Biochimica et Biophysica Acta 1840 (2014) 1441–1453

Contents lists available at ScienceDirect

Biochimica et Biophysica Acta

journal homepage: www.elsevier.com/locate/bbagen

Review

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

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ARTICLE INFO

*Article history:*
Received 30 May 2013
Received in revised form 28 August 2013
Accepted 3 September 2013
Available online 7 September 2013

*Keywords:*
Mitochondrion
MicroRNA
Cancer
TCA cycle
Reprogramming of metabolism

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

☆ This article is part of a Special Issue entitled Frontiers of Mitochondrial Research.

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http://dx.doi.org/10.1016/j.bbagen.2013.09.002

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 [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 miRNAs 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

other cellular compartments than the cytosol. Nuclear encoded miRNAs have been found associated with the mitochondrial outer membrane (MOM) [35]. This compartment may provide a platform for the assembly of signalling complexes that play an important role in the regulation of transcriptional repression.

It has been reported that MiR-181c, encoded in the nucleus, matures in the cytoplasm and then translocates into mitochondria of cardiac myocytes. This miRNA can enter and target the mitochondrial genome, ultimately causing re-modelling of complex IV (CIV) and mitochondrial dysfunction [36]. Mitochondria harbour their own genetic system that may be a potential site for miRNA-mediated post-transcriptional regulation. For example, miRNAs have been identified in mitochondria purified from rat liver [5,6], and localisation of pre-miRNAs and mature miRNAs has been demonstrated for mitochondria isolated from human muscle cells [37]. Whether mitochondrial miRNAs are transported into the mitochondrion or are endogenously synthesised remains unknown and should be further investigated. Several arguments support the miRNA import hypothesis. A possible link between mitochondria and RNAi came from co-immunoprecipitation of human AGO2 with mitochondrial $tRNA^{Met}$ [38]. This suggests that components involved in the mitochondrial RISC assembly, in particular AGO2, may be involved in the transport of miRNAs to mitochondria. In mitochondria, post-transcriptional regulation via miRNAs would provide a sensitive and rapid mechanism that will adjust the expression of the mitochondrial genome in relation to the conditions and metabolic demands of the cell.

A recent study, aimed to investigate the possible link between miRNAs and mitochondria in human cells, identified 13 miRNAs significantly enriched in mitochondria purified from HeLa cells that have been referred to as mito-miRNAs and that are coded for in the nucleus [39]. For example, apart from its role in the cytosol, MiR-494 is likely to have a function in mitochondria due to its localisation to this organelle. Conceivably, hsa-MiR-1974, hsa-MiR-1977 and hsa-MiR-1978 are considered non-canonical miRNAs because they map to mitochondrial tRNA and rRNA genes. The actual mitochondrial localisation of certain miRNAs implies that small RNA-mediated processes may regulate mitochondrial biogenesis and function. Details of which miRNAs modulate the mitochondrial function are rather obscure.

## 4. MiRNAs and mitochondrial metabolism

A fundamental function of mitochondria is to produce ATP via OXPHOS to supply energy for a variety of cellular functions. MiR-15b, MiR-16, MiR-195 and MiR-424 have emerged as regulators of the ATP levels, all sharing the same 'seed' sequence. Their over-expression suppresses ATP levels and affects the mitochondrial integrity. The target of these miRNAs is the ADP-ribosylation factor-like 2 (ARL2) mRNA; knock-down of ARL2 by siRNA also resulted in reduced ATP level and degeneration of mitochondria [40]. Conditions that induce up-regulation of MiR-15b as well as several other miRNAs that share the same 'seed' sequence, such as MiR-195, resulted in the mitochondrial degeneration and reduction in ATP levels in cardiomyocytes. MiR-338 is another miRNA that modulates OXPHOS and the mitochondrial function, since it targets cytochrome c oxidase IV (COX IV) mRNA [41]. In neurons, MiR-338 over-expression by transfection resulted in decreased COX IV and reduced ATP. In contrast, expression of anti-miRNA oligonucleotides increased COX IV levels and improved OXPHOS. It was therefore concluded that over-expression of MiR-338 jeopardises the mitochondrial function.

Mitochondrial glutaminase (GLS) is important for mitochondrial metabolism since it converts glutamine to glutamate that is further catabolised by the tricarboxylic acid (TCA) cycle for the production of ATP. Glutamate also serves as a substrate for glutathione synthesis. Proliferating cells are known to utilise glutamine as a major source of energy, 'nitrogen' for biosynthetic pathways and 'carbon' for anabolic processes [42,43]. Recent reports show that transcriptional regulation of the Myc oncogene is coordinated with the expression of genes that promote cells to engage in excessive glutamine catabolism, which exceeds the cellular requirement for protein and nucleotide biosynthesis [42]. Such Myc-dependent glutaminolysis results in re-programming of the mitochondrial metabolism to depend on glutamine catabolism and to maintain cellular viability and TCA cycle anaplerosis. Concomitantly, this stimulation of mitochondrial glutamine metabolism leads to reduced glucose-derived metabolites to enter the TCA cycle and decreased contribution of glucose to the mitochondria-dependent synthesis of phospholipids [44]. MiR-23a and MiR-23b have been demonstrated to target mitochondrial GLS, since they can directly repress GLS levels [43]. Myc, in turn, up-regulates GLS by suppressing the expression of MiR-23a/b.

Early studies have demonstrated the formation of mitochondrial ROS under hypoxia, and these radicals have been suggested as 'sensors' of oxygen deficiencies [45]. Cells sense and respond to the shortage of oxygen by activating the transcription factor hypoxia-inducible factor-1 (HIF-1) and HIF-2 to evoke adaptive responses. Mitochondria are at the center of hypoxia sensing and function as the response relay system. In hypoxic cancer cells, mitochondria re-direct the TCA cycle intermediates to preserve their biosynthetic function. Persistent HIF activation lowers the entry of the mitochondria 'electron-delivering' compounds to reduce the TCA cycle fuelling and β-oxidation, attenuates the expression of the electron transport chain (ETC) components, limits the mitochondria-associated biosynthetic pathways, and provokes their removal by autophagy [46]. MiR-210, significantly up-regulated during hypoxic stress in many cell types [47–49], has been reported to be involved in repressing mitochondrial respiration and the ensuing down-stream events [50,51]. Following an exposure to hypoxia, cellular metabolism shifts from OXPHOS to glycolysis. MiR-210 contributes to the metabolic shift by down-regulating several steps of mitochondrial metabolism and, in particular, the ETC complexes.

