Gene Expression **2024** vol. 000(000) | 000–000 Epub
DOI: 10.14218/GE.2023.00134

Review Article

Deciphering the Role of Mitochondrial DNA Targeted Therapy in Hepatic Cell Carcinoma

Madhunika Agrawal[1] and Satyam Kumar Agrawal[2*]

[1]*Cellsinvitro Lifesciences Pvt. Ltd., SAS Nagar, Punjab, India;* [2]*Centre for in vitro Studies and Translational Research, Chitkara School of Health Sciences, Chitkara University, Rajpura, Punjab, India*

Received: October 04, 2023 | **Revised:** November 03, 2023 | **Accepted:** March 12, 2024 | **Published online:** May 28, 2024

Abstract

Liver cancer, also identified as hepatic cell carcinoma, is the fifth most prevalent kind of malignancy globally and the fourth foremost cause of cancer-associated mortality. The development and progression of liver cancer are complex processes that involve multiple genetic and environmental factors. As the diagnosis of liver cancer is still worse, with late-stage patients facing a less than 20% 5-year survival rate, there is a critical need for the development of new and effective therapeutic approaches for liver cancer. Mitochondrial alterations and mitochondrial DNA (mtDNA) mutations have long been associated with cancer pathogenesis, including liver cancer. These alterations not only disrupt cellular bioenergetics but also deteriorate the situation by modifying tumor suppressors and oncogenic proteins. Excessive reactive oxygen species generation and flaws in mitochondrial enzymes are among the factors responsible for mitochondrial dysfunction. Additionally, perturbed microRNA levels have also been linked to mtDNA dysfunction and reactive oxygen species generation. Various pharmacological approaches to target mitochondrial dysfunction and mtDNA mutations in cancer have been proposed as potential therapeutic strategies. These approaches include targeting the electron transport chain, which is responsible for the production of adenosine triphosphate in the mitochondria, or transcriptional inhibition of various proteins involved in the mitochondrial biogenesis pathway. Overall, mtDNA is a crucial component of the cell, and alterations in mtDNA make it an attractive target for therapeutic interventions. Hence, we advocate that understanding the role of mtDNA in cancer pathogenesis is important for the development of targeted therapies for these disorders.

Introduction

Mitochondria are cellular organelles that are present in the majority of eukaryotic cells. They play an important role in energy production, metabolism, and cell signaling. Eukaryotic mitochondria have their own DNA, which is referred to as mitochondrial DNA (mtDNA), and it encodes various critical proteins involved in oxidative phosphorylation and the synthesis of adenosine triphosphate (ATP).[1] mtDNA is a tiny, circular, double-stranded genome, measuring about 16.6 kbp in humans, and is maternally inherited,[2] passed from mother to child. This is due to the fact that the egg cell supplies the growing embryo with the vast majority of its cytoplasm, including its mitochondrion.

Keywords: Mitochondrial DNA; Mitochondrial biogenesis; Anticancer agents; AMPK signaling; Hepatic cell carcinoma; Natural products.

***Correspondence to:** Satyam Kumar Agrawal, Centre for in vitro Studies and Translational Research, Chitkara School of Health Sciences, Chitkara University, Rajpura 140401, Punjab, India. ORCID: https://orcid.org/0000-0001-8127-6380. Tel: +91-89-20139002, E-mail: satyamka@gmail.com; satyam.ka@chitkara.edu.in

How to cite this article: Agrawal M, Agrawal SK. Deciphering the Role of Mitochondrial DNA Targeted Therapy in Hepatic Cell Carcinoma. *Gene Expr* 2024;000(000):000–000. doi: 10.14218/GE.2023.00134.

The mtDNA is partitioned into two distinct strands, namely the heavy (H) strand and the light (L) strand, which may be differentiated based on their nucleotide content. The H-strand has higher guanine content, while the L-strand shows a higher cytosine content. mtDNA is structured into three regions: the coding region, the D-loop region, and the non-coding region. The coding region contains 37 genes responsible for encoding proteins, transfer RNAs (tRNAs), and ribosomal RNAs (rRNAs), crucial for mitochondrial protein translation. These proteins derived from mtDNA are essential for the electron transport chain (ETC), where they regulate the oxidative respiratory chain and preserve its functional integrity. The D-loop region serves as the regulatory area of mtDNA, hosting replication origins and transcription promoters. The non-coding region, between the coding and D-loop regions, contains regulatory elements important for the replication and transcription of mtDNA.[3] mtDNA is a kind of DNA that exists in multiple copies within cells. Their number of copies may range from 100 to 10,000, and this variation is influenced by cellular energy needs governed by mitochondrial biogenesis.[4] The process encompasses mitochondria proliferation which includes the development of inner and outer mitochondrial membranes, the initiation and movement of proteins encoded by nuclear genes, and the

replication of mtDNA. The whole process is governed by several components, particularly peroxisome proliferator-activated receptor γ coactivator-1α (PGC-1α), nuclear respiratory factors (NRFs), and mitochondrial transcription factor A signaling pathways.[5] Mitochondrial transcription factor A, a member of the high mobility group box subfamily, is the principal nuclear protein that interacts with the promoter region of mitochondria and regulates the replication of mtDNA.[6] PGC-1α, a member of the transcriptional cofactor family, operates as a facilitator of mitochondrial biogenesis, exerting a positive regulatory effect. NRF-1/2 continues to function as a transcription factor downstream of PGC-1α, regulating mitochondrial proteins encoded by mtDNA. Several crucial upstream regulators, such as AMPK (AMP-activated protein kinase), and downstream effectors including sirtuins (SIRT), NRF-1/2, mTOR, and HIF-1α, have been shown to activate PGC-1α, promoting mitochondrial biogenesis.[5,7,8] AMPK is responsible for keeping a check on mitochondrial homeostasis by direct phosphorylation of the mitochondrial fission factor, controlling mitochondrial fission through dynamin-like protein-1.[9] AMPK is considered a tumor suppressor by negatively regulating aerobic glycolysis, known as the Warburg effect, in cancer cells, ultimately suppressing tumor growth.[10] Given the pivotal role of mitochondrial damage in the initiation and progression of many diseases, comprehensive knowledge of the underlying processes and regulatory signals involved in biogenesis is essential. Therefore, this review will concentrate on the significance of mtDNA in the context of liver cancer. It will also summarize how mtDNA genes, proteins, and microRNA (miRNA) are implicated in mtDNA dysfunction. Additionally, the study will discuss existing research on phytochemicals and chemicals targeting mitochondrial biogenesis and mtDNA disruption in liver cancer.

