular metabolism and provide support for rapid ATP generation to maintain the energy status, increased biosynthesis of macromolecules, and maintenance of the appropriate redox status [8]. The best characterised metabolic phenotype that distinguishes cancer from normal cells is glycolysis (Warburg effect). Cancer cells metabolise glucose to lactate under aerobic conditions, despite the fact that this metabolic pathway is much less energy-efficient compared to oxidative phosphorylation (OXPHOS). Alterations in oncogenes and tumour-suppressor genes are involved in the metabolic switch of cancer cells to aerobic glycolysis, increased glutaminolysis, and fatty acid biosynthesis. The altered metabolism of tumour cells may be a potential means to evade programmed cell death in order to favour survival and growth. MiRNAs mediate fine-tuning of genes involved directly or indirectly in cancer metabolism. Therefore, the modulation of the level of miRNAs may provide a new therapeutic approach to cancer treatment. In this review we discuss the regulatory role of miRNAs in controlling mitochondrial signalling pathways. We also consider the role of metabolic-related miRNA in tumour suppression and their therapeutic potential in cancer treatment.

2. Biogenesis and function of MiRNAs

MiRNAs are a class of short non-coding RNAs with post-transcriptional regulatory functions. They serve as 'master regulators' controlling the activity of multiple genes. Gene coding for MiRNAs are scattered in all chromosomes in humans except for the Y chromosome. Approximately 50% of known miRNAs are found in clusters 1, 3 and 26, and they are transcribed as polycistronic primary transcripts [9]. The miRNAs in a given cluster are often related to each other, suggesting that the gene cluster is a result of gene duplication. A miRNA gene cluster also often contains unrelated miRNAs. Most miRNA-coding genes are located in intergenic regions, but they are also found within exonic or intronic regions in either sense or antisense orientation [10].

The biogenesis of miRNAs is controlled by two RNase-dependent processing steps that convert a long primary transcript into a mature miRNA. First, primary miRNAs (pri-miRNAs) are processed by the Drosha-containing complex, i.e. the RNase III-like enzyme and DGCR8 (DiGeorge syndrome critical region gene 8), to stem-loop pre-miRNAs that are then further processed by the second RNase, Dicer, to short double-strand duplexes. Eventually, one of the functional strands in the resulting duplex is preserved, being integrated in the RNA-induced silencing complex (RISC) of proteins, and acts as a 'guide' strand for specific recognition. A number of RNA-binding proteins, such as hnRNPs (heterogeneous nuclear ribonucleoproteins) A1, Lin28, Smad proteins and the KSRP protein (KH-type splicing regulatory protein) have been shown to positively or negatively regulate miRNA production [reviewed in 11]. Drosha itself can regulate the level of the 'microprocessor complex' by cleaving hairpins in the 3′-UTR and the coding region of the DGCR8 mRNA, whereby destabilising the mature transcript and leading to a decrease in the DGCR8 protein [12,13]. This suggests that a balance between the levels of the microprocessor and its regulator proteins is essential for the physiological homeostasis.

An open question remains regarding miRNA biogenesis and its subcellular localisation and transport. Nucleocytoplasmic transport (especially export) is critical in the expression and functions of RNAs [14,15]. It is possible that this process is similar to that of mRNAs. Thus, pre-mRNAs are retained in the nucleus until splicing is successfully carried out, so that only correctly processed mRNAs can pass the 'quality control' and become available for cytoplasmic translation [16,17]. Biogenesis of the uridylate-rich small nuclear RNAs (UsnRNAs) is also closely linked to the nucleocytoplasmic transport. The UsnRNAs, with the exception of U6 and U6atac, must first be exported to the cytoplasm where the assembly of small nuclear ribonucleic particles (snRNP) is initiated [18,19]. Following modifications and core assembly, the UsnRNAs are re-imported to the nucleus to complete snRNP assembly and to participate in pre-mRNA splicing [20,21]. Studies of the localisation and transport of miRNAs are likely to reveal important aspects of miRNA expression and function.

Mature miRNAs associate with Argonaute (AGO) proteins to form the core of the RISC, which is the basis for the subsequent RNA interference (RNAi). RNAi occurs upon pairing of one of the two miRNA strands, associated with an AGO protein, with target sites in an mRNA, thereby affecting its stability/translation [22,23]. Mammalian cells contain four AGO proteins (AGO1–4), which have been shown to function in translational repression [24], but only AGO2 can catalyse the cleavage of the target transcript [25]. Furthermore, knock-down and knock-out AGO2 experiments in human cells and in mice, respectively, suggest that this protein has specific functions that may not be complemented by the other three AGO proteins. Initially, mature miRNAs and AGO2 were believed to accumulate and function exclusively in the cytosol and/or in unstructured cytosolic foci, such as the P-bodies and stress granules [26,27]. However, more recent evidence shows that they can also localise to and function within different cellular compartments. To date, miRNAs and AGO2 have been found to localise to the nucleus [28–30] and to multi-vesicular bodies [31]. Interestingly, ~90% of extracellular MiRs are packaged with (lipo)proteins (i.e. AGO2, high-density lipoprotein, RNA-binding proteins) and ~10% are wrapped in small membranous particles (i.e. exosomes, microvesicles, and apoptotic bodies). It is believed that these extracellular miRNAs mediate cell-to-cell communications [32].

MiRNAs are conserved among the species, expressed in different tissues and cell types and involved in almost every biological process, including cell cycle, growth, apoptosis, differentiation and stress response, and exerting a finely tuned regulation of gene expression by targeting multiple molecules. As a consequence of the widespread range of processes they are able to modulate, it is not surprising that miRNA deregulation is a hallmark of several pathological conditions, including cancer [33]. Recent studies have shown that miRNAs control different aspects of energy metabolism including insulin production and signalling, glucose transport and metabolism, or lipid homeostasis [1,34]. Mitochondrial function is fundamental to metabolic homeostasis. Alterations of mitochondrial function are related to a variety of pathological process and diseases.

3. MiRNAs in mitochondria

The regulation of mitochondrial function is critically determined by proteins encoded by both nuclear and mitochondrial genomes. Replication and transcription of mitochondrial (mt) DNA is initiated from a small non-coding region, the D-loop, and is regulated by nuclear-encoded proteins that are post-translationally imported into mitochondria. The transcription and translation of mtDNA as well as the processing of mitochondrial transcripts requires several types of non-coding RNAs, which can be either mitochondrially encoded or transcribed within the nucleus and subsequently localised to mitochondria. Recent studies have reported that certain miRNAs localise to and function in

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