The effect of MiR-210 on the metabolic function is also linked to its direct repression of the iron sulphur cluster assembly proteins ISCU1 and ISCU2. Upon identifying the ISCU1/2 proteins as MiR-210 targets, it has been shown that HIF-expressing tissues feature increased levels of MiR-210, decreased expression of ISCU1/2, and consequently the disruption of the integrity of the iron–sulphur clusters. In turn, due to repressing ISCU1/2 during hypoxia, MiR-210 decreases the activity of iron–sulphur enzymes that control the mitochondrial metabolism, including complex I (CI) and aconitase. Consequently, MiR-210 represses mitochondrial respiration, which, in the presence of normal oxygen, leads to decreased ATP levels. In contrast, during hypoxia, miR-210 appears to increase ATP levels [50] and optimise energy production in the hypoxic cell via the Pasteur effect [52].

The repressive effect of MiR-210 on the ETC also impacts on mitochondrial ROS production, a consequence of electron leakage. Indeed, MiR-210 expression increases oxidative stress under normoxic conditions and this is, in part, mediated by ISCU [50,51]. However, conflicting results have been reported for hypoxia. In cancer cell lines, miR-210 alleviated the hypoxia-induced ROS formation. On the other hand, hypoxia exposure did not induce significant changes in ROS production in normal endothelial cells, which increased when miR-210 was inhibited. This discrepancy underlies the differences between normal and cancer cells. Other relevant mitochondrial targets of MiR-210 have been reported to be the NADH dehydrogenase (ubiquinone) 1-α sub-complex 4, a subunit of CI [53], and succinate dehydrogenase subunit D, a subunit of CII [54]. An additional target is glycerol-3-phosphate dehydrogenase (GPD), the catalyst of the glycerol phosphate shuttle, which transfers electrons from cytoplasmic NADH to the ETC. Collectively, MiR-210 suppresses the mitochondrial respiration and enhances glycolysis, which leads to a decrease in the ATP level.

Very recently, MiR-126 has been found to induce a metabolic shift in tumours toward a more glycolytic phenotype. MiR-126 suppresses the mitochondrial respiratory activity in malignant mesothelioma (MM) cells and stimulates glycolysis in response to the inhibition of mitochondrial oxygen consumption, indicative of a compensatory process

(Fig. 1A). This process is coupled to the glycolytically derived pyruvate that enters a truncated TCA cycle, where citrate is preferentially exported to the cytosol via the tricarboxylate transporter [55]. Once in the cytosol, citrate is cleaved by ATP citrate lyase (ACL) to produce cytosolic acetyl-CoA used for endogenous synthesis of fatty acids, cholesterol, and isoprenoids, as well as acetylation reactions that modify proteins. It has been postulated that MiR-126 affects the mitochondrial citrate metabolism by inhibiting the Akt pathway to restore the TCA cycle for the synthesis of ATP. Thus, this mechanism favours glucose oxidation to produce cellular energy rather than converting it into other macromolecules for cellular biosynthesis (Fig. 1B–D).

## 5. Mitochondrial dysfunction and MiRNAs

There is considerable evidence for mitochondrial dysfunction in association with insulin resistance, obesity, diabetes and cancer [56–60]. Mitochondrial dysfunction has been shown to compromise insulin signalling via serine phosphorylation of the insulin receptor substrate-1 (IRS1) [61]. The contribution of mitochondrial dysfunction to the impairment in insulin metabolic signalling has been also suggested by results of gene array analysis showing that the repression of genes regulating mitochondrial ATP production is associated with insulin resistance and type 2 diabetes mellitus. Moreover, reduction in the oxidative capacity of the ETC has been manifested in obese, insulin-resistant persons as well as diabetic patients. Genetic and environmental factors, oxidative stress, and alterations in mitochondrial biogenesis can adversely affect the mitochondrial function, leading to insulin resistance and various pathological conditions such as the cardiorenal syndrome and type 2 diabetes.

Several miRNAs have been implicated in the metabolic homeostasis deregulation, as follows from loss-of-function studies in mice [1,62]. Both MiR-378 and MiR-378* participate in the regulation of mitochondrial metabolism and energy homeostasis in mice via the transcriptional network controlled by the peroxisome proliferator-activated receptor γ co-activator-1 (PGC-1β) [63]. Mice lacking MiR-378 and MiR-378* are resistant to high-fat diet-induced obesity, and exhibited enhanced mitochondrial fatty acid metabolism and elevated oxidative capacity of insulin-target tissues. On the other hand, up-regulation of MiR-378* in cancer cells has been proposed to mediate increased lactate production owing to the shift from oxidative to glycolytic metabolism, which is associated with tumourigenesis [64].

Mitochondrial dysfunction resulting from genetic alteration (mtDNA depletion) or metabolic inhibition (rotenone toxicity) induces insulin resistance in hepatocytes via reduced expression of the IRS1 protein. Furthermore, it has been found that mitochondrial dysfunction induces the expression of several miRNAs thought to target the IRS1 3′UTR. Of these, MiR-126 is actively involved in the development of insulin resistance as it directly targets IRS1 [65,66]. MiR-126 acts as a metabolic