mtDNA in disease pathology

mtDNA is a significant part of cellular bioenergy synthesis and metabolic reactions, and thus alterations in mtDNA have been associated with various diseases, particularly mitochondrial diseases. Mitochondrial diseases include a collection of disorders resulting from genetic abnormalities in either mtDNA or nuclear DNA, which subsequently impair mitochondrial functionality. These disorders are characterized by persistent depletion of cellular energy, leading to noticeable disease traits due to the inability to fulfill cellular energy requirements. The clinical range of mitochondrial disease is rather wide, often involving tissues with higher metabolic requirements, such as the central nervous system and cardiac tissue.[11] Moreover, empirical evidence has shown that alterations in mtDNA have a significant role in the pathogenesis of several diseases, including neurological disorders, diabetes, and the aging process.[12,13] mtDNA alterations have been extensively involved in the etiology of Parkinson's disease, Alzheimer's disease, and Huntington's disease.[14] These mutations can affect mitochondrial function, leading to increased oxidative stress, which contributes to the development of these disorders. Besides, these alterations can significantly impact cellular functioning and downstream events too. The comprehension of the involvement of mtDNA in the pathophysiology of diseases has significant importance in the advancement of focused therapeutic approaches for various ailments. Further, it has been shown that modifications in mtDNA have a significant impact on the proliferation, advancement, and metastasis of cancer cells, as well as their interactions with the immune system and the tumor microenvironment (TME).[15] Mutations in mtDNA have been observed in various cancer types, including breast, ovarian, colon, and rectum cancer, affecting the functionality of the ETC and leading to oxidative phosphorylation dysfunction and increased reactive oxygen species (ROS) production, promoting cancer development. Moreover, it has been observed that mutations in mtDNA contribute to the development of several types of cancer in different organs, including the lungs, stomach, and liver. In addition to mutations, changes in mtDNA copies are linked to the cancer occurrence and unfavorable prognosis across diverse cancer types, such as lung, ovarian, and breast cancer. Additionally, mtDNA damage and apoptosis are interconnected processes (Fig. 1). Apoptotic factor APAF-1 can inhibit anti-apoptotic proteins, allowing pro-apoptotic factors to cause membrane permeabilization in the outer mitochondrial membrane, leading to cytosolic release of oxidized mtDNA. This exudence of distorted mtDNA can trigger various receptors, including Toll-like receptors and inflammasomes.[15] mtDNA defects can also result in reduced levels of respiratory complexes along with ATP content, which leads to mitigation of cell growth and induction of apoptotic activities.[16] Hence mtDNA defects contribute to mitochondrial deregulation, leading to increased inflammation, apoptosis, and ultimately, cell death.[17]

mtDNA alteration in liver cancer

Hepatic cell carcinoma (HCC), also known as liver cancer, ranks as the fifth most prevalent form of cancer on a global scale.[18] Furthermore, it stands as the fourth leading cause of cancer-related mortality.[19] The etiology and pathogenesis of liver cancer are complex, involving multiple genetic and environmental factors. The prognosis for liver cancer is characterized by a lower survival rate in cases of advanced-stage illness, owing to the poor prediction of liver cancer propagation.[20] Hence, it is essential to prioritize the development of novel and efficacious therapeutic strategies for the treatment of liver cancer. HCC, the predominant type of principal liver cancer, often exhibits abnormalities in mtDNA. The two most prevalent forms of mtDNA abnormalities seen in HCC are somatic point mutations and depletions.[21] Previous studies have shown a notable association between decreased mtDNA quantity in HCC and adverse clinical factors, including increased tumor dimensions, liver cirrhosis, and reduced 5-year survival rate.[22] Furthermore, numerous experimental and clinical investigations have shown a significant correlation between mtDNA and HCC. The precise mechanism behind the contribution of mtDNA alterations and mitochondrial deregulation to the advancement of HCC remains incompletely elucidated. Hypermethylated mtDNA is reported to deregulate mitochondrial genes and metabolism in liver cancer cell lines.[23] Frequent occurrences of mutations in the D-loop regulatory region of mtDNA have been observed in HCC patients.[24] Furthermore, the presence of circulating mtDNA in the bloodstream is related to the disease.[25] These alterations may impact the expression and functionality of mitochondrial proteins that are implicated in oxidative phosphorylation and ATP production.[22,26] Consequently, this can result in metabolic reprogramming and changes in energy metabolism within cancerous cells.

Furthermore, mitochondrial dysfunction resulting from mtDNA mutations may contribute to DNA damage, genomic instability, and altered signaling pathways that promote cancer cell survival and proliferation. Moreover, mitochondrial dysfunction may also affect the immune response and contribute to tumor immune evasion.[27] Nevertheless, a growing body of evidence suggests that the unintended release of mtDNA into the cytoplasm serves as a damage-associated molecular pattern, activating the innate immune response and eliciting an inflammatory reaction.[28] The in-

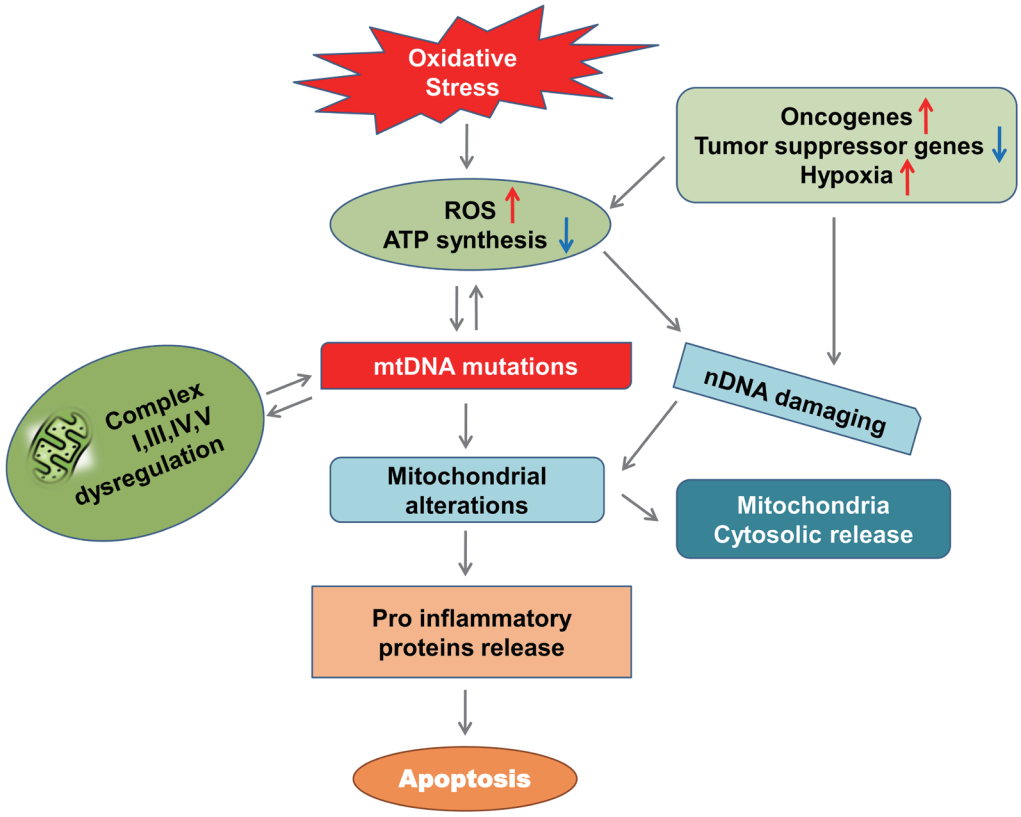

Fig. 1. Schematic illustration of mtDNA damage and apoptotic activation. ATP, adenosine triphosphate; mtDNA, mitochondrial DNA; nDNA, nuclear DNA; ROS, reactive oxygen species.

nate immune system functions as the first barrier against infections, effectively identifying and removing them, playing a crucial role in preserving internal equilibrium and being strongly associated with the initiation of inflammation. The potential activation of self-mediated innate immune responses cannot be disregarded when considering the release of mtDNA by liver cells, which have the largest mitochondrial content in the human body, under pathological situations.[28]

