review

MitomiRs: their roles in mitochondria and importance in cancer cell metabolism

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Radiol Oncol 2021; 55(4): 379-392.

Received 10 August 2021
Accepted 28 September 2021

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Disclosure: No potential conflicts of interest were disclosed.

Background. MicroRNAs (miRNAs) are short non-coding RNAs that play important roles in almost all biological pathways. They regulate post-transcriptional gene expression by binding to the 3'untranslated region (3'UTR) of messenger RNAs (mRNAs). MitomiRs are miRNAs of nuclear or mitochondrial origin that are localized in mitochondria and have a crucial role in regulation of mitochondrial function and metabolism. In eukaryotes, mitochondria are the major sites of oxidative metabolism of sugars, lipids, amino acids, and other bio-macromolecules. They are also the main sites of adenosine triphosphate (ATP) production.

Conclusions. In the review, we discuss the role of mitomiRs in mitochondria and introduce currently well studied mitomiRs, their target genes and functions. We also discuss their role in cancer initiation and progression through the regulation of mRNA expression in mitochondria. MitomiRs directly target key molecules such as transporters or enzymes in cell metabolism and regulate several oncogenic signaling pathways. They also play an important role in the Warburg effect, which is vital for cancer cells to maintain their proliferative potential. In addition, we discuss how they indirectly upregulate hexokinase 2 (HK2), an enzyme involved in glucose phosphorylation, and thus may affect energy metabolism in breast cancer cells. In tumor tissues such as breast cancer and head and neck tumors, the expression of one of the mitomiRs (miR-210) correlates with hypoxia gene signatures, suggesting a direct link between mitomiR expression and hypoxia in cancer. The miR-17/92 cluster has been shown to act as a key factor in metabolic reprogramming of tumors by regulating glycolytic and mitochondrial metabolism. This cluster is deregulated in B-cell lymphomas, B-cell chronic lymphocytic leukemia, acute myeloid leukemia, and T-cell lymphomas, and is particularly overexpressed in several other cancers. Based on the current knowledge, we can conclude that there is a large number of miRNAs present in mitochondria, termed mitomiR, and that they are important regulators of mitochondrial function. Therefore, mitomiRs are important players in the metabolism of cancer cells, which need to be further investigated in order to develop a potential new therapies for cancer.

Key words: microRNAs; mitomiR; mitochondria; cancer; cancer cell metabolism

Introduction

MicroRNAs (miRNAs) are short non-coding RNAs (ncRNAs) of ~18-25 nucleotides that are present in all eukaryotic cells and play important roles in almost all biological signaling pathways.[1–4] Since the discovery of the first miRNA (lin-4) in *C. elegans*[5], approximately 2000 miRNAs have been annotated in the human genome.[6] Data from genomic studies show that most miRNAs are highly conserved, making them very interesting targets for studying various disease states.[7] They regulate post-transcriptional gene expression by binding to the 3'UTR of messenger RNAs.[8–14] A single miRNA

can regulate many mRNA targets, and conversely, a single mRNA target can be regulated by many miRNAs.[15–17] Therefore, by regulating these fundamental target genes, miRNAs have been implicated in signaling pathways to modulate a large set of important biological processes such as cell proliferation[12], metastasis[18], apoptosis[19], senescence[12], differentiation[20], autophagy[21], and immune response[22]. Moreover, miRNAs have been found to be dysregulated in many pathological conditions, such as neurodegenerative diseases[23], cardiovascular diseases[24], and cancer.[25–28]

More recently, miRNAs have been found to be specifically present in mitochondria. These mitochondrial miRNAs were named "mitomiR".[7,29–32] Most of them have a nuclear origin, but some mitomiRs originate from mRNA molecules derived from the mitochondrial genome. The association of mitomiRs with mitochondria is species- and cell type-specific.[7,33] They have been found in mitochondria in various tissues and cells and are thought to have different thermodynamic properties than miRNAs.[7,34] Mitochondria have a discrete and unique pool of mitomiRs, which has been demonstrated with various experiments.[29]

For the first time, in 2011, Barrey and co-workers demonstrated the presence of pre-miRNAs (precursor-miRNAs) in mitochondria and postulated that some pre-miRNA sequences could be processed into mature miRNAs that could immediately become active on mitochondrial transcripts or exported to the cytosol to disrupt genomic mRNA.[35] Barrey's group screened for 742 miRNAs using qRT-PCR and showed that 243 miRNAs had significant expression in mitochondrial RNA samples isolated from human myotubes by *in situ* hybridization. This study was the first to provide evidence that pre-miRNAs can be localized in mitochondria. Subsequently, a number of studies have identified "signatures" of miRNAs localized to mitochondria through various experimental approaches. Mercer *et al.*[15] examined the human mitochondrial transcriptome and demonstrated that 3 miRNAs (miR-146a, miR-103, and miR-16) have quite high expression in the intermembrane region compared to the matrix. Latronico and Condorelli[36] found 15 nuclear-encoded miRNAs in mitochondria isolated from rat liver, 20 miRNAs from mouse liver mitochondria, and 13 miRNAs from HeLa cells (isolated from human cervical cancer) by microarray. Some other groups identified novel mitomiRs from HEK293 cells (isolated from human embryonic kidneys)[37], 143B cells (isolated from human bone marrow)[38], mouse heart[39] and HeLa cells.[37,40]

MitomiRs have been shown to be important regulators of mitochondrial function.[35,38,41] The regulation of mitochondria by mitomiRs influences the development of many diseases caused by mitochondrial dysfunction, which is responsible for the pathophysiology of numerous diseases, such as cardiovascular and neurodegenerative diseases, diabetes, obesity, and cancer.[42]

In the first part of this review article, we describe the biosynthesis of mitomiRs and the transport mechanisms from mitomiRs to mitochondria. The next part is dedicated to the role of these small molecules in mitochondria and the presentation of some important mitomiRs, their target genes and functions. In the last part of the review, we discuss the functions of mitomiRs in cancer cell metabolism and introduced mitomiRs in the context of cancer.