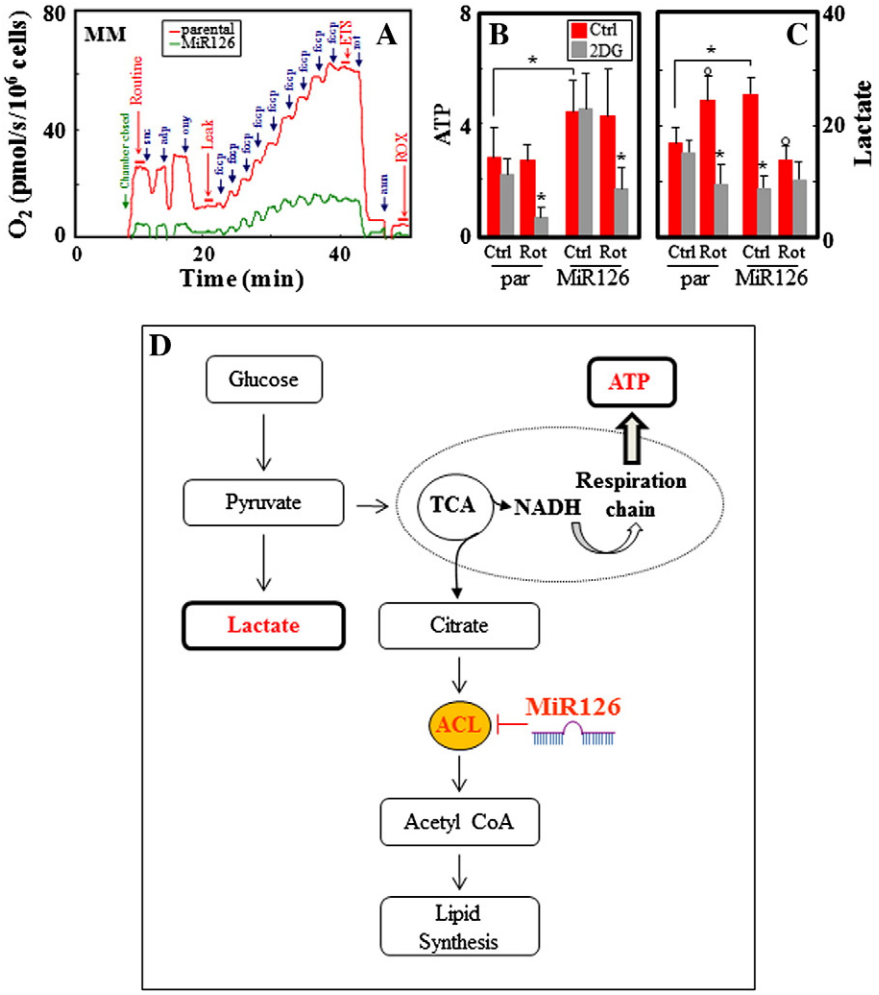


**Fig. 1.** MiR-126 alters respiration and the bioenergetic profile of MM cells. Parental H28 cells ($H28^{par}$) and their MiR-126-transfected counterparts ($H28^{MiR126}$) were evaluated for mitochondrial respiration (A), and ATP (nmol/mg protein) (B) and lactate (nmol/mg protein) levels (C) in the presence of rotenone (20 μM) and 2-deoxyglucose (2DG, 5 mM). Ectopic MiR-126 was found to suppress mitochondrial respiration and maximise ATP production via glycolysis. Comparisons among groups were determined by *t*-student test, the symbol '*' indicates significant differences, symbol '°' significance compared to control with $p < 0.05$. D) Scheme of the proposed function of MiR-126. MiR-126 affects the mitochondrial citrate metabolism by inhibiting the ACL to restore the TCA cycle for the synthesis of ATP via OXPHOS.

regulator, its up-regulation being associated with the inhibition of Akt signalling [67,68].

An important component of cellular metabolism is the production of ROS [69], which are generated by mitochondria as well as NADPH oxidases (NOX). They can cause damage to membranes and can be mutagenic. Emerging data suggest that conditions of cellular stress can alter the biogenesis of miRNAs, the expression of their targets and the activity of miRNA–protein complexes. The level of miRNA-mediated repression depends not only on the ratio of a particular mRNA target relative to the miRNA, but also on the amount of other miRNAs present in the transcriptome targeted by the same miRNAs. Consistent with this, expression of a number of miRNAs is rapidly modulated by stress, as documented for cells exposed to UV radiation [70].

Exposure to $H_2O_2$ has been reported to alter the miRNA expression profile. For example, MiR-126 is induced by oxidative stress, exerting a protective function by inducing the expression of antioxidant enzymes (Tomasetti et al., submitted). Over-expression of MiR-145 significantly inhibited $H_2O_2$-induced cellular apoptosis, ROS production, mitochondrial structure disruption as well as the activation of key signalling proteins in the mitochondrial apoptotic pathway [71]. Haque et al. [72] demonstrated that a sub-lethal dose of $H_2O_2$ up-regulated the expression of MiR-30b, which inhibited the expression of catalase (CAT). MiR-335 and MiR-34a were found to inhibit the expression of superoxide dismutase-2 (SOD2) and thioredoxin reductase 2 (Txnrd2) by binding to the 3'-UTR of each mRNA, respectively. Overexpression of MiR-335 and MiR-34a induced premature senescence of mesangial cells via suppression of SOD2 and Txnrd2 with a concomitant increase in ROS [73]. Furthermore, MiRNA-320a has been found responsive to oxidative stress and involved in the regulation of glycolysis [74]. Regulation of miRNA processing therefore plays an important role in the response to environmental stress and, consequently, stress-related pathologies.

## 6. MitoRNAs and mitochondrial dynamics

Mitochondrial dynamics and turnover are crucial for cellular homeostasis and differentiation. Mitochondria form dynamic networks that are necessary for the maintenance of the organelle fidelity [75,76]. Mitochondrial biogenesis involves the growth and division of pre-existing mitochondria. It requires the coordination of several distinct processes: 1) asymmetric formation of both the inner and outer mitochondrial membranes; 2) synthesis of mitochondrial proteins; 3) synthesis and import of proteins encoded by the nuclear genome; 4) replication of mtDNA; and 5) mitochondrial fusion and fission. Mitochondrial fission requires the activation of the dynamin-related protein-1 (Drp-1) [77], a GTPase that causes the scission of the mitochondrial outer membrane, resulting in the fission of mitochondrial tubules into individual fragments. Drp-1 activity is also linked to cytochrome c release and caspase activation [78].

Mitochondria are under constant cycles of fission and fusion. If subjected to stress, they may be removed by autophagy, and their biogenesis might, in turn, increase to meet the energetic demands. The rates of mitochondrial fission and fusion respond to changes in the metabolism, such that mitochondria become 'more fused' when they are forced to rely on OXPHOS by withdrawing glucose as the carbon source. Fusion is also enhanced by the induction of autophagy, increasing the reliance on OXPHOS via enhanced metabolism of lipids and protein. Alternatively, increased fusion may be necessary to maximise the fidelity of OXPHOS by stimulating the complementation among mitochondria. Autophagy may also evoke a specific stress response referred to as 'stress-induced mitochondrial hyperfusion' or it may inhibit fission to protect mitochondria from autophagic catabolism when their function needs to be preserved [79]. These effects are consistent with a model in which mitochondrial dynamics helps maximise the capacity for OXPHOS under conditions of stress.

MiRNAs have been shown to be involved in the regulation of mitochondrial dynamics. MiR-30 family members have been reported to regulate apoptosis by targeting the mitochondrial fission machinery, inhibiting mitochondrial fission by suppressing the expression of p53 and its downstream target Drp-1 [80]. More recently, MiR-494, whose location is predominantly mitochondrial, has been found to modulate mitochondrial biogenesis by down-regulating the mitochondrial transcriptional factor A (TFAM) and the nuclear transcription factor Forkhead box J3 (FOXJ3) during myocyte differentiation and skeletal muscle adaptation to physical exercise [81].

The removal of damaged mitochondria by autophagy, a process referred to as mitophagy, is critical for maintaining proper cellular functions. Mitophagy regulates the number of mitochondria to match the metabolic or developmental demands [82], and is also a part of the quality control based on the removal of malfunctional mitochondria [82–84]. Malfunctional/damaged mitochondria are removed by autophagy, a process requiring two steps: induction of general autophagy and priming of damaged mitochondria for selective recognition. Recent reports reveal that mitochondrial priming is mediated by the PINK1-Parkin signalling pathway (Parkin-dependent) or by the mitophagic receptors Nix and BNIP3 (Parkin-independent). The induction of canonic autophagy requires ATG proteins and, furthermore, it involves mTOR suppression in response to ROS generated by damaged mitochondria and ATP depletion. Upon mitochondrial membrane depolarisation, PINK1 triggers Parkin translocation from the cytosol to mitochondria where it promotes ubiquitination of the MOM proteins, which may either be degraded by the proteasome or serve as binding partners for the p62 protein. This protein then acts as an adaptor molecule directly interacting with the LC3 protein to recruit autophagosomal membranes to the mitochondria. For Parkin-independent mechanism, damaged mitochondria (particularly under hypoxic condition) increase the expression of the FUNDC1 and Nix proteins, which may in turn recruit autophagosomes to mitochondria by direct interaction with LC3. BNIP3 and Nix (upregulated by oxidative stress and/or hypoxia) can induce mitophagy by triggering mitochondrial depolarisation, which is known to induce mitochondrial removal by autophagy [85].