Additionally, AMPK is reported to promote innate immunity and antiviral defense by modulating the signaling pathway of Stimulator of Interferon Genes (STING).[29] Emerging research has shown that the use of STING pathway agonists for regulatory purposes might potentially counteract the progression of HCC and serve as a viable therapeutic approach.[30] Undoubtedly, the cGAS-STING pathway plays a crucial role in the evolution of HCC. A publication highlights an association between diminished levels of STING in tumor tissues and worse prognosis among patients with HCC.[31] Moreover, the re-establishment of *IFI16* expression in neoplastic cells significantly facilitates tumor regression, although this process may be somewhat hindered by the suppression of p53 signaling activation or the induction of the inflammasome.[32] Along with this, the Toll-like receptor 9 has received significant attention in the field of liver diseases, with several studies dedicated to its investigation. Hypoxia-induced translocation of mtDNA and high mobility group box 1 into the cytoplasm of cancer cells activates Toll-like receptor 9, promoting tumor cell proliferation.[33] AMPK upregulation was seen as involved in increasing the drug sensitization in HCC.[34] Its activation was observed as a result of the knockdown of a critical enzyme of the pentose phosphate pathway, 6-phosphogluconate dehydrogenase, in HCC.[35] In general, the involvement of mtDNA modifications in HCC is intricate and diverse, necessitating further investigation to comprehensively comprehend the fundamental processes and establish efficacious treatment approaches focusing on liver cancer's mitochondrial function.

mtDNA genes and their implications in liver cancer

Mitochondria are considered the powerhouse of cells and are involved in various metabolic and cell survival activities, as they possess their own genome. This genome contains 13 polypeptides associated with the ETC, along with 22 tRNA genes and 2 rRNA genes, facilitating protein synthesis inside the mitochondria. The remaining protein subunits participate in the ETC complexes. A study was conducted on a significant liver cancer C57BL/6J mice strain model to access the whole genomic sequence. The overall length of the mitogenome was determined to be 16,308 bp, bearing protein-coding genes (13), rRNA genes (2), tRNA genes (22), and the D-loop region (1). Additionally, evidence of mutations was documented.[36] Similar sequencing details were also reported by Zhang *et al*.[37]

The ATP synthase enzyme complex plays a pivotal role in the synthesis of ATP, which serves as the principal form of cellular energy. Situated inside the internal mitochondrial membrane, the ATP synthase enzyme plays a crucial role in the ultimate stage of oxidative phosphorylation, converting glucose and other nutrients into ATP, the primary energy currency of the cell.[38] Recent scien-

tific research has revealed that ATP synthase has a significant role in cancer development and progression. Overexpression of ATP synthase has been associated with increased cancer cell growth, propagation, metastasis, and resistance to chemotherapy. Furthermore, ATP synthase is involved in modulating the TME. The involvement of ATP synthase in the control of the pH of the TME has been determined, which potentially influences the growth and viability of cancerous cells. Additionally, ATP synthase has been implicated in modulating immune response inside the TME has also been shown. Cytochrome c oxidase (COX) serves as a crucial component of the mitochondrial ETC, playing a pivotal role in ATP synthesis and the formation of ROS.[39] During the process of apoptosis, it oxidizes cytochrome c for its cytosolic release, and thus its dysfunction may impact the apoptotic process. NADH dehydrogenase, referred to as complex I, is an essential component of the mitochondrial ETC. The process under consideration is accountable for transferring electrons from NADH to ubiquinone. This electron transport results in the creation of a proton gradient along the inner mitochondrial membrane. This proton gradient is responsible for facilitating ATP synthesis. Additionally, this process has been linked to the formation of hepatic malignancies.[40] The mitochondrial ribosome consists of two distinct subunits, the large subunit (mtLSU) and the small subunit (mtSSU), composed of rRNA and protein components. Dysregulation of mitochondrial ribosome biogenesis and function has been observed in the pathogenesis and advancement of liver cancer.[41] Mitochondrial transfer RNA (mt-tRNA) is responsible for delivering amino acids to the ribosome during protein synthesis,[42] and mutations in mt-tRNA are linked to diverse mitochondrial diseases, including certain forms of liver disease.

ATP synthase

The administration of Etomoxir resulted in an elevated oxidative state and impaired mitochondrial metabolic system, as evidenced by a notable reduction in reduced glutathione, the reduced/oxidized glutathione ratio, mitochondrial membrane potential, and ATP levels. Additionally, there was a concurrent increase in oxidized glutathione and superoxide generation.[43] The role of ATP synthase in cancer development was also substantiated by preparing an antisense of hAS-e and exploring its potential to inhibit cell proliferation via the MAPK pathway.[44] Chrysophanol-induced necrotic cellular death was associated with depletion in ATP levels, resulting in a reduction of energy production.[45] The synthetic metal complexes (1 and 2) derived from α-N-heterocyclic thiosemicarbazones were shown to cause mitochondrial damage, ROS generation, a decrease in ATP content, and mitochondrial membrane depolarization.[46]

COX

The role of COX has been explored from a very early time. Long back in 1990, thyroid hormone and/or dexamethasone were given for the treatment of hepatoma. This experimentation led to an estimated 4-fold upregulation of various RNAs ciphered in the mitochondrial genomic sequence, which includes subunit II of COX. The study inferred the potent role of modification in transcription and RNA stability in elevated mitochondrial genomic expression.[47] Then, in 1998, hepatitis B virus (HBV) infection and its role in altering cellular metabolic energy, resulting in mitochondrial dysfunction were associated with the *ATP synthase 6* and *COX III* genes.[48] A study conducted on HL-7702 cells displayed that co-localization of *HBx* and *COX III* resulted in an increase in mitochondrial function as well as ROS production, which was further linked to hepatic cell carcinogenesis associated with HBV.[49] Single nucleotide polymorphisms (SNPs) aggregated mtDNA is disposed to cancer development. Wang *et al*.[50] have studied SNPs in the COX genes of the mtDNA coding region in hepatic cancer patients and healthy controls. The 9545G allele was observed to be a risk for malignant conditions. Recently, Zhao and his co-workers have investigated the amplified expression of several genes, including from the COX family (*mt-CO1*) in HCC. A positive correlation between elevated gene expression and poor survival rates was observed.[51] A 9-base pair deletion polymorphism in mitochondrial CoII/tRNA was studied to have pathogenic activities in hepatic carcinoma.[52]

mt-rRNA

Polymorphism in 12S rRNA (mt-RNR1) G709A has been considered to have an impact on metastasis-free survival and general survival. A study was conducted on a patient suffering from cirrhosis in HBV-related hepatic carcinoma, where a direct association between hexokinase 2 expression and poor diagnosis in liver cancer patients was observed.[53]

mt-tRNA

Nucleotide sequencing of mt-tRNA^{Asp} in Morris hepatoma-5123D was explored in 1981, revealing many abnormal characteristics accompanying the normal features when compared to healthy controls. Lacking GC loop adjoining to loop-IV was observed. Further, several loci such as 4, 16, 23, and 24 were totally different in the hepatoma model.[54] Several insertions (AH-130 tRNA^{Cys}) and deletions (YS tRNA^{Tyr}, AH-7974-tRNA^{Trp}) in tRNA mutations have already been documented in the past when compared to tumor and healthy rat models (Yoshida Sarcoma's, Ascites hepatoma; AH-7974 & AH-130).[55] Alterations in mt-tRNA-modifying enzymes have been reported to be connected with certain disorders such as MTU1 in cases of acute infantile liver failure.[56] Very recently, Zhao and his co-workers in 2023 have conducted a study to unravel the role of tRNA methyltransferase 5 in liver cancer progression. It was observed that mitigating tRNA methyltransferase 5 has a suppressive effect on the hypoxia-inducible factor-1 (HIF-1) signaling pathway by amplifying the oxygen quantity of cells, as well as decreasing doxorubicin resistance in liver cells.[57]

Role of miRNA in the functional activity of mitochondrial proteins

miRNAs are small non-coding RNAs with a length of about 19–25 nucleotides and are involved in many physiological and pathological cellular processes. They can act as both oncogenic and tumor suppressor miRNAs, controlling the proliferation, dissemination, and propagation of cancer cells (Table 1).[58–66]

Natural products targeting mitochondrial proteins in liver cancer

The promising prospective of plant-based natural products to be explored as potent novel anticancer agents has always been an area of interest for researchers.[67,68] Phytochemicals specifically targeting mitochondrial proteins included in mitochondrial biogenesis and mutation have been summarized below. A schematic illustration of targeted proteins has been depicted in Figure 2.