Biosynthesis of miRNA/mitomiRs

Most miRNAs/mitomiRs are produced via the canonical biosynthetic pathway, which involves transcription by RNA polymerase II (Pol II) to produce a primary transcript (pri-miRNA/mitomiR). The primary transcript is first cleaved in the nucleus by the nuclear heterodimer Drosha/DGCR8 (DiGeorge syndrome chromosomal region 8), which cleaves the pri-miRNA/mitomiR and produces a pre-miRNA/mitomiR with a hairpin structure that is much more stable than the pri-miRNA/mitomiR due to its characteristic hairpin loop structure.[43] Exportin 5 (EXP5) and GTP-binding nuclear protein (RANGTP) then form a transport machinery to export the pre-miRNA from the nucleus to the cytoplasm. After export to the cytoplasm, the pre-miRNA/mitomiR is further cleaved by the enzyme Dicer to form a double-stranded RNA (dsRNA) duplex (Figure 1). Only a single strand of the dsRNA duplex forms the mature miRNA/mitomiR and is incorporated into the RNA-induced silencing complex (RISC), which directs the binding of Argonaute (AGO) proteins in the RISC to the 3'UTR of the target mRNA to either repress protein translation or promote mRNA degradation.[43–45] After incorporation into RISC, mature miRNA/mitomiRs are transported into mitochondria, back to nucleus by importin 8 (IPO-8) or extracellular environment (Figure 1).[46,47]

In addition to the canonical miRNAs/mitomiRs biosynthesis pathway, there are also non-canonical, Drosha/DGCR8-independent and Dicer-independent biosynthesis pathways. Prominent

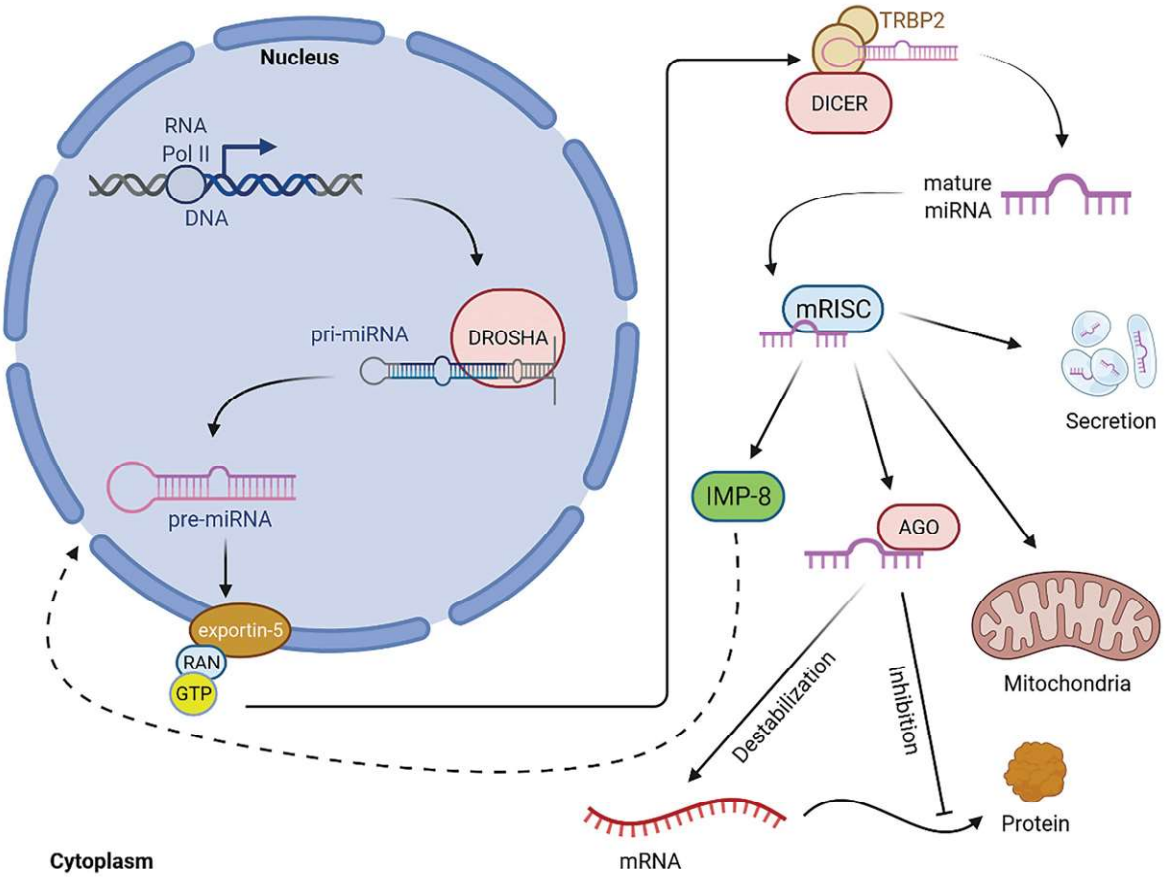

FIGURE 1. Canonical biosynthesis of miRNAs/mitomiRs (adopted from [29,43,45]). Mature miRNA can be transported into any part of the cell; but miRNA/mitomiR regulation is possible only after incorporation into RISC. (AGO2 = argonaute 2; DGCR8 = DiGeorge critical region 8; EXP5 = exportin 5; GTP = guanosine triphosphate; IMP8 = importin 8; mRISC = RNA induced silencing complex loaded with mature miRNA; POLII = DNA polymerase II; RANGTP = binding nuclear protein RAN; RISC = RNA-induced silencing complex; TRBP2 = RISC-loading complex subunit TRBP2).

classes of Drosha/DGCR8-independent miRNAs/mitomiRs are the "mirtrons" derived from introns that, once spliced, function as pre-miRNAs and thus do not require cleavage by Drosha/DGCR8 and can be immediately exported to the cytoplasm for processing by Dicer. MiRNAs/mitomiRs can also be processed from hairpins generated directly by Pol II at specific transcription start sites. These pre-miRNAs are capped and exported via the exportin 1 (EXP1) pathway. The Dicer-independent miRNAs/mitomiRs biosynthesis pathway involves the unusually short hairpin of miR-451, which is directly cleaved by argounaute 2 (AGO2).[45]

MitomiRs transport to mitochondria

The discovery of mitomiRs raised the question of elucidating the underlying molecular mechanisms of their transport into mitochondria. Due to their size and charged nature, mitomiRs are unlikely to cross membranes under their own power. The molecular mechanisms of mitomiR transport into mitochondria may vary between species and are not well understood.[29]

Some proposals have been published on AGO2 as a potential mitomiR import protein.[7,29,48] Due to its RNA-binding ability and dual localization in the cytosol and mitochondria, AGO2 might be involved in the trafficking of mitomiRs.[7] Shepherd *et al.*[49] showed that the exoribonuclease polyribonucleotide nucleotidyltransferase (PNPT1/ PNPase) has a major role in the import of mitomiRs. Therefore, PNPase could be part of an alternative, AGO2-independent, uptake pathway of mitochondrial miRNA. Furthermore, a possible mechanism could involve the voltage-dependent anion-selective channel protein (VDAC).[34] Several studies have suggested that the instability of RISC in the

cytoplasm promotes miRNA translocation to mitochondria, but the molecular components that facilitate this translocation process are not fully understood. Furthermore, the concept that mammalian mitochondria can import cytosolic ncRNAs may facilitate research in another exciting area, long ncRNAs. Clearly, these translocation mechanisms and the identification of pathway components for mitochondrial targeting require further studies.[7]