Recent studies have suggested a role for miRNAs in autophagy, including MiR-101 [86], MiR-204 [87] and MiR-30a [88]. MiRNAs target transcripts of autophagy-related proteins, thereby suppressing their function in autophagy with pathological consequences [89]. For example, down-regulation of MiR-34b/c is an early event in Parkinson's disease [90]. In some tumours, the level of MiR-21 is increased, and it suppresses the expression of PTEN, which regulates the mitophagy-associated PINK1 [91]. Mitophagy has been characterised as a HIF-dependent mechanism [92]. Finally, Hypoxic repression of the mitochondrial function by MiR-210 and ISCU1/2 may trigger mitophagy.

## 7. MicroRNAs and mitochondria-induced cell death

Defective mitochondria can be toxic by generating excessive amounts of ROS, by consuming ATP and by interfering with other metabolic processes. Low levels of damage may be corrected by complementation via the mitochondrial fusion. Damaged mitochondria will affect other mitochondria if they become part of the mitochondrial network unless they are eliminated by autophagy. High levels of ROS can induce apoptosis by affecting mitochondria. Stimulation of the intrinsic mitochondrial apoptotic pathway by ROS and mitochondrial DNA damage promotes the MOM permeabilisation and cytosolic translocation of cytochrome c, the AIF or the Smac/Diablo proteins that trigger/promote caspase-dependent or -independent apoptotic cascade of reactions [93]. In caspase-dependent signalling, cytochrome c forms the apoptosome complex that induces the cleavage of the downstream effector caspases. Additionally, Smac/Diablo antagonises the inhibitory effects of the inhibitor of apoptosis proteins, which promotes caspase activation.

AIF mediates caspase-independent signalling through cytosol-to-nuclear translocation and induction of nuclear chromatin condensation and DNA fragmentation [94]. The mechanism regulating apoptogenic processing and translocation of mitochondrial AIF into the cytoplasm is complex and not fully understood. Permeabilisation of mitochondria, irrespective of whether it occurs before or after AIF cleavage, is an obligatory event in the AIF-mediated apoptotic signalling. Mitochondrial permeabilisation and mitochondrial release of apoptogenic factors are regulated by the permeability transition pore (PTP), a megapore spanning the inner and outer mitochondrial membrane composed by cyclophilin, VDAC and the adenine nucleotide translocase [95]. Mitochondrial apoptogenic factors can be released through the pores in the MOM formed by the pro-apoptotic Bcl-2 family members. Key members of the anti-apoptotic (e.g. Bcl-2, Bcl-$x_L$, and Mcl-1) and pro-apoptotic (e.g. Bax, Bak, Bad, Bim, and Bid) Bcl-2 family of proteins are major players in the MOM permeabilisation and apoptotic susceptibility [96].

MiR-15a and the MiR-16-1 induce apoptosis by regulating the mitochondrial function by affecting multiple oncogenic activities including those of Bcl-2 and Mcl-1. Furthermore, MiR-15a promotes mitochondrial dysfunction resulting in cytochrome c release into the cytoplasm and the dissipation of the mitochondrial membrane potential ($\Delta\Psi_{m,i}$) [97]. MiR-210, MiR-181, and the muscle-specific MiR-1 have been reported to be increased upon apoptotic stimulation and found to be associated with the release of cytochrome c from mitochondria and with $\Delta\Psi_{m,i}$ decrease [98–100]. Recently, several miRNAs have been demonstrated to regulate the expression of members of the Bcl-2 family. MiR-195, MiR-24-2 and MiR-365-2 act as negative regulators of Bcl-2 by direct binding to their cognate sites in the 3′-UTR of the human Bcl-2 mRNA [101–103]. Over-expression of these miRNAs induced dissipation of $\Delta\Psi_{m,i}$ and the release of cytochrome c [104]. Furthermore, MiR-135a, considerably down-regulated in malignant gliomas and correlated with the pathological grading of the neoplasia, can induce mitochondria-dependent apoptosis of malignant gliomas by regulating various genes including STAT6, SMAD5 and BMPR2, as well as affecting the downstream signalling events [105].

Several miRNAs inhibit apoptosis by exerting a protective role. MiR-145 protects from the activation of the mitochondrial apoptotic pathways in cardiomyocytes under oxidative stress by direct targeting of BH3-only proteins such as BNIP3. This protein primarily localising to the MOM functions not only as a sensor of mitochondria to oxidative stress in the cytoplasm, but also as an effector of mitochondria-mediated apoptosis. BNIP3 transduces the apoptotic signals via the activation of pro-apoptotic Bax/Bak proteins, neutralising the anti-apoptotic BH1-4 proteins, and promoting mitochondrial membrane depolarisation by inducing the formation of the PTP. In addition, ectopic expression of MiR-125b partially restored cell viability and inhibited apoptosis induced by temozolomide and camptothecin (CPT), which are promising chemotherapeutic agent for glioblastomas [106,107]. It has been demonstrated that CPT induces apoptosis in cancer cells by MiR-125b-mediated mitochondrial pathways via targeting the 3′-UTRs of Bak1, Mcl-1, and p53 mRNAs [107]. These data clearly document the regulatory role of miRNAs in apoptosis modulation, both positive and negative. The miRNAs involved in mitochondrial biological function are summarised in Table 1.