Curcumin, a natural polyphenol from *Curcuma longa*,[69] exerted mitochondrial as well as nuclear DNA damage in human liver carcinoma (HepG2) cells in a dose-dependent mode.[70] Curcumin also demonstrated an inhibitory effect on hepatic cancer cells through the mitigation of ATP synthase activity.[71] Resveratrol, a

Table 1. Effect of microRNAs upon various genes/proteins in hepatic cell carcinoma

MicroRNA	Targeted sites	Effect	Model used for the study	Reference
MiR-34a, miR-26a & miR-145 upregulation	Sirtuins	p53 – mediated suppression of SIRT1, 2, 6, & 7 after transcription; PGC-1α/β mediated regulation of SIRT3, 4 & 5 while transcription	Livers of telomerase knockout male G4 mice (mice between the age of 8 and 16 weeks	58
MiR-181a-5p	Mitochondrial DNA genes of the COX family (*mt-CYB & mt-CO2*)	MMP decreased, increased expressions of HK-2 & GLUT-1 enhanced release of glucose and lactic acid, Lactate dehydrogenase upregulated	SMMC-7721, HepG2 cells, 4-week-old nude mice	59
MiR-199a-5p downregulation	ANRIL (A long non-coding RNA) served as a competitive endogenous RNA that stimulated the production of *ARL2* through miR-199a-5p/ARL2 axis.	Increased mtDNA copy numbers, as well as ATP synthesis	Huh7, SMMC7721, HepG2, Hep3B cells; LO2 (Normal human hepatic cells)	60
MiR-329-3p upregulation	Lysine (K)-specific demethylase 1A	Demethylation of myocyte-specific enhancer factor-2, activation of Programmed Death Ligand-1 expression	HepG2, SMMC7721; H22 (murine cancer); LO2 (Normal Human Hepatic cells)	61
MiR-342-3p upregulation	*MCT-1*	Decrease in cell proliferation, migration, colony formation	LT2/MYC animal tumor model	62
MiR-214 upregulation	*Wnt2a*	Decrease in cell proliferation	SMMC-7721, HepG2, Hep3B; HL-7702 (Normal Human Hepatic cells)	63
MiR-552-5p downregulation	MiR-552-5p interaction to 3′ UTR of ACSL4 mRNA	Glutathione quantity was reduced, intracellular Fe^{2+} amount was enhanced, increased ACSL4 expression, overexpression of ZNF8, and increased ferroptosis	Huh-7, Hep3B cells	64
Mir-122 upregulation	Decrease in *PKM2* expression	Glycolytic activity suppression, reduction in lactate generation, and elevated oxygen consumption	HepG2, Hep3B, Huh-7, MHCC97L, MHCC97H, Hepatic cancer cells from primary tumor (H2P) and its matched metastasis (H2M)	65
Mir-520 downregulation	*PFKP* regulation	TAT-activated regulatory DNA-binding protein repression impaired glycolysis	Female athymic nude mice	66

ACSL4, Acyl-CoA synthetase long-chain family member 4; ARL-2, ADP-ribosylation factor-like 2; ATP, adenosine triphosphate; GLUT-1, glucose transporter 1; HK-2, hexokinase 2; MCT-1, monocarboxylic acid transporter 1; Mt-CYB, mitochondrially encoded cytochrome B; PFKP, platelet isoform of phosphofructokinase; PGC-1, peroxisome proliferator-activated receptor-gamma coactivator-1; PKM2, pyruvate kinase isoenzyme type M2; SIRT, sirtuin; TAT, trans-activator of transcription; UTR, untranslated region; ZNF8, zinc finger protein 8.

well-known and extensively studied polyphenol, demonstrated the induction of apoptosis in HepG2 cells via upregulating phospho-AMPK expressions while simultaneously downregulating surviving signaling.[72] Resveratrol also demonstrated tumor suppressor liver kinase B1 mediated SIRT1 phosphorylation and activation.[73] A combinatorial exposure of resveratrol and sorafenib exerted a significant antiproliferative effect in mice-bearing HepG2 xenograft. The pathway involved was PKA/AMPK/eEF2K in HepG2 and Huh7 cells.[74] Likewise, Xu *et al*.[75] have studied recently the synergistic efficacy of resveratrol along with fibroblast growth factor 1 in hepatic cancer cells. An AMPK/NRF2-mediated noticeable repression of oxidative stress and liver dysfunction was observed in both *in vitro* and *in vivo* models.[75] PLGA nanoparticles of resveratrol were also studied and observed to have an enhanced activity profile compared to their native form. They were found to alleviate lipid formation and reduce cancer cells more efficiently.[76]

Berberine, isolated from the rhizome of *Rhizoma coptidis*, is known to induce up-regulation of p38 MAPK signaling in both hepatic cancer cells (HepG2, MHCC97-L) via stimulation of the AKT/Beclin-1/mTOR-signaling transduction pathway.[77] Further, berberine and rotenone were explored for their effect on mitochondrial pathways in C57BL/6J male rats as well as primary hepatocytes isolated from rats. It was observed that berberine has a repressive effect on mitochondrial swelling and mitochondrial complex I. Additionally, it was found to mitigate ATP and citrate synthesis. Furthermore, mtDNA copy numbers were also found to be reduced in hepatic cancer cells.[78] Higher expressions of AMPK by downregulating mTOR 4E-binding protein-1 along with repressing mRNA translation in p53-negative Hep 3B cells

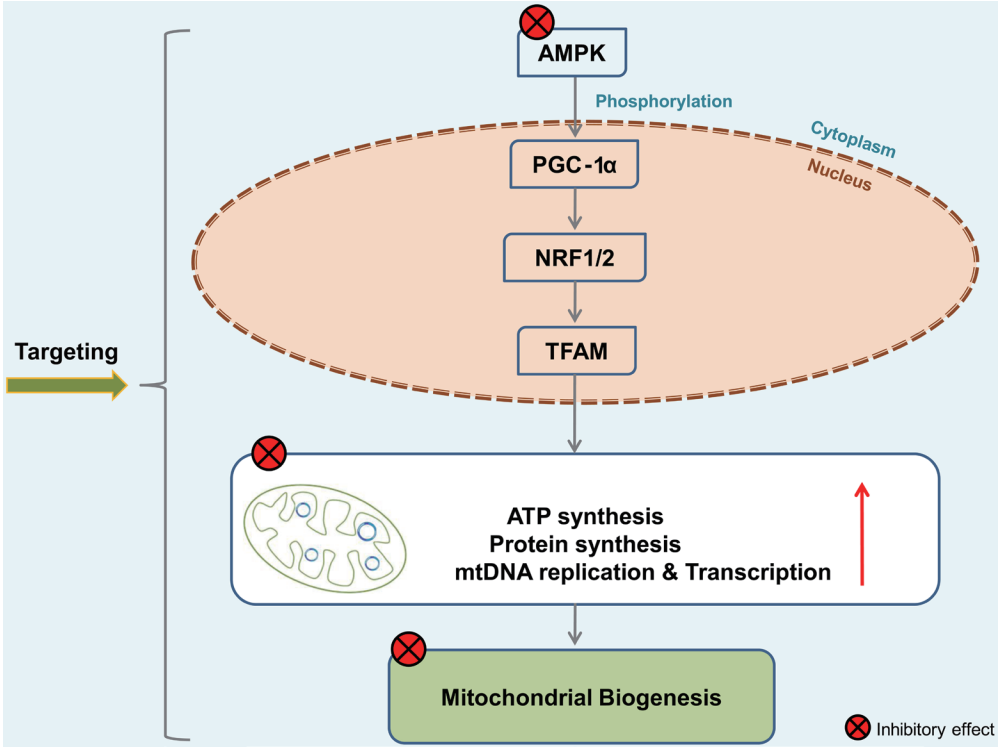

Fig. 2. Schematic illustration of targeted proteins during de-regulated Mitochondrial Biogenesis. AMPK, adenosine monophosphate-activated protein kinase; ATP, adenosine triphosphate; mtDNA, mitochondrial DNA; NRF, nuclear respiratory factor; PGC-1α, peroxisome proliferator-activated receptor γ coactivator-1α.