Roles of mitomiRs in mitochondria

Mitochondria are semi-autonomous cell organelles with their own DNA (mtDNA) encoding 22 tRNAs, 2 rRNAs, and 13 polypeptides. These polypeptides and those encoded by nuclear genes, form 4 protein complexes of the electron transport chain (ETC). Mitochondria are constantly dividing and fusing, and the balance between mitochondrial fission and fusion influences mitochondrial morphology, whose dynamics and turnover are critical for cellular homeostasis and differentiation.[50] Several proteins are involved in the regulation of mitochondrial dynamics. Deregulation of mitochondrial dynamics is not only associated with deregulation of mitochondrial function, but is also closely related to several biological processes such as proliferation, cell death, apoptosis and production of reactive oxygen species (ROS), since mitochondria are the major sites of oxidative metabolism of sugars, lipids, amino acids and ATP production.[1,51–53]

It's also worth noting that the mitochondrial matrix has its own set of environmental variables. Because of its thioester bond, acetyl-coenzyme A (acetyl-CoA) is a very abundant metabolite in mitochondria and functions as a powerful acetylation reagent. Protein lysine acetylation and succinylation are caused by acetyl-CoA and mitochondrial matrix pH concentrations. Non-enzymatic acetylation occurs often in mitochondria.[54] The most of mitochondrial proteins have acetyl groups, which is consistent with this hypothesis. Non-enzymatic acetylation of RNA molecules, including miRNAs, is a logical possibility for mitochondrial modification. An acetyl group covalently attached to a miRNA might change its mRNA recognition behavior. If it happens at the 2 OH group of ribose needed for the cleavage process, it could inhibit spontaneous bond cleavage and therefore increase the half-life of mRNA. Furthermore, post-transcriptional alterations can result in structural changes[55] as well as changed interactions with other RNA molecules or proteins.[56]

As stated, mitomiRs are regulators of mitochondrial function, as shown in the following examples. *In silico* analysis identified miR-378, miR-24, and miR-23b in liver mitochondria (Table 1) and these mitomiRs have been shown to regulate systemic energy homeostasis, oxidative capacity, ROS, and mitochondrial lipid metabolism.[35,57–62] Several reports have indicated that miRNAs such as miR-1291, miR-138, miR-150, miR-199a, and miR-532-5p can alter the expression of some important glycolytic enzymes (Table 1).[4,63–70] miR-29a, miR-29b and miR-124 (Table 1) regulate the expression of monocarboxylate transporter 1 (SLC16A1) in pancreatic beta cells.[71] miR-33a/b has been shown to regulate lipid metabolism by targeting the cholesterol transporter ATP-binding cassette transporter (ABCA1).[72] miR-143 and miR-24 have also been shown to regulate mitochondrial lipid metabolism (Table 1).[73,74] On the other hand, miR-204 accelerates fatty acid oxidation by inhibiting acetyl-coenzyme A carboxylase (ACC).[75] Ahmad *et al.* (2011) showed that miR-200 is associated with the regulation of phosphoglucose isomerase (PGI), which is an important factor in glycolysis and glucogenesis. Overexpression of miR-338 leads to downregulation of the protein level of cytochrome c oxidase IV and reduces mitochondrial oxygen consumption and ATP production.[77,78] Similarly, overexpression of miR-181c decreases mt-COX1 protein and causes remodeling of the complex IV (*in vitro*)[48] and a dysfunctional complex IV (*in vivo*)[79], along with increased production of ROS. It has also been reported that miR-210 modulates the function of the complex IV by targeting the nuclear-encoded mRNA, COX10.[80,81] It has also been reported that miR-15b, miR-16, miR-195 and miR-338 (Table 1) regulate ATP production by targeting several nuclear genes that play important roles in ETC.[77,82,83] miR-101-3p regulates the expression of ATP synthase subunit beta (ATP5B) in ETC (Table 1).[84] In addition, miR-210-5p reduces the expression of iron-sulfur cluster assembly enzyme (ISCU) under hypoxic conditions, which affects the proteins containing iron-sulfur clusters (Fe-S).[85] It has also been reported that miR-29a-3p[86] is involved in ß-oxidation of lipids (Table 1) and that miR-19b negatively regulates mitochondrial fusion by downregulating mitofusin 1 (MFN1).[87]

The microRNAs listed in Table 1 significantly affect mitochondrial regulation and function, which is why they are classified in the group of mitomiRs, which are crucial regulatory molecules