**Table 1**
MiRNAs involved in mitochondrial function.

| ATP level | Target | Reference |
|---|---|---|
| MiR-15 | ARL2 | [40] |
| MiR-16 | ARL2 | [40] |
| MiR-195 | ARL2 | [40] |
| MiR-424 | ARL2 | [40] |
| MiR-338 | COX IV | [41] |
| *Mitochondrial metabolism* | | |
| MiR-23a/b | GLS | [43] |
| MiR-210 | ETC components | [51] |
| MiR-378 | PGC1β | [63] |
| MiR-126 | IRS-1 | [65,66] |
| *Inhibition of mitochondrial ROS* | | |
| MiR-126 | CAT/SOD2 | [68] |
| MiR-145 | Bnip3 | [71] |
| MiR-335 | SOD2/Txnrd2 | [73] |
| MiR-34a | SOD2/Txnrd2 | [73] |
| *Mitochondria dynamics* | | |
| MiR-30 | Mitochondrial fusion | [80] |
| MiR-494 | Mitochondrial biogenesis | [81] |
| *Mitophagy* | | |
| MiR-101 | | [86] |
| MiR-204 | | [87] |
| MiR-30a | | [88] |
| MiR-21 | PINK1 | [91] |
| MiR-126 | | [68] |
| *Apoptosis* | | |
| MiR-15a | Bcl-2/Mcl-1 | [174] |
| MiR-16-1 | Bcl-2/Mcl-1 | [174] |
| MiR-210 | Bcl-2 | [98] |
| MiR-1 | Bcl-2 | [175] |
| MiR-195 | Bcl-2 | [101] |
| MiR-365 | Bcl-2 | [102] |
| MiR-24 | Bcl-2/XIAP | [103] |

ARL2 = ADP-Ribosylation factor-like 2; COX IV = cytochrome c oxidase IV; GLS = mitochondrial glutaminase; ETC = electron transport chain, PGC1β = peroxisome proliferator activated receptor-γ coactivator 1; IRS-1 = insulin receptor substrate-1; Txnrd2 = thioredoxin reductase 2; SOD2 = superoxide dismutase; CAT = catalase; XIAP = X-linked inhibitor of apoptosis protein.

## 8. MiRNAs link metabolic reprogramming to oncogenesis: micromanagement of tumour suppression

Cancer cells undergo fundamental changes in their metabolism to support rapid growth, adapt to alternative nutrient resources, and compete for these supplies with surrounding normal cells. One of the prominent characteristics of proliferating tumour cells is their capacity to sustain high rates of glycolysis for ATP generation, irrespective of oxygen availability. This phenomenon is known as the Warburg effect, and cancer biology research has revealed some of the molecular mechanisms responsible for the cancer phenotype [8]. The Warburg effect was originally proposed to be a result of an impairment of oxidative metabolism [108]. However, in contrast to earlier perceptions about 'mitochondrial dysfunction' as a key driver of such glycolytic 'decoupling', a growing body of evidence shows that, in fact, oxidative phosphorylation is preserved, albeit at a lower level, in many if not all types of cancer cells [109–111]. Although lactate dehydrogenase A (LDHA) is overexpressed in several tumours [112–114] it has been shown that LDH-silenced cancer cells maintain a substantial capacity to produce ATP by OXPHOS [115].

Tumours are often characterised by a general decline in miRNA expression [116], and recent evidence indicates their tumour suppressive role. Accordingly, the interrelation between deregulated miRNAs and imbalanced signalling pathways largely contributes to abnormal cell metabolism and carcinogenesis. Major factors and pathways involved in metabolic reprogramming include Akt, HIF-1α and c-MYC.

Activation of the phosphatidyl inositol-3 kinase (PI3K)/Akt pathway is one of the most common events in spontaneous human cancers, and it has been shown to alter metabolism and to promote the flow of precursors into anabolic pathways [117]. Akt stimulates glycolysis by increasing the expression and membrane translocation of glucose transporters, and by phosphorylating key glycolytic enzymes, such as hexokinase [118], via the inhibition of the Forkhead box O-class 1 (FOXO1) transcription factor. FOXO1 is a key downstream effector of the Akt pathway, functionally inactive due to its phosphorylation by activated Akt in a variety of cancers. FOXO1 inactivation favours enhanced cell survival, cell proliferation, and susceptibility to stress, while its

activation leads to apoptosis, cell cycle arrest and stress resistance in various tissues [119–121].

MiR-126, frequently lost in colorectal cancers [122], gastric cancer [123], lung cancers [124], breast cancer [125], and MM [126], impedes tumour cell growth by targeting the p85b subunit of PI3K [122]. MiR-126 negatively regulate IRS1 [65], an important inhibitor of FOXO1 via its Akt-mediated phosphorylation [127,128]. Ectopic MiR-126 has been found to re-activate FOXO1 via the inhibition of the IRS1/Akt pathway. Consistent with this, MiR-126 induced nuclear translocation of FOXO1 in both non-malignant mesothelial and MM cells (Fig. 2A, B), resulting in increased expression of genes involved in the glucose metabolism and mitochondrial function [68]. MiR-126 induced the expression of phosphoenolpyruvate carboxykinase (PCK1), which is a main control point for the regulation of gluconeogenesis (Fig. 2C). Substrate level phosphorylation occurs in glycolysis, where phosphoenol pyruvate is converted to pyruvate, which then enters the TCA cycle. Under these conditions, increased glycolysis is an important early compensatory mechanism to produce ATP (Fig. 2D). Further, cells expressing MiR-126 feature high levels of mitochondrial SOD2 and CAT, also regulated by FOXO1 [68,121]. Enhanced ROS production in cancer drives the onset of aerobic glycolysis, with lactate and ketone production promoting mitochondrial biogenesis and anabolic growth of tumour cells. Alleviation of mitochondrial oxidative stress via enhanced expression of antioxidant enzymes targeted to mitochondria was found to be sufficient to lower tumour severity and to considerably reduce the tumour burden, linking MiR-126 to the suppression of the onset and progression of cancer.

Akt activates ACL, promoting the conversion of mitochondria-derived citrate to acetyl-CoA for lipid synthesis [129]. Therefore, the re-programming of mitochondrial citrate metabolism is a central aspect of the PI3K/Akt activity [130]. It has been demonstrated that cells under conditions of hypoxia with defective mitochondria primarily utilise glutamine to generate citrate and lipids through reductive carboxylation (RC) of α-ketoglutarate by isocitrate dehydrogenase-1 (IDH1) or -2 (IDH2) [131,132]. There is evidence to support the hypothesis that RC may be triggered by deficient pyruvate oxidation in the mitochondria and the subsequent reduction in citrate levels. Restoration of citrate levels was found to inhibit RC and suppress the growth of renal carcinoma cells as mice xenografts [133]. Indeed, low citrate levels were found in MM, suggesting that these cells use glutamine metabolism through RC to support the efficient carbon utilisation for anabolism and growth. Ectopic expression of MiR-126 restored the low citrate levels by inhibiting ACL. This mechanism favours glucose oxidation to produce energy rather than convert it into precursors for cellular biosynthesis [68].