were observed by exposing the cells to Epigallocatechin gallate, a major catechin in green tea.[79] Epigallocatechin gallate treatment given to both male and female BALB/c nude mice injected with doxorubicin-resistant BEL-7404 cells also resulted in a subsequent decrease in cellular growth and proliferation with a simultaneous decrease in drug efflux and an increase in doxorubicin sensitivity in cells.[80] AMPK activation is reported to repress mTOR by suppressing HIF-1α expressions, which is one of the key regulators of glycolysis in the cells.[81] Quercetin (a promising flavonoid) and gemcitabine/doxorubicin combination, in doxorubicin-resistant BEL-7404 cells, demonstrated an increase in sensitization of cells to the drug. There was downregulation of drug-resistant proteins and HIF-1α, with an increase in apoptotic activities.[82] Restoration of SIRT6 followed by repression of Frizzled-4 and H3K9AC proteins were observed in hepatic cancer cell lines (HepG2, HuH-6, and HepT1) by treating them with quercetin.[83] Kaempferol, a known flavonol, was studied in SK-HEP-1 (human liver carcinoma cells) and found to cause G2/M repression and a decrease in expressions of CDK1/cyclin B via the AKT/AMPK signaling pathway.[84,85] The antioxidant potential of kaempferol was also explored in HepG2 cells to find out that it could amplify the NRF2 expression and subsequently stimulate its target genes (*SOD1* and *GPX3*).[86] Apigenin, when given together with the known anticancer drug paclitaxel to HepG2 cells, exerted enhanced anticancer profiling of paclitaxel via reducing HIF-1α and phosphorylated as well as whole AKT.[87] AMPK activation and repression of downstream mTOR/NF-κBp65 interaction were also observed with Naringenin treatment of fatty acids-induced damages in HepG2 cells.[88] A distinctive anthocyanin pigment, Cyanidin-3-O-β-glucoside, was examined and observed to exert its significant effects on AMPK activation and fatty acid oxidation in HepG2 cells. Further, an increase in CPT-1 expression was also observed.[89] Anthracenone T-514, abundantly available in *Karwinski aparvifolia*, demonstrated mitochondrial alterations prior to apoptosis. This was substantiated by increased expressions of cytochrome oxidase, p53, and decreased PCNA levels. Simultaneously, proapoptotic biomarkers were upregulated with the downregulation of anti-apoptotic Bcl-2 proteins.[90] Cryptotanshinone, a diterpene extracted from *Salvia miltiorrhiza* Bunge, demonstrated an activated pAMPK/SIRT1/NRF2 pathway in HepG2 and AML-12 cells.[91] AMPK activation and inhibition of HCC *in vitro* and in C57BL/6 mice were observed with dietary intake of Genistein.[92] Carnosol, a phenolic diterpene, inhibited the proliferation, propagation, and metastasis of HepG2 and Huh7 cell lines by activating the AMPK-p53 pathway.[93]

Miscellaneous

A PLGA nanoformulation named PPCu, comprising Cu12Sb4S13 in hyperthermic conditions, was observed to induce apoptosis by downregulating *mt-CO1* through the RAS/MAPK/MT-CO1 signaling pathway, eventually resulting in mitochondrial deregulation and consequent apoptosis.[94] Metformin, working as an adjuvant to sorafenib, helped repress sorafenib resistance in Huh7 and Hep3B hepatic cancer cell lines via AMPK/CEBPD-mediated autophagy.[95] Very recently, metformin was investigated to affect deregulated glycolysis, ROS generation, and apoptotic activities via AMPK/p38MAPK upregulation.[96] Diallyl trisulfide, a powerful antioxidant, displayed activation of the AMPK/SIRT1 signaling pathway in the HepG2 cell line.[97] A combination of Empagliflozin with metformin was investigated in male albino mice, and an

AMPK-dependent inhibition of NFκβ, p38, and ERK1/2 proteins was observed.[98] An antiplatelet drug cilostazol inhibited hepatic cancer cell proliferation via AMPK and AKT/ERK signaling.[99] Propofol inhibited HepG2 proliferation by activating the AMPK signaling.[100]

Conclusions

This review discusses current advancements in mtDNA modifications and the dynamics of mitochondria in the progression of cancer malignancy. In addition, we recapitulated potential regulatory mechanisms involved in mitochondrial dysfunction-induced mitochondrial retrograde signaling pathways, including molecules derived from mitochondria such as ROS and mtDNA. The significance of mtDNA is paramount in the initiation and progression of several clinical disorders, with a specific focus on cancer-related conditions. The presence of mtDNA mutations has been identified as a triggering factor in tumor formation, indicating a causal connection. The concept of targeting mtDNA has surfaced as a prospective strategy within the realm of cancer therapy. Understanding the processes behind the cellular responses to mtDNA damages plays a pivotal role in advancing mtDNA-targeted therapeutic interventions, predicting treatment effectiveness, and assessing possible drug resistance. In recent years, notable advancements have been made in mtDNA-targeted treatment, including various mitochondrial proteins. Several therapeutic candidates are undergoing clinical trials for various cancers, although there is a lack of clinical studies assessing the efficacy of mitochondrial-targeted therapy in HCC. The activation or inactivation of AMPK, in a context-dependent way, may be used to develop innovative treatment regimens to mitigate cancer development. Despite the intricate and multifaceted nature of the mechanism of action shown by these metabolites, it is crucial to further progress plant cancer research beyond its current applications. The objective of this work is to provide valuable insights that might inform future research and facilitate the development of innovative medications and methods targeting mtDNA.

Acknowledgments

None.

Funding

None.

Conflict of interest

Madhunika Agrawal is Director at Cellsinvitro Lifesciences Pvt. Ltd. The authors declare that there is no conflict of interests.

Author contributions

Conceptualization (MA, SKA), writing and original draft preparation (MA), reviewing and editing (MA, SKA). All authors have made a significant contribution to this study and have approved the final manuscript.

References

[1] Filograna R, Mennuni M, Alsina D, Larsson NG. Mitochondrial DNA copy number in human disease: the more the better? FEBS Lett 2021;595(8):976–1002. doi:10.1002/1873-3468.14021, PMID:33314045.

[2] Zhang W, Wu F. Effects of adverse fertility-related factors on mitochondrial DNA in the oocyte: a comprehensive review. Reprod Biol Endocrinol 2023;21(1):27. doi:10.1186/s12958-023-01078-6, PMID:36932444.