TABLE 1. Summary of microRNAs and their roles in mitochondria

miR	miR accession number	Target genes	Gene accession number	Function	Functional pathway	Location	Species	References
miR-378	MI0000795	Crat	ENSMUSG00000026853	Downregulation	Mitochondrial oxidative metabolism	Mitochondria in liver cells	Mouse	Carrer *et al.*, 2012[59]
miR-24	MI0000080	H2ax	ENSMUSG00000049932	Downregulation	Insulin signaling pathway	Mitochondria in liver cells	Human	Jeong *et al.*, 2017[61]
miR-23b	MI0000439	GLS	ENSG00000115419	Downregulation	Glutamine metabolism	LMitochondria in liver cells	Human	Gao *et al.*, 2009[60]
miR-1291	MI0006353	SLC2A1 CPT1C ESRRA ASS1 GLUT1	ENSG00000117394 ENSG00000169169 ENSG00000173153 ENSG00000130707 ENSG00000117394	Downregulation Downregulation Downregulation Downregulation Downregulation	Mitochondrial metabolism	Mitochondria in renal cells	Human	Yamasaki *et al.*, 2013; Chen *et al.*, 2020, Tu *et al.*, 2020[63–65]
miR-138	MI0000455	PDK1	ENSG00000152256	Downregulation	Glucose metabolism	Mitochondria in cardiac cells	Human	Zhu *et al.*, 2017[66]
miR-150	MI0000920 MI0000479	Slc2a4 SLC2A1	ENSRNOG00000017226 ENSG00000117394	Downregulation Downregulation	Metabolism	Mitochondria in cardiac cells	Rat Human	Ju *et al.*, 2020[67] Li *et al.*, 2017[68]
miR-199a	MI0000941 MI0000242	Slc2a4 Hk2 HK2	ENSRNOG00000017226 ENSRNOG00000006116 ENSG00000159399	Upregulation Upregulation Upregulation	Expression of glucose transporters	Mitochondria in muscle cells Mitochondria in liver cells	Rat Human	Esteves *et al.*, 2018, Yan *et al.*, 2014, Guo *et al.*, 2015[4,69,70]
miR-532-5p	MI0006154	Slc2a4 Hk2	ENSRNOG00000017226 ENSRNOG00000006116	Upregulation Upregulation	Expression of glucose transporters	Mitochondria in muscle cells	Rat	Esteves *et al.*, 2018[70]
miR-29a	MI0000576	Slc16a1	ENSMUSG00000032902	Downregulation	Mitochondrial oxidative metabolism	Mitochondria in pancreatic beta-cells	Mouse	Pullen *et al.*, 2011[71]
miR-29b	MI0000143	Slc16a1	ENSMUSG00000032902	Downregulation	Mitochondrial oxidative metabolism	Mitochondria in pancreatic beta-cells	Mouse	Pullen *et al.*, 2011[71]
miR-124	MI0000716	Slc16a1	ENSMUSG00000032902	Downregulation	Mitochondrial oxidative metabolism	Mitochondria in pancreatic beta-cells	Mouse	Pullen *et al.*, 2011[71]
miR-33a/b	a-MI0002684, b-MI0007603	CROT CPT1A HADHB PRKAA1 ABCA1 SREBF1 FASN ACLY ACACA	ENSANAG00000028065 ENSANAG00000017356 ENSANAG00000027802 ENSANAG00000032687 ENSANAG00000033387 ENSANAG00000021477 ENSANAG00000032055 ENSANAG00000036009 ENSANAG00000035253	Downregulation Downregulation Downregulation Downregulation Downregulation Upregulation Upregulation Upregulation Upregulation	Lipid metabolism	Mitochondria in liver cells	Monkey	Rayner *et al.*, 2011[72]
miR-143	MI0000916 MI0000459	Map2k5 APOL6	ENSRNOG00000007926 ENSG00000221963	Downregulation Downregulation	Adipogenesis Adpiogenesis	Mitochondria in adipose cells Mitochondria in adipose cells	Rat Human	Chen *et al.*, 2014[73] Ye *et al.*, 2013[74]
miR-204	MI0000284	ACACB	ENSG00000076555	Downregulation	Lipid metabolism	Mitochondria in adipose cells	Human	Civelek *et al.*, 2013[75]
miR-200	MI0000737	ZEB1 ZEB2	ENSG00000148516 ENSG00000169554	Upregulation Upregulation	Lipid metabolism	Mitochondria in breast cells	Human	Ahmad *et al.*, 2011[76]
miR-338	MI0000618	COXIV	ENSRNOG00000007827	Downregulation	Mitochondria oxidative metabolism	Mitochondria in neural cells	Rat	Aschrafi *et al.*, 2008[77]
miR-181c	MI0000924	COX1	ENSRNOG00000034234	Downregulation	Mitochondria oxidative metabolism	Mitochondria in cardiac cells	Rat	Das *et al.*, 2012[88]
miR-210	MI0000268	ISCU	ENSG00000136003	Downregulation	Mitochondria oxidative metabolism	Mitochondria in placenta cells	Human	Colleoni *et al.*, 2013; Qiao *et al.*, 2013[81,85]
miR-15b	MI0000843	Arl2 Bcl2	ENSRNOG00000021010 ENSRNOG00000002791	Downregulation Downregulation	ATP production	Mitochondria in cardiac cells	Rat	Nishi *et al.*, 2010[82]
miR-16	MI0000844	Bcl2 Arl2	ENSRNOG00000002791 ENSRNOG00000021010	Downregulation Downregulation	ATP production	Mitochondria in cardiac cells	Rat	Nishi *et al.*, 2010[82]
miR-195	MI0000939	Arl2	ENSRNOG00000021010	Downregulation	ATP production	Mitochondria in cardiac cells	Rat	Nishi *et al.*, 2010[82]
miR-29a-3p	MI0000576	Foxa2	ENSMUSG00000037025	Upregulation	Lipid metabolism	Mitochondria in liver cells	Mouse	Kurtz *et al.*, 2014[86]
miR-19b	MI0000074	MFN1	ENSG00000171109	Downregulation	Apoptosis	Mitochondria in bone cells	Human	Li *et al.*, 2014[87]
miR-101-3p	MI0000103	ATP5B	ENSG00000110955	Silencing	Mitochondria metabolism	Mitochondria in heLa cells	Human	Zheng *et al.*, 2011[84]