Hypoxia selects cells with a fundamental metabolic adaptation in which glycolysis is uncoupled from the mitochondrial respiratory

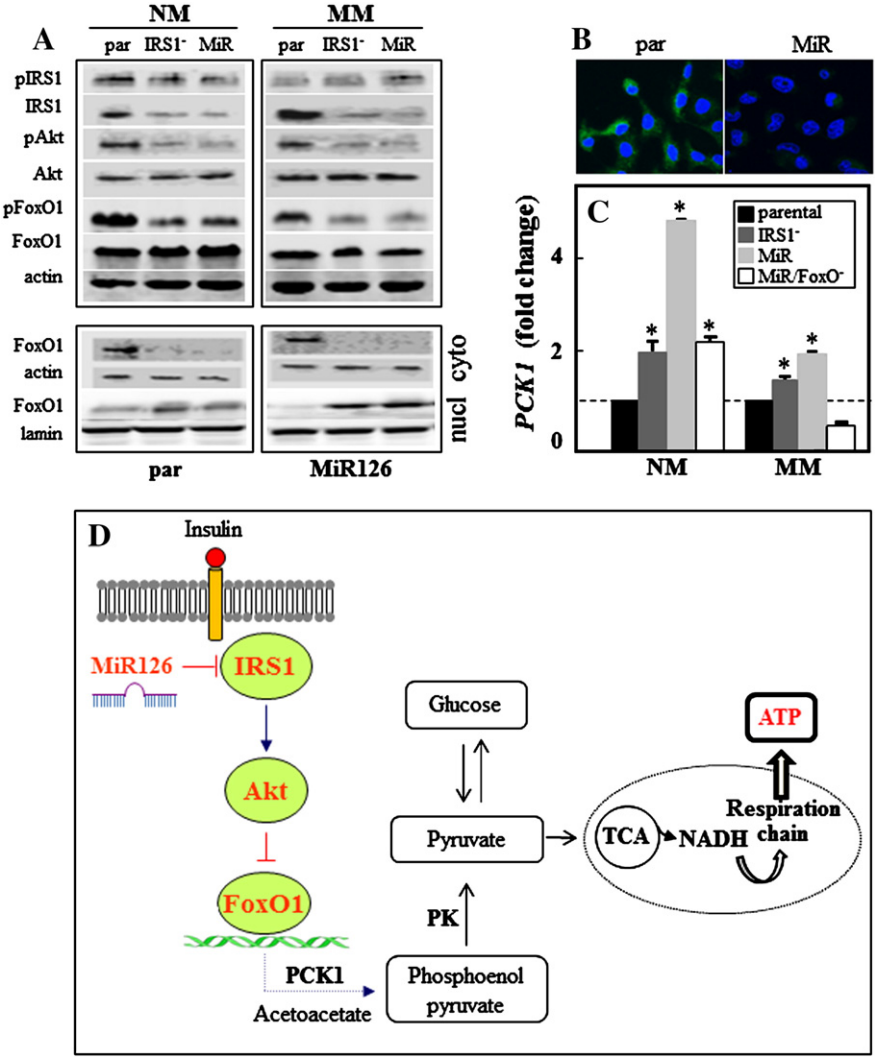


**Fig. 2.** MiR-126 alters the IRS1 pathway-dependent mitochondrial function and expression of FOXO1-dependent genes. MiR-126 was found to suppress IRS1 with ensuing inhibition of Akt (A). This causes the failure of FOXO1 sequestration in the cytosol (B), which results in the increase in the gluconeogenesis gene PCK1 (C) yielding increased pyruvate accompanied by a glycolytic shift. Panel D depicts the proposed role of MiR-126. IRS1$^{-}$ = IRS1-silenced, FOXO1$^{-}$ = FOXO1-silenced. '*' indicates significantly different values with $p < 0.05$.

chain and becomes the main ATP source [134]. Under hypoxic conditions, the hypoxia-inducible factor (HIF) is stabilised and translocated into the nucleus, activating a broad array of downstream genes involved in glycolysis, lactate production and lactate/proton extrusion, angiogenesis, metastasis, and iron metabolism [135]. In addition, several studies have shown that *de novo* synthesis of HIF is regulated through oxygen-independent mechanisms involving oncogene activation (e.g., p53, PTEN, and VHL) and a variety of growth factors (e.g., HGF and EGF) [136,137,138]. The transcription factors HIF-1α and HIF-2α have been established as master regulators of the hypoxic programme and tumour phenotype [139]. HIF-1α plays a key role in the re-programming of cancer metabolism by activating transcription of genes encoding glucose transporters and glycolytic enzymes, which take up glucose and convert it to lactate. Pyruvate represents a critical metabolic control point, as it can be converted to acetyl-CoA by pyruvate dehydrogenase (PDH) for entry into the TCA cycle or it can be converted to lactate by LDHA. Pyruvate dehydrogenase kinase (PDK), which phosphorylates and inactivates the catalytic domain of PDH, is activated by HIF-1α [140,141]. As a result of PDK activation, pyruvate is actively shunted away from the mitochondria, which reduces the flux through the TCA cycle, thereby reducing delivery of NADH and $FADH_2$ to the ETC. MYC, which is activated in 40% of human cancers, cooperates with HIF-1α to activate transcription of PDK to amplify the hypoxic response [142]. This orchestrated upregulation of several enzymes ensures the diversion of pyruvate to lactate production.

Several miRNAs that mediate metabolism re-programming can contribute to HIF-1α expression and stabilisation [143], (Fig. 3). In chronic lymphocytic leukemia (CLL), the stabilisation of HIF-1α under normoxia is mediated by MiR-92-1, which targets the von Hippel–Lindau (VHL) tumour suppressor [144], an E3 ubiquitin ligase involved in the HIF-1α degradation in the presence of oxygen. Under of decreased oxygen availability, MiR-424 upregulation in endothelial cells stabilises HIF-1α through the targeting of cullin 2, a scaffolding protein critical to the assembly of the ubiquitin ligase system [145]. Recently, MiR-126 was found to induce HIF-1α activation and stabilisation. The stability and transcriptional activity of HIF-1α and HIF-2α is regulated by two oxygen-dependent events that are catalysed by three HIF prolyl 4-hydroxylases (PHDs) and one HIF asparaginyl hydroxylase. Certain TCA cycle intermediates and related compounds have recently been reported to inhibit activities of PHDs [146]. Fumarate and succinate were identified as inhibitors of all three PHDs, as well as oxaloacetate and citrate [147]. Although the PHDs are the proximal regulators of HIF-1α protein stabilisation, considerable evidence has emerged to suggest that mitochondrial ETC is involved in oxygen sensing and would therefore respond to changes in oxygen levels [45].