[3] Abhyankar A, Park HB, Tonolo G, Luthman H. Comparative sequence analysis of the non-protein-coding mitochondrial DNA of inbred rat strains. PLoS One 2009;4(12):e8148. doi:10.1371/journal.pone.0008148, PMID:19997590.

[4] Abd Radzak SM, Mohd Khair SZN, Ahmad F, Patar A, Idris Z, Mohamed Yusoff AA. Insights regarding mitochondrial DNA copy number alterations in human cancer (Review). Int J Mol Med 2022;50(2):104. doi:10.3892/ijmm.2022.5160, PMID:35713211.

[5] Fang X, Wu H, Wei J, Miao R, Zhang Y, Tian J. Research progress on the pharmacological effects of berberine targeting mitochondria. Front Endocrinol (Lausanne) 2022;13:982145. doi:10.3389/fendo.2022.982145, PMID:36034426.

[6] Kozhukhar N, Alexeyev MF. 35 Years of TFAM Research: Old Protein, New Puzzles. Biology (Basel) 2023;12(6):823. doi:10.3390/biology12060823, PMID:37372108.

[7] Chun Y, Kim J. AMPK-mTOR Signaling and Cellular Adaptations in Hypoxia. Int J Mol Sci 2021;22(18):9765. doi:10.3390/ijms22189765, PMID:34575924.

[8] Huang H, Wang L, Qian F, Chen X, Zhu H, Yang M, *et al*. Liraglutide via Activation of AMP-Activated Protein Kinase-Hypoxia Inducible Factor-1α-Heme Oxygenase-1 Signaling Promotes Wound Healing by Preventing Endothelial Dysfunction in Diabetic Mice. Front Physiol 2021;12:660263. doi:10.3389/fphys.2021.660263, PMID:34483951.

[9] Herzig S, Shaw RJ. AMPK: guardian of metabolism and mitochondrial homeostasis. Nat Rev Mol Cell Biol 2018;19(2):121–135. doi:10.1038/nrm.2017.95, PMID:28974774.

[10] Faubert B, Boily G, Izreig S, Griss T, Samborska B, Dong Z, *et al*. AMPK is a negative regulator of the Warburg effect and suppresses tumor growth in vivo. Cell Metab 2013;17(1):113–124. doi:10.1016/j.cmet.2012.12.001, PMID:23274086.

[11] Wallace DC, Fan W, Procaccio V. Mitochondrial energetics and therapeutics. Annu Rev Pathol 2010;5:297–348. doi:10.1146/annurev.pathol.4.110807.092314, PMID:20078222.

[12] Klein HU, Trumpff C, Yang HS, Lee AJ, Picard M, Bennett DA, *et al*. Characterization of mitochondrial DNA quantity and quality in the human aged and Alzheimer's disease brain. Mol Neurodegener 2021;16(1):75. doi:10.1186/s13024-021-00495-8, PMID:34742335.

[13] Bazzani V, Equisoain Redin M, McHale J, Perrone L, Vascotto C. Mitochondrial DNA Repair in Neurodegenerative Diseases and Ageing. Int J Mol Sci 2022;23(19):11391. doi:10.3390/ijms231911391, PMID:36232693.

[14] Franco-Iborra S, Vila M, Perier C. Mitochondrial Quality Control in Neurodegenerative Diseases: Focus on Parkinson's Disease and Huntington's Disease. Front Neurosci 2018;12:342. doi:10.3389/fnins.2018.00342, PMID:29875626.

[15] Lin Y, Yang B, Huang Y, Zhang Y, Jiang Y, Ma L, *et al*. Mitochondrial DNA-targeted therapy: A novel approach to combat cancer. Cell Insight 2023;2(4):100113. doi:10.1016/j.cellin.2023.100113, PMID:37554301.

[16] Nadalutti CA, Ayala-Peña S, Santos JH. Mitochondrial DNA damage as driver of cellular outcomes. Am J Physiol Cell Physiol 2022;322(2):C136–C150. doi:10.1152/ajpcell.00389.2021, PMID:34936503.

[17] Heilig R, Lee J, Tait SWG. Mitochondrial DNA in cell death and inflammation. Biochem Soc Trans 2023;51(1):457–472. doi:10.1042/BST20221525, PMID:36815695.

[18] Asafo-Agyei KO, Samant H. Hepatocellular Carcinoma. Treasure Island (FL): StatPearls Publishing; 2023.

[19] Oh JH, Jun DW. The latest global burden of liver cancer: A past and present threat. Clin Mol Hepatol 2023;29(2):355–357. doi:10.3350/cmh.2023.0070, PMID:36891606.

[20] Trivedi NK, Tiwari RG, Anand A, Gautam V, Witarsyah D, Misra A. Application of Machine Learning for Diagnosis of Liver Cancer. 2022 International Conference Advancement in Data Science, E-learning