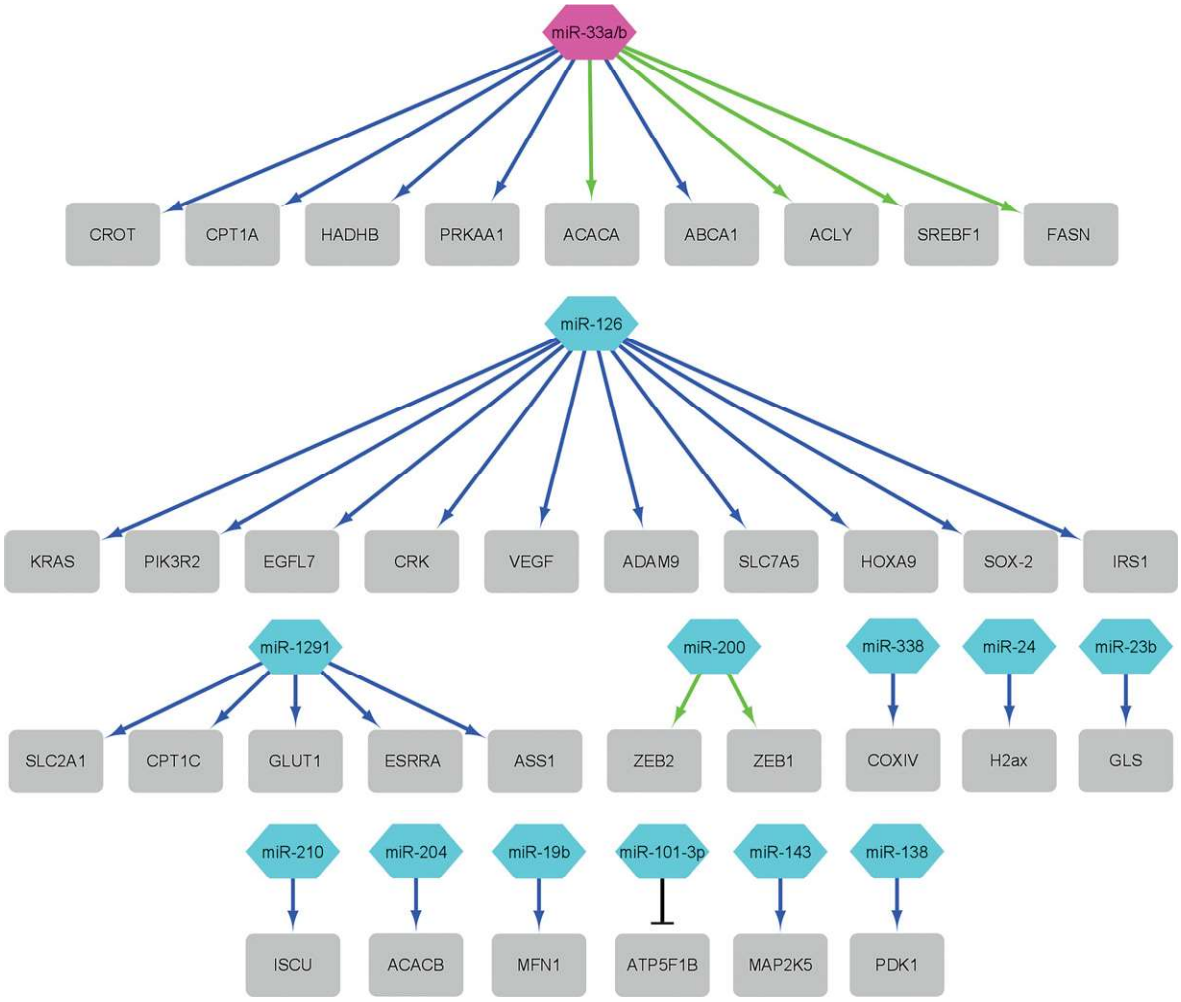

FIGURE 2. The network of the mitomiRs and their target genes (grey rectangle) in primates (data from Table 1). Blue arrows present downregulation, green arrows present upregulation and black T-line present silencing. Purple octagon shape presents monkey miRNA and cyan hexagon presents human miRNAs (figure constructed with Cytoscape Network Data Integration, Analysis, and Visualization in a Box V3.8.2).

of mitochondrial function and regulation of metabolism. In the figures (Figure 2 and Figure 3), we have shown how these mitomiRs are linked to their target genes in primates (Figure 2) and rodents (Figure 3).

In primates, there is no regulation of the same genes by different mitomiRs from Table 1 (Figure 2). Moreover, most mitomiRs target one gene and only a few mitomiRs target a larger number of genes and in most cases mitomiRs downregulate genes.

In contrast to primates, in rodents, some genes are regulated by different mitomiRs (Figure 3). The mitomiRs miR-15b and miR-16 both regulate the *Arl2* gene[82], which is a nucleotide-binding gene, and the *Bcl2* gene, which regulates apoptosis. In addition, the mitomiRs miR-199a[69,70] and miR-532-5p[70] both regulate the *Hk2* gene, which has an important function in regulating glucose metabolism, and the *Slc2a4* gene, which is a glucose transmembrane transporter. It can be concluded that there is a greater overlap of mitomiRs in rodents than in primates. In most cases, mitomiRs downregulate genes.

From the figures (Figure 2 and Figure 3), we can summarize that some mitomiRs and their target genes are related in primates and rodents. MitomiR miR-199a[69,70] regulates the same gene in both primates and rodents (Figure 3), the gene *Hk2*, which has an important function in regulating glucose metabolism. MiR-143[73,74] regulates the same gene *MAP2K5* (Figure 3), which has an important

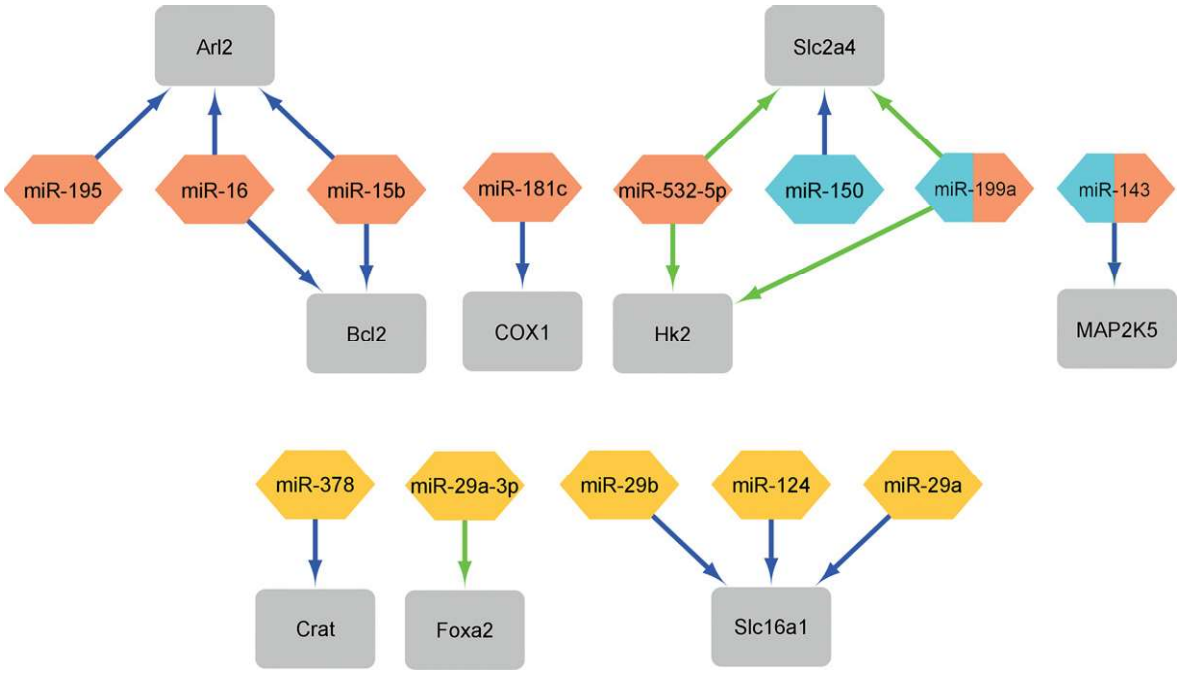

FIGURE 3. The network of the mitomiRs and their target genes (grey rectangle) in rodents (data from Table 1). Blue arrows present downregulation and green arrows present upregulation. Orange diamond shape presents rat miRNAs, yellow rectangle presents mouse miRNAs and cyan hexaon presents human miRNAs. miR-199a and miR-143 show that this two miRNAs regulate (figure constructed with Cytoscape Network Data Integration, Analysis, and Visualization in a Box V3.8.2).

function in signal cascade involved in growth factor stimulated cell proliferation and muscle cell differentiation.