Several studies have reported that genetic and pharmacological inhibition of the ETC, including rotenone (which inhibits complex I), myxothiazol and stigmatellin (which inhibit complex III), block the hypoxic stabilisation of HIF-1α [148–150]. It was found that cells depleted of their mitochondrial DNA ($\rho^0$ cells) failed to stabilise HIF-1α in response to hypoxia, suggesting that hypoxic stabilisation of HIF-1α requires functional ETC [150]. ROS have been proposed to participate in the signal transduction process mediating the stabilisation of the transcription factor HIF-1α during hypoxia. In moderate hypoxia,

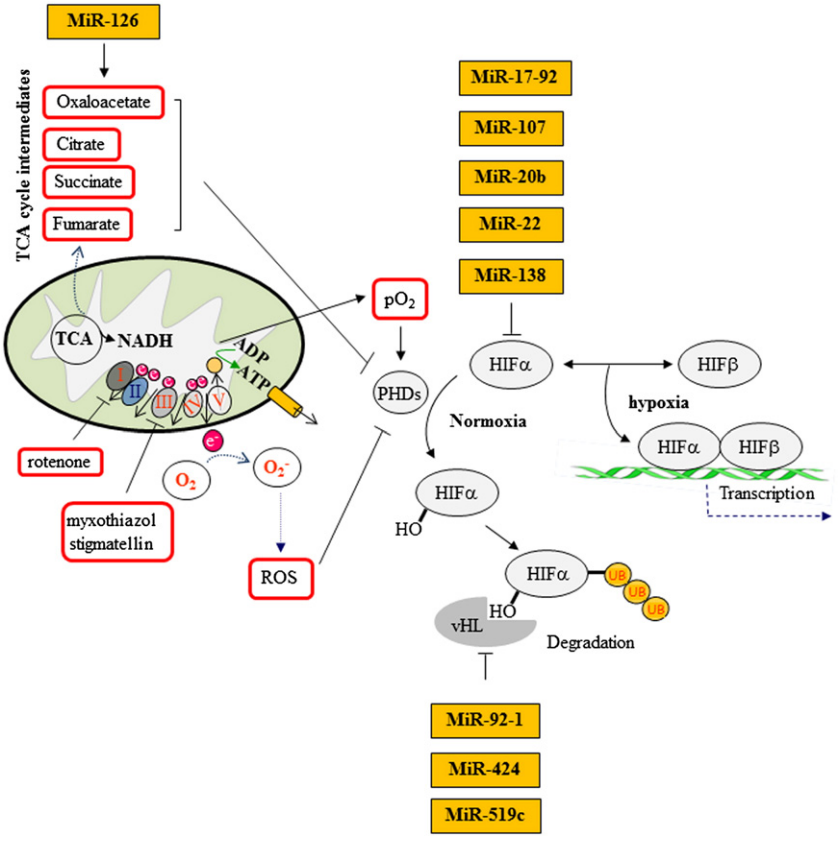


**Fig. 3.** Regulation of the transcription factor HIF by mitochondrial-signalling and miRNAs. HIF is a heterodimeric complex formed by two subunits, HIFβ, which is constitutively expressed, and HIFα, which is highly susceptible to oxygen-dependent degradation. In the presence of oxygen (normoxia), HIFα is hydroxylated on proline residues by prolyl hydroxylase domain proteins (PHDs). This oxygen dependence allows HIFα recognition by the von Hippel–Lindau (VHL) protein, which targets HIFα for proteasome degradation. In hypoxia, PHDs are inhibited, since they utilize oxygen as a co-substrate. Inhibition of electron transfer chain (ETC) at complex I and complex III can cause a build-up of TCA cycle intermediates and ROS that inhibit PHDs, stabilizing HIF-1α. Alternatively, mitochondria signal to PHDs indirectly through their consumption of oxygen. The decreased mitochondrial oxygen consumption due to ETC inhibition could help maintain cytosolic $pO_2$ and, consequently, PHD activity. MiRNA-targeting elements of mitochondria and the hypoxia-signalling pathway are involved in HIFα stabilization and its transcriptional activity.

mitochondria stimulate the production of cellular ROS, which inhibit PHD activity and HIF-1α degradation [147]. However, controversy exists regarding HIF regulation by mitochondrial ROS. It has been suggested that mitochondria signal to PHDs indirectly through their consumption of oxygen and not through ROS production. The decreased mitochondrial oxygen consumption due to ETC inhibition could help maintain cytosolic $pO_2$ and, consequently, PHD activity [147]. Despite recent advances in our knowledge of HIF regulation by mitochondrial ROS, many questions remain unanswered. Most probably, a combination of oxygen, iron, TCA cycle intermediates and ROS signalling might optimise hydroxylation of the HIF-1α protein [151] (Fig. 3).

Several studies prove that HIF is a critical down-stream target of the VHL-tumour suppressor and established that activation of HIF target genes can promote tumourigenesis *in vivo* [152–154]. It has been demonstrated that a specific set of miRNAs is involved in the inhibition of HIF-1α expression. It was reported that the MiR-17–92 cluster, MiR-107, MiR-20b and MiR-22 modulate tumour growth by inhibiting HIF-1α expression [155–158]. In addition, MiR-519c-over-expressing cells exhibited dramatically reduced HIF-1α levels, followed by suppressed tumour angiogenesis, growth, and metastasis [159]. More recently, MiR-138 was found to directly target HIF-1α reversing the HIF-1α-mediated induction of ovarian cancer cell invasion [160].

Although HIF-1α is usually thought to promote tumour growth, there is precedence for its function as a tumour suppressor. HIF-1α expression correlates with the early stage of tumour formation or decreased patient mortality in certain cancers [161]. Conceivably, some of the genes that are preferentially activated by HIF-1α decrease renal carcinoma aggressiveness. In this context, three genes induced by HIF-1α activation such as TXNIP, KCTSII and PLAGLI have been implicated as tumour suppressors [162–164]. Moreover, HIF-1α is engaged in collateral signalling pathways such as those involving c-MYC and NOTCH. HIF-1α, via a variety of mechanisms, can inhibit c-MYC activity under specific circumstances [165,166].