and Information Systems (ICADEIS). Bandung, Indonesia; 2022:1–5. doi:10.1109/ICADEIS56544.2022.10037379.
[21] Okochi O, Hibi K, Uemura T, Inoue S, Takeda S, Kaneko T, *et al*. Detection of mitochondrial DNA alterations in the serum of hepatocellular carcinoma patients. Clin Cancer Res 2002;8(9):2875–2878. PMID:12231530.
[22] Hsu CC, Lee HC, Wei YH. Mitochondrial DNA alterations and mitochondrial dysfunction in the progression of hepatocellular carcinoma. World J Gastroenterol 2013;19(47):8880–8886. doi:10.3748/wjg.v19.i47.8880, PMID:24379611.
[23] Mposhi A, Cortés-Mancera F, Heegsma J, de Meijer VE, van de Sluis B, Sydor S, *et al*. Mitochondrial DNA methylation in metabolic associated fatty liver disease. Front Nutr 2023;10:964337. doi:10.3389/fnut.2023.964337, PMID:37305089.
[24] Campo DS, Nayak V, Srinivasamoorthy G, Khudyakov Y. Entropy of mitochondrial DNA circulating in blood is associated with hepatocellular carcinoma. BMC Med Genomics 2019;12(Suppl 4):74. doi:10.1186/s12920-019-0506-7, PMID:31167647.
[25] Nomoto S, Yamashita K, Koshikawa K, Nakao A, Sidransky D. Mitochondrial D-loop mutations as clonal markers in multicentric hepatocellular carcinoma and plasma. Clin Cancer Res 2002;8(2):481–487. PMID:11839667.
[26] Lee HY, Nga HT, Tian J, Yi HS. Mitochondrial Metabolic Signatures in Hepatocellular Carcinoma. Cells 2021;10(8):1901. doi:10.3390/cells10081901, PMID:34440674.
[27] Klein K, He K, Younes AI, Barsoumian HB, Chen D, Ozgen T, *et al*. Role of Mitochondria in Cancer Immune Evasion and Potential Therapeutic Approaches. Front Immunol 2020;11:573326. doi:10.3389/fimmu.2020.573326, PMID:33178201.
[28] Xu D, Tian Y, Xia Q, Ke B. The cGAS-STING Pathway: Novel Perspectives in Liver Diseases. Front Immunol 2021;12:682736. doi:10.3389/fimmu.2021.682736, PMID:33995425.
[29] Prantner D, Perkins DJ, Vogel SN. AMP-activated Kinase (AMPK) Promotes Innate Immunity and Antiviral Defense through Modulation of Stimulator of Interferon Genes (STING) Signaling. J Biol Chem 2017;292(1):292–304. doi:10.1074/jbc.M116.763268, PMID:27879319.
[30] Thomsen MK, Skouboe MK, Boularan C, Vernejoul F, Lioux T, Leknes SL, *et al*. The cGAS-STING pathway is a therapeutic target in a preclinical model of hepatocellular carcinoma. Oncogene 2020;39(8):1652–1664. doi:10.1038/s41388-019-1108-8, PMID:31740782.
[31] Pu Z, Liu J, Liu Z, Peng F, Zhu Y, Wang X, *et al*. STING pathway contributes to the prognosis of hepatocellular carcinoma and identification of prognostic gene signatures correlated to tumor microenvironment. Cancer Cell Int 2022;22(1):314. doi:10.1186/s12935-022-02734-4, PMID:36224658.
[32] Lin W, Zhao Z, Ni Z, Zhao Y, Du W, Chen S. IFI16 restoration in hepatocellular carcinoma induces tumour inhibition via activation of p53 signals and inflammasome. Cell Prolif 2017;50(6):e12392. doi:10.1111/cpr.12392, PMID:28990231.
[33] Liu Y, Yan W, Tohme S, Chen M, Fu Y, Tian D, *et al*. Hypoxia induced HMGB1 and mitochondrial DNA interactions mediate tumor growth in hepatocellular carcinoma through Toll-like receptor 9. J Hepatol 2015;63(1):114–121. doi:10.1016/j.jhep.2015.02.009, PMID:25681553.
[34] Bort A, Sánchez BG, Mateos-Gómez PA, Vara-Ciruelos D, Rodríguez-Henche N, Díaz-Laviada I. Targeting AMP-activated kinase impacts hepatocellular cancer stem cells induced by long-term treatment with sorafenib. Mol Oncol 2019;13(5):1311–1331. doi:10.1002/1878-0261.12488, PMID:30959553.
[35] Chen H, Wu D, Bao L, Yin T, Lei D, Yu J, *et al*. 6PGD inhibition sensitizes hepatocellular carcinoma to chemotherapy via AMPK activation and metabolic reprogramming. Biomed Pharmacother 2019;111:1353–1358. doi:10.1016/j.biopha.2019.01.028, PMID:30841449.
[36] Lu S, Gao YY, Shao WY, Han GY, Ding WZ, Huang XL. Complete mitochondrial genome sequence and mutations of the liver cancer model inbred C57BL/6 mice strain. Mitochondrial DNA A DNA Mapp Seq Anal 2016;27(3):1999–2000. doi:10.3109/19401736.2014.974160, PMID:25350733.
[37] Zhang S, Jiang Z, Zhang S, Xia M, Tian F, Tian H. The complete mitochondrial genome of a chronic hepatitis associated liver cancer LEC rat strain. Mitochondrial DNA A DNA Mapp Seq Anal 2016;27(3):1928–1929. doi:10.3109/19401736.2014.971291, PMID:25329295.
[38] Neupane P, Bhuju S, Thapa N, Bhattarai HK. ATP Synthase: Structure, Function and Inhibition. Biomol Concepts 2019;10(1):1–10. doi:10.1515/bmc-2019-0001, PMID:30888962.
[39] Mansilla N, Racca S, Gras DE, Gonzalez DH, Welchen E. The Complexity of Mitochondrial Complex IV: An Update of Cytochrome c Oxidase Biogenesis in Plants. Int J Mol Sci 2018;19(3):662. doi:10.3390/ijms19030662, PMID:29495437.
[40] Sharma LK, Lu J, Bai Y. Mitochondrial respiratory complex I: structure, function and implication in human diseases. Curr Med Chem 2009;16(10):1266–1277. doi:10.2174/092986709787846578, PMID:19355884.
[41] Rebelo-Guiomar P, Pellegrino S, Dent KC, Sas-Chen A, Miller-Fleming L, Garone C, *et al*. A late-stage assembly checkpoint of the human mitochondrial ribosome large subunit. Nat Commun 2022;13(1):929. doi:10.1038/s41467-022-28503-5, PMID:35177605.
[42] He Q, He X, Xiao Y, Zhao Q, Ye Z, Cui L, *et al*. Tissue-specific expression atlas of murine mitochondrial tRNAs. J Biol Chem 2021;297(2):100960. doi:10.1016/j.jbc.2021.100960, PMID:34265302.
[43] Merrill CL, Ni H, Yoon LW, Tirmenstein MA, Narayanan P, Benavides GR, *et al*. Etomoxir-induced oxidative stress in HepG2 cells detected by differential gene expression is confirmed biochemically. Toxicol Sci 2002;68(1):93–101. doi:10.1093/toxsci/68.1.93, PMID:12075114.
[44] Ying H, Yu Y, Xu Y. Antisense of ATP synthase subunit e inhibits the growth of human hepatocellular carcinoma cells. Oncol Res 2001;12(11-12):485–490. doi:10.3727/096504001108747495, PMID:11939412.
[45] Ni CH, Chen PY, Lu HF, Yang JS, Huang HY, Wu SH, *et al*. Chrysophanol-induced necrotic-like cell death through an impaired mitochondrial ATP synthesis in Hep3B human liver cancer cells. Arch Pharm Res 2012;35(5):887–895. doi:10.1007/s12272-012-0514-z, PMID:22644856.
[46] Zhang S, Zhao J, Guo Y, Hu J, Chen X, Ruan H, *et al*. Thiosemicarbazone N-Heterocyclic Cu(II) complexes inducing nuclei DNA and mitochondria damage in hepatocellular carcinoma cells. J Inorg Biochem 2022;236:111964. doi:10.1016/j.jinorgbio.2022.111964, PMID:36027842.
[47] Van Itallie CM. Thyroid hormone and dexamethasone increase the levels of a messenger ribonucleic acid for a mitochondrially encoded subunit but not for a nuclear-encoded subunit of cytochrome c oxidase. Endocrinology 1990;127(1):55–62. doi:10.1210/endo-127-1-55, PMID:1694499.
[48] Kuo KW, Yang PY, Huang YS, Shieh DZ. Variations in gene expression and genomic stability of human hepatoma cells integrated with hepatitis B virus DNA. Biochem Mol Biol Int 1998;44(6):1133–1140. doi:10.1080/15216549800202212, PMID:9623767.
[49] Zou LY, Zheng BY, Fang XF, Li D, Huang YH, Chen ZX, *et al*. HBx co-localizes with COXIII in HL-7702 cells to upregulate mitochondrial function and ROS generation. Oncol Rep 2015;33(5):2461–2467. doi:10.3892/or.2015.3852, PMID:25778742.
[50] Wang H, Xu J, Li D, Zhang S, Guo Z. Identification of sequence polymorphisms in the mitochondrial cytochrome c oxidase genes as risk factors for hepatocellular carcinoma. J Clin Lab Anal 2018;32(3):e22299. doi:10.1002/jcla.22299, PMID:28703354.
[51] Zhao X, Chen J, Yin S, Shi J, Zheng M, He C, *et al*. The expression of cuproptosis-related genes in hepatocellular carcinoma and their relationships with prognosis. Front Oncol 2022;12:992468. doi:10.3389/fonc.2022.992468, PMID:36313717.
[52] Ren W, Li Y, Li R, Feng H, Wu S, Mao Y, *et al*. Mitochondrial intergenic COII/tRNA(Lys) 9-bp deletion, a biomarker for hepatocellular carcinoma? Mitochondrial DNA A DNA Mapp Seq Anal 2016;27(4):2520–2522. doi:10.3109/19401736.2015.1036256, PMID:26017042.
[53] Lin YH, Chu YD, Lim SN, Chen CW, Yeh CT, Lin WR. Impact of an MT-RNR1 Gene Polymorphism on Hepatocellular Carcinoma Progression and Clinical Characteristics. Int J Mol Sci 2021;22(3):1119. doi:10.3390/ijms22031119, PMID:33498721.
[54] Agrawal HP, Randerath K, Randerath E. Tumor mitochondrial transfer ribonucleic acids: the nucleotide sequence of Morris hepatoma 5123D mitochondrial tRNA GUC Asp. Nucleic Acids Res 1981;9(11):2535–2541. doi:10.1093/nar/9.11.2535, PMID:6912441.