MitomiRs in cancer

Traditional cancer traits include ten biological capabilities gained during the multistage development of human tumors.[89] These ten traditional cancer traits include resistance to cell death, induction of angiogenesis, maintenance of proliferative signaling, evasion of growth suppressors, activation of invasion and metastasis, facilitation of replicative immortality, altered metabolism, evasion of destruction by the immune system, tumor-promoting inflammation, and genome instability (Figure 4).[89,90]

An important feature of cancer is the presence of the Warburg effect. Under aerobic conditions, normal cells generate ATP primarily in the mitochondrial oxidative phosphorylation process (OXPHOS), which utilizes the products of glycolysis and the Krebs cycle. Under anaerobic conditions, relatively little pyruvate, the end product of glycolysis, is added to the Krebs cycle and is instead converted to lactate. However, this metabolic conversion of glucose appears to be energetically detrimental. In tumor cells, ATP deficiency can be compensated to some extent by upregulation of glycolysis.[91] Interestingly, it has been observed that many cancer cells prefer glycolysis over OXPHOS even in the presence of an adequate amount of oxygen. This abnormal energy metabolism is known as the Warburg effect. Reduced OXPHOS and enhanced aerobic glycolysis are the main manifestations of reprogramming of glucose metabolism in tumor cells.[1,92] Albeit the specific causes and utilitarian outcomes of this metabolic switch are as yet unclear, there is a developing agreement that the impact of Warburg effect is certifiably not an inconsequential result of carcinogenesis, yet is imperative for cancer cells to keep up with their proliferative potential and is driven by a few elements.[92–94]

It has been confirmed that abnormal expression of mitomiRs in mitochondria is related to the occurrence of cancer features.[95] Moreover, mitomiRs play an essential role in the control of cancer cell metabolism by regulating mRNA expression. They regulate several oncogenic signaling pathways and

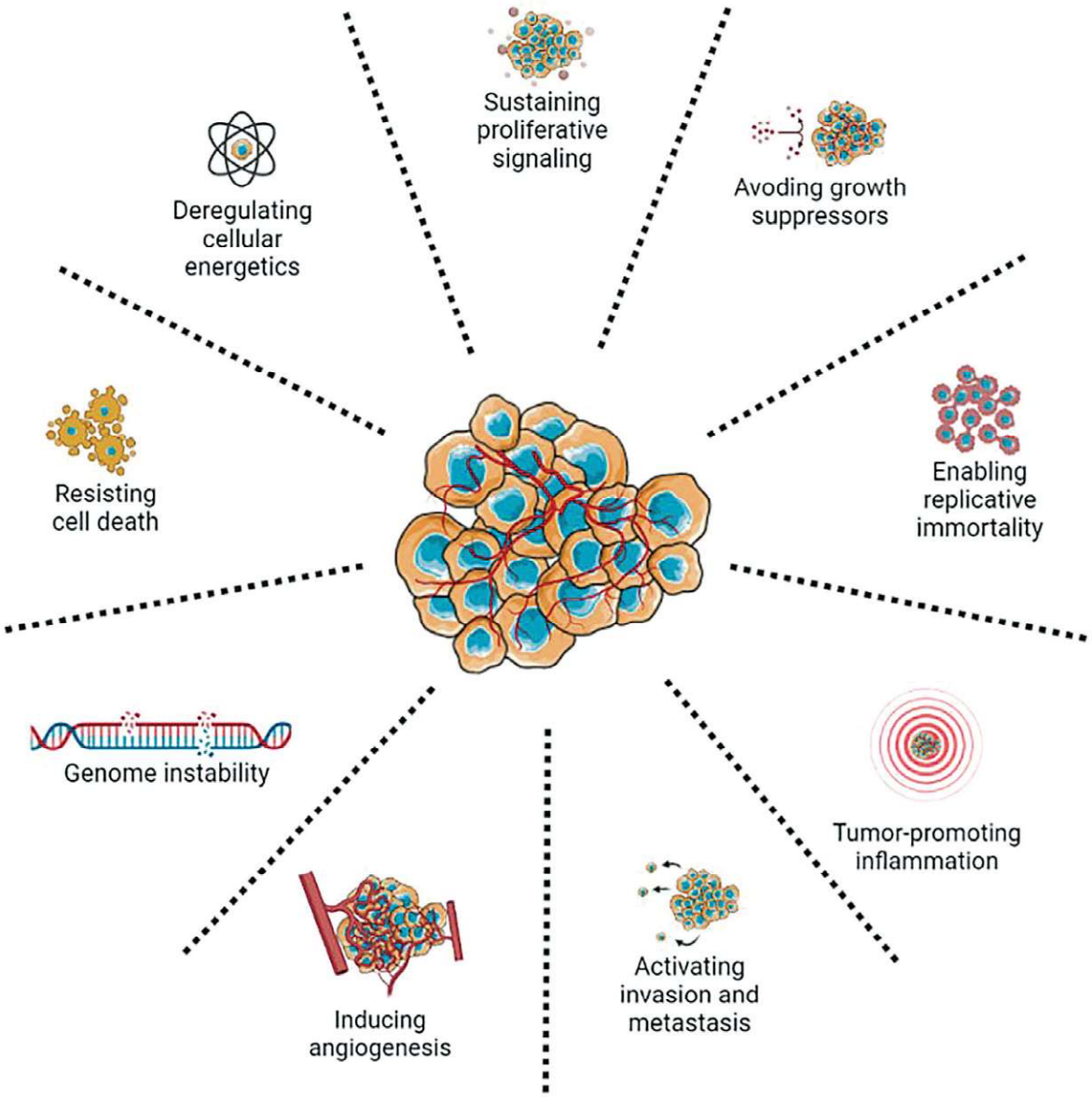

FIGURE 4. Traditional cancer traits.[89]

target key transporters or enzymes in cellular metabolism. In addition, they may have a function as tumor suppressors that inhibit tumor cell proliferation or as oncogenes that induce tumorigenesis.[96–98] MitomiRs can be isolated from any tissue or body fluid of any organism to study the level of expression in the organism in a diseased state, and thus can function as novel prognostic and predictive biomarkers.[99]

The first evidence of miRNA involvement in human cancers was provided in a study of chronic lymphocytic leukemia (CLL).[100] MiR-15a and miR-16-1 localized to 13q14 were reported to be frequently deleted and/or reduced in patients with B-cell chronic lymphocytic leukemia. This finding provided the first evidence that miRNAs may be involved in the pathogenesis of human cancers, as deletion of chromosome 13q14 resulted in the loss of these two miRNAs. MiR-15a induces apoptosis by regulating mitochondrial function and affecting the activity of Bcl-2 and Mcl-1 in human (Table 2). In addition, miR-15a causes mitochondrial dysfunction, leading to the release of cytochrome c into the cytoplasm and depletion of mitochondrial membrane potential.[101] MiR-15a and miR-16a have been shown to be ATP modulators correlated with