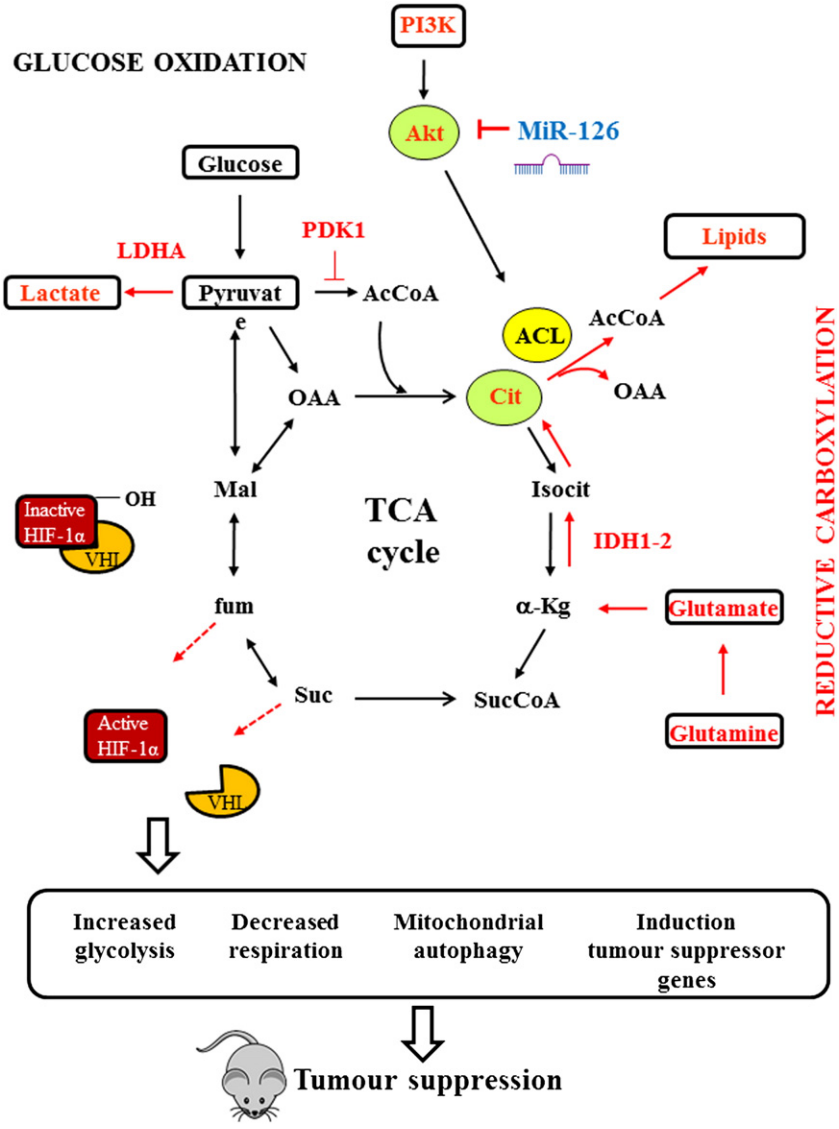


**Fig. 4.** MiR-126 affects the mitochondrial metabolism inducing tumour suppression. Tumour cells convert glutamine to generate glutamate that is catabolised by the TCA cycle for the production of citrate and ATP through reductive carboxylation (RC) of α-ketoglutarate (αKg) by isocitrate dehydrogenase 1–2 (IDH1–2) (red pathway). In the cytosol, citrate (Cit) is cleaved by ACL to produce acetyl-CoA (AcCoA) used for endogenous synthesis of lipids. MiR-126 affects the mitochondrial citrate metabolism by inhibiting the Akt pathway to restore the TCA cycle for the synthesis of ATP. This mechanism favours glucose oxidation to produce cellular energy rather than converting it into other macromolecules for cellular biosynthesis (black pathway). MiR-126 was found to induce HIF-1α activation and stabilisation. Intermediates of the TCA cycle such as fumarate (fum), succinate (suc), oxaloacetate (OAA) and citrate were identified as inhibitors of prolyl hydroxylase domain protein (PHD) enzymes that are required for the binding of the von Hippel–Lindau (VHL) protein, which recruits a ubiquitin ligase that target HIF-1α for proteasomal degradation. Activated HIF-1α induces transcription of target genes encoding proteins involved in the metabolic re-programming of cancer cells leading to tumour suppression.

HIF-1α also activates transcription of the gene encoding the BH3 protein BNIP3, which induces selective mitochondrial autophagy by competing with Beclin 1 for binding to Bcl-2, thereby liberating Beclin-1 to trigger autophagy [92]. BNIP3-induced autophagy was originally associated with hypoxic cell death, but studies with HIF-1α-null mouse embryonic fibroblasts have revealed that mitochondrial autophagy is an adaptive response that maintains cell viability under conditions of prolonged hypoxia.

Taken together, the prototypic tumour suppressor MiR-126 has been found to be induced by hypoxia, which in turn activates and stabilises HIF-1α. This phenomenon has a profound effect on cancer cell metabolism resulting in the inhibition of RC favouring glucose oxidation to produce energy. Robust formation of autophagic vacuoles was observed in cancer cells over-expressing MiR-126 as a result of their aberrant bioenergetic status. Ectopic MiR-126 induces the loss of malignancy and the failure of MM cells to induce tumours; these events were not observed in HIF-non-responsive malignant cells and in MM cells lacking Beclin-1, a critical regulator of autophagosome formation. The proposed molecular mechanism how MiR-126 suppresses tumour initiation is summarised in Fig. 4.

## 9. Conclusions and further perspectives: targeting potential of metabolism-related miRNAs

Clinical evidence has linked cell metabolism with cancer outcome. Together, these observations have raised interest in targeting metabolic enzymes for cancer therapy, but they have also raised concerns that these therapies could have deleterious effects on normal cells. It has been identified through gain- and loss-of-function studies that miRNA deregulation could have therapeutic effects by suppressing the growth of cancer cells without affecting the physiology of normal cells [167]. These findings provide direct evidence that synthetic miRNA mimics can be systemically delivered to the mammalian cells and support the development of miRNA-based therapeutics [168]. The potential therapeutic application of miRNAs would include two strategies. One strategy is directed toward the gain-of-function approach and aims to inhibit oncogenic miRNAs by using miRNA antagonists, such as anti-miRNAs, locked-nucleic acids (LNA) or the so called antagomiRs. The second strategy, miRNA replacement, involves the re-introduction of a tumour suppressor miRNA mimetic to restore a loss-of-function phenotype. However, there are some concerns about potential toxicity, especially under conditions where the therapeutic delivery of miRNA mimetics will also lead to the accumulation of the exogenous miRNA in normal cells. These toxic effects might be the result of overloading RISC with the exogenous miRNA, thereby competing with endogenous miRNAs necessary for normal cellular physiology, and/or hyper-activating cellular pathways that will also reduce the viability of normal cells. While these suppositions are well-founded and ought to be considered, *in vivo* evidence for toxicity induced by miRNA mimics is still lacking. Mouse studies that evaluated the therapeutic delivery of tumour suppressor miRNAs failed to reveal adverse events associated with the miRNA and suggest that delivery of miRNA was well tolerated by normal tissues [169–171].

One recent study proposed the systemic delivery of miRNA mimics in complex with neutral lipid emulsion, showing therapeutic benefits in lung cancer mouse model [172]. The main challenge for successful translation into the clinic remains *in vivo* delivery which will be the focus of future therapeutic development efforts to harness the full potential of miRNAs. Notwithstanding these reservations, the miRNA-based therapy, in particular given the multiple targets for each miRNA, offers some promise. This can be reconciled with a recent finding that considerable intra-tumoural heterogeneity exists, suggesting that established tumour therapies may be efficient only in a portion of the tumour, while its other parts will 'escape' [173].

## Acknowledgements

This work was supported in part by grants from the National Health and Medical Research Council, Australian Research Council, Clem Jones Foundation and the Czech Science Foundation (P301/10/1937) to J.N., and by the Griffith University Fellowship to L.F.D.

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