[55] Taira M, Yoshida E, Kobayashi M, Yaginuma K, Koike K. Tumor-associated mutations of rat mitochondrial transfer RNA genes. Nucleic Acids Res 1983;11(6):1635–1643. doi:10.1093/nar/11.6.1635, PMID:6300770.
[56] Suzuki T, Yashiro Y, Kikuchi I, Ishigami Y, Saito H, Matsuzawa I, *et al*. Complete chemical structures of human mitochondrial tRNAs. Nat Commun 2020;11(1):4269. doi:10.1038/s41467-020-18068-6, PMID:32859890.
[57] Zhao Q, Zhang L, He Q, Chang H, Wang Z, Cao H, *et al*. Targeting TRMT5 suppresses hepatocellular carcinoma progression via inhibiting the HIF-1α pathways. J Zhejiang Univ Sci B 2023;24(1):50–63. doi:10.1631/jzus.B2200224, PMID:36632750.
[58] Amano H, Chaudhury A, Rodriguez-Aguayo C, Lu L, Akhanov V, Catic A, *et al*. Telomere Dysfunction Induces Sirtuin Repression that Drives Telomere-Dependent Disease. Cell Metab 2019;29(6):1274–1290.e9. doi:10.1016/j.cmet.2019.03.001, PMID:30930169.
[59] Zhuang X, Chen Y, Wu Z, Xu Q, Chen M, Shao M, *et al*. Mitochondrial miR-181a-5p promotes glucose metabolism reprogramming in liver cancer by regulating the electron transport chain. Carcinogenesis 2020;41(7):972–983. doi:10.1093/carcin/bgz174, PMID:31628462.
[60] Li K, Zhao B, Wei D, Cui Y, Qian L, Wang W, *et al*. Long non-coding RNA ANRIL enhances mitochondrial function of hepatocellular carcinoma by regulating the MiR-199a-5p/ARL2 axis. Environ Toxicol 2020;35(3):313–321. doi:10.1002/tox.22867, PMID:31670868.
[61] Wang Y, Cao K. KDM1A Promotes Immunosuppression in Hepatocellular Carcinoma by Regulating PD-L1 through Demethylating MEF2D. J Immunol Res 2021;2021:9965099. doi:10.1155/2021/9965099, PMID:34307695.
[62] Komoll RM, Hu Q, Olarewaju O, von Döhlen L, Yuan Q, Xie Y, *et al*. MicroRNA-342-3p is a potent tumour suppressor in hepatocellular carcinoma. J Hepatol 2021;74(1):122–134. doi:10.1016/j.jhep.2020.07.039, PMID:32738449.
[63] Yang Y, Zhao Z, Hou N, Li Y, Wang X, Wu F, *et al*. MicroRNA-214 targets Wnt3a to suppress liver cancer cell proliferation. Mol Med Rep 2017;16(5):6920–6927. doi:10.3892/mmr.2017.7483, PMID:28901526.
[64] Yang H, Sun W, Bi T, Sun J, Lu Z, Li J, *et al*. ZNF8-miR-552-5p Axis Modulates ACSL4-Mediated Ferroptosis in Hepatocellular Carcinoma. DNA Cell Biol 2023;42(6):336–347. doi:10.1089/dna.2022.0582, PMID:37126948.
[65] Inomata Y, Oh JW, Taniguchi K, Sugito N, Kawaguchi N, Hirokawa F, *et al*. Downregulation of miR-122-5p Activates Glycolysis via PKM2 in Kupffer Cells of Rat and Mouse Models of Non-Alcoholic Steatohepatitis. Int J Mol Sci 2022;23(9):5230. doi:10.3390/ijms23095230, PMID:35563621.
[66] Park YY, Kim SB, Han HD, Sohn BH, Kim JH, Liang J, *et al*. Tat-activating regulatory DNA-binding protein regulates glycolysis in hepatocellular carcinoma by regulating the platelet isoform of phosphofructokinase through microRNA 520. Hepatology 2013;58(1):182–191. doi:10.1002/hep.26310, PMID:23389994.
[67] Thind TS, Rampal G, Agrawal SK, Saxena AK, Arora S. Evaluation of cytotoxic and radical-scavenging activities of root extracts of Schleichera oleosa (Lour.) Oken. Nat Prod Res 2012;26(18):1728–1731. doi:10.1080/14786419.2011.606547, PMID:21988592.
[68] Agrawal SK, Agrawal M, Sharma PR, Ahmad K, Shawl AS, Arora S, *et al*. Anagallis arvensis Induces Apoptosis in HL-60 Cells Through ROS-Mediated Mitochondrial Pathway. Nutr Cancer 2021;73(11-12):2720–2731. doi:10.1080/01635581.2020.1856893, PMID:33305590.
[69] Badavath VN, Jayaprakash V, Mondal SK, Arora S, Acevedo O, Thakur A, *et al*. Pharmacokinetic Studies of Curcumin Based Pyrazoline MAO Inhibitors. J Pharm Tech Res Management 2020;8(2):85–89. doi:10.15415/jptrm.2020.82012.
[70] Cao J, Jia L, Zhou HM, Liu Y, Zhong LF. Mitochondrial and nuclear DNA damage induced by curcumin in human hepatoma G2 cells. Toxicol Sci 2006;91(2):476–483. doi:10.1093/toxsci/kfj153, PMID:16537656.
[71] Rawat D, Shrivastava S, Naik RA, Chhonker SK, Mehrotra A, Koiri RK. An Overview of Natural Plant Products in the Treatment of Hepatocellular Carcinoma. Anticancer Agents Med Chem 2018;18(13):1838–1859. doi:10.2174/1871520618666180604085612, PMID:29866017.
[72] Tameda M, Sugimoto K, Shiraki K, Inagaki Y, Ogura S, Kasai C, *et al*. Resveratrol sensitizes HepG2 cells to TRAIL-induced apoptosis. Anticancer Drugs 2014;25(9):1028–1034. doi:10.1097/CAD.0000000000000128, PMID:24892721.
[73] Huang Y, Lu J, Zhan L, Wang M, Shi R, Yuan X, *et al*. Resveratrol-induced Sirt1 phosphorylation by LKB1 mediates mitochondrial metabolism. J Biol Chem 2021;297(2):100929. doi:10.1016/j.jbc.2021.100929, PMID:34216621.
[74] Gao M, Deng C, Dang F. Synergistic antitumor effect of resveratrol and sorafenib on hepatocellular carcinoma through PKA/AMPK/eEF2K pathway. Food Nutr Res 2021;65:3602. doi:10.29219/fnr.v65.3602, PMID:34776832.
[75] Xu X, Liu Q, Li J, Xiao M, Gao T, Zhang X, *et al*. Co-Treatment With Resveratrol and FGF1 Protects Against Acute Liver Toxicity After Doxorubicin Treatment via the AMPK/NRF2 Pathway. Front Pharmacol 2022;13:940406. doi:10.3389/fphar.2022.940406, PMID:36110535.
[76] Wan S, Zhang L, Quan Y, Wei K. Resveratrol-loaded PLGA nanoparticles: enhanced stability, solubility and bioactivity of resveratrol for non-alcoholic fatty liver