TABLE 2. Summary of mitomiRs with roles in cancer

miR	miR accession number	Target genes	Gene accession number	Function	Functional pathway	Type of cancer	Species	References
miR-210	MI0000286	HIF-1	ENSG00000258777	Upregulation	Hypoxia	Breast cancer, neck and head cancer, lung cancer	Human	Qin *et al.*, 2014; Gee *et al.*, 2010; Puissegur *et al.*, 2011[109–111]
		ISCU	ENSG00000136003	Upregulation				
		COX10	ENSG00000006695	Upregulation				
		SDHD	ENSG00000204370	Upregulation				
		NDUFA4	ENSG00000189043	Upregulation				
miR-200a	MI0000342	TFAM	ENSG00000108064	Downregulation	Mitochondrial biogenesis, cancer metabolism	Breast cancer	Human	Yao *et al.*, 2014[112]
miR-155	MI0000681	HK2	ENSG00000159399	Upregulation	Glucose phosphorylation	Breast cancer	Human	Fang *et al.*, 2012; Jiang *et al.*, 2012[104]
miR-124	MI0000443	PKM	ENSG00000067225	Upregulation	Glucose metabolism	Colorectal cancer	Human	Sun *et al.*, 2012[105]
miR-137	MI0000454	PKM	ENSG00000067225	Upregulation	Glucose metabolism	Colorectal cancer	Human	Sun *et al.*, 2012[105]
miR-340	MI0000802	PKM	ENSG00000067225	Upregulation	Glucose metabolism	Colorectal cancer	Human	Sun *et al.*, 2012[105]
miR-326	MI0000808	PKM2	ENSG00000067225	Downregulation	Glucose metabolism	Glioblastoma	Human	Kefas *et al.*, 2010[106]
miR-181-5p	MIMAT0000256	RASSF6	ENSG00000169435	Downregulation	Mitogen-activated protein kinase (MAPK) signaling pathway	Gastric cancer, cervical cancer	Human	Mi *et al.*, 2017; Zhuang *et al.*, 2017[108,113]
		INPP5A	ENSG00000068383	Downregulation				
miR-92a-1	MI0000093	BCL2L11	ENSG00000153094	Downregulation	Apoptosis	Lymphoma	Human	Mogilyansky and Rigoutsos, 2013[94]
miR-126	MI0000471	PIK3R2	ENSG00000105647	Downregulation	Inflammation, angiogenesis	Breast cancer cells	Human	Zhu *et al.*, 2011[114]
		PLK2	ENSG00000260410	Downregulation		Acute leukaemia cells		Li *et al.*, 2008[115]
		EGFL7	ENSG00000172889	Downregulation		Oral squamous cells		Sasahira *et al.*, 2012[116]
		CRK	ENSG00000167193	Downregulation		Lung cancer cells		Crawford *et al.*, 2008[117]
		ADAM9	ENSG00000168615	Downregulation		Melanoma cancer cells		Felli *et al.*, 2013[118]
		HOXA9	ENSG00000078399	Downregulation		Acute leukaemia cells		Shen *et al.*, 2008[119]
		IRS1	ENSG00000169047	Downregulation		Breast cancer cells		Zhang *et al.*, 2008[120]
		SOX-2	ENSG00000242808	Downregulation		Gastric cancer cells		Otsubo *et al.*, 2011[121]
		SLC7A5	ENSG00000103257	Downregulation		Lung cancer cells		Miko *et al.*, 2011[122]
		VEGFA	ENSG00000150630	Downregulation		Oral squamous cells		Sasahira *et al.*, 2012[116]
		MMP7	ENSG00000137673	Downregulation		Melanoma cancer cells		Felli *et al.*, 2013[118]
miR-15a	MI0000069	BCL-2	ENSG00000171791	Downregulation	Apoptosis, ATP production	B-cell chronic lymphocytic leukemia	Human	Gao *et al.*, 2010[101]
		MCL-1	ENSG00000143384	Downregulation				
		COX4I2	ENSG00000131055	Downregulation				Siengdee *et al.*, 2010[102]
		COX6A2	ENSG00000156885	Downregulation				
		NDUFB7	ENSG00000099795	Downregulation				
		NDUFV1	ENSG00000167792	Downregulation				
		NDUFS4	ENSG00000164258	Downregulation				
miR-16a	MI0000070	COX4I2	ENSG00000131055	Downregulation	Apoptosis, ATP production	B-cell chronic lymphocytic leukemia	Human	Siengdee *et al.*, 2010[102]
		COX6A2	ENSG00000156885	Downregulation				
		NDUFB7	ENSG00000099795	Downregulation				
		NDUFV1	ENSG00000167792	Downregulation				
		NDUFS4	ENSG00000164258	Downregulation				

cytochrome c oxidase subunit 4I2 (Cox4i2), subunit 6A2 (Cox6a2), NADH:ubiquinone oxidoreductase subunit B7 (Ndufb7), NADH:ubiquinone oxidoreductase core subunit V1 (Ndufv1) and NADH:ubiquinone oxidoreductase subunit S4 (Ndufs4) expression.[102]

Glycolysis is the initial step in glucose catabolism, and occurs outside of the mitochondria in the cytoplasm. In the context of miRNAs affecting cell metabolism, miR-155 (Table 2) was found to indirectly upregulate hexokinase 2 (HK2), a glucose phosphorylation enzyme that might affect energy consumption in breast cancer cells. Mir-143 appears to be one of two potential pathways regulating miR-155-dependent HK2 regulation.[103,104] Alternative splicing of pyruvate kinase isoenzyme (PKM), whose splicing proteins are regulated by miR-124, miR-137, and miR-340, is another pathway regulating glucose metabolism (Table 2). This miRNA-dependent regulation of PKM is able to influence colorectal cancer growth and counteract the Warburg effect.[105] In addition, pyruvate kinase (PK) is a direct target of the tumor suppressor miR-326, making it a potential glucose metabolism regulator.[94,106,107]

In hepatocellular carcinoma, reduced mRNA levels were detected in 11 of the 13 genes encoded in the mtDNA, including the genes encoding cytochrome B (mt-CYB) and cytochrome C oxidase II (mt-CO2).[108] When miR-181a-5p expression was increased, the levels of mt-CYB and mt-CO2 were reduced in hepatocellular carcinoma cells, while mitochondrial membrane potential (MMP) maintained by electron transfer chain was reduced. *In vivo* experiments, which were done by Zhuang *et al.*[108], have shown to have caused glucose metabolism to reprogram and stimulated tumor growth and early lung metastasis in patients with hepatocellular carcinoma.

Several studies reported that miR-126 has an important role in different human cancers (Table 2) such as breast, lung, gastric cancers, melanoma cancer and acute leukaemia. Tomasetti *et al.*[83] reported that miR-126 affects mitochondrial energy metabolism, resulting in malignant mesothelioma tumor suppression. This mitomiR reduce mitochondrial respiration and promote glycolysis in H28 cells, associated with IRS1 modulate ATP-citrate lyase deregulation. This leads to an increase in ATP and citrate production which is linked with reducing Akt signaling and inhibiting cytosolic sequestration of Forkhead box O1 (FoxO1), which promote the expression of genes involved in gluconeogenesis and oxidative stress defense.[83]

Hypoxia has previously been related to altered mitomiR expression, with hypoxia-regulated mitomiRs being found to play a key role in cell survival in oxygen-depleted settings.[123] MiR-210 is one of the mitomiRs that is continuously increased in normal and transformed cells during hypoxia, suggesting that miR-210 plays a role in cells' adaptive response to hypoxia.[109] MiR-210 expression corresponds with hypoxia gene signatures in tumor tissues such as breast and head and neck cancers, demonstrating a direct connection between miR-210 expression and hypoxia in cancer.[110] MiR-210 has been researched extensively and has a number of functionally significant targets in cell cycle control, cell survival, differentiation, angiogenesis, and metabolism.[123] Cell metabolism switches from mitochondrial OXPHOS to glycolysis under hypoxic environments. HIF-1, a hypoxia-inducible factor that upregulates the expression of most glycolytic enzymes as well as pyruvate dehydrogenase kinase while downregulating mitochondrial respiration, plays a key role in this action. Previous research has looked into how miR-210 regulates mitochondrial metabolism under hypoxia. MiR-210 target iron-sulfur cluster assembly proteins (ISCU1/2) and inhibit the activity of iron-sulfur proteins that govern mitochondrial metabolism, such as complex I and aconitase, resulting in lower OXPHOS.[123] It acts directly on cytochrome c oxidase assembly factor heme A:farnesyltransferase (COX10), succinate dehydrogenase complex subunit D (SDHD), and NADH dehydrogenase (ubiquinone) 1 alpha subcomplex 4 (NDUFA4) in regulating mitochondrial activity.[123] Another study found an abnormal mitochondrial phenotype in A549 lung cells overexpressing miR-210, and mRNA expression profile analysis connecting miR-210 to mitochondrial dysfunction.[112] Interestingly, HIF is rapidly destroyed upon reoxygenation of hypoxic cells due to miR-210's high stability, whereas miR-210 stays stable to maintain the glycolytic phenotype. Under normal conditions, this slows mitochondrial metabolism and may contribute to the Warburg effect in cancer cells. This result supports miR-210's involvement in regulating mitochondrial metabolism and promoting cancer cells' adaptability to hypoxic environments.

Another important mitomiR is miR-200, which has been identified as involved in tumor progression.[124,125] One of miR-200 targets, is transcription factor mitochondria (TFAM) which is one of the most important proteins regulating mitochondrial biogenesis. TFAM has been described as a functional target of miR-200 in breast cancer cells.[113] Its

transcription factor activity is required for mtDNA replication and transcription. In addition to its function in replication and transcription, the presence of TFAM is necessary for mtDNA maintenance.[126] It has also been implicated as a primary architectural protein of the mitochondrial genome by packaging mtDNA. In addition, TFAM expression has been reported to be involved in tumor progression, cancer cell growth, and chemoresistance.[127]

Regarding the role of miRNAs in cancer and metabolism, the miR-17/92 cluster is one of the best characterized oncogenic miRNAs. This cluster is also known as oncomiR-1, and there is growing evidence of its oncogenic potential.[93] It has been shown that miR-17/92 suppresses apoptosis and was originally found amplified in B-cell lymphomas, where ectopically overexpressed truncated versions lacking miR-92a-1 were shown to possess oncogenic properties.[110] The MiR-17/92 cluster is deregulated in B-cell lymphomas, T-cell lymphomas, B-cell chronic lymphocytic leukemia, and acute myeloid leukemia. This cluster is particularly overexpressed in several other cancers, including osteosarcoma, neuroblastoma, cervical, pancreatic, breast, lung, colorectal, ovarian, kidney, and liver cancers.[93,105] Izreig *et al*.[128] reported that this miRNA cluster is a key factor in metabolic reprogramming of tumors. If oncomiR-1 is absent in Myc+ tumor cells, there is a global decrease in glycolytic and mitochondrial metabolism. If increased oncomiR-1 expression is present, this is sufficient for increased nutrient utilization by tumor cells. Deletion of miR-17/92 promoted changes in gene expression in Myc+ lymphoma which results in global decrease in metabolic pathways including glycolysis, the Krebs cycle, components of the electron transfer chain, amino acid metabolism, the pentose phosphate pathway, serine biosynthesis and nucleotide biosynthesis.[128]

Conclusions

MiRNAs have been found in the mitochondria of many cell types, as shown by an increasing number of studies and they were named mitomiRs. In general, mitomiR populations differ in various tissues and under different pathological circumstances, implying that mitomiR populations are regulated by mechanisms that remain to be discovered. Based on the available information, we can deduce that there are a significant number of miRNAs which are present in mitochondria.[7,29–33]

In our review, we have shown that various mitomiRs play a role in the initiation and progression of cancer via the regulation of mitochondria. They are involved in the Warburg effect, which is necessary for cancer cells to maintain their proliferative capacity.[91] MitomiRs also upregulate HK2, a glucose phosphorylation enzyme, in an indirect manner, which may impact energy consumption in breast cancer cells.[103,104] Expression of one of the mitomiRs (miR-210) corresponds with hypoxia gene signatures in tumor tissues such as breast cancer and head and neck cancers, demonstrating a clear connection between mitomiR expression and hypoxia in cancer.[108,109,121] MiRNAs have emerged in the last decade as key regulators in cancer-related processes and are classified as either oncogenic or tumor suppressive miRNAs. The miR-17/92 cluster was first discovered to be amplified in diffuse cell lymphoma and B-cell lymphoma. This mitomiR cluster suppresses apoptosis and may act as an oncogene in B-cell lymphomas, B-cell chronic lymphocytic leukemia, acute myeloid leukemia, and T-cell lymphomas. It is also overexpressed in numerous other malignancies. This cluster is a key factor in metabolic reprogramming of tumors by regulating glycolytic and mitochondrial metabolism. Tumor-targeting treatments based on mitomiRs are emerging as a novel diagnostic and therapeutic tool.[94,106,111,128]

Future perspectives

We have shown that mitomiRs are important players in mitochondria of cancer cell that need to be further investigated to develop a new potential therapies for cancer. Numerous studies that have been published in recent years give promising predictions that mitomirRs will receive more attention in the context of their role in cancer as possible biomarkers or targets for treatment.

Acknowledgement

This work was financially supported by the Slovenian Research Agency (ARRS grant # P3-0003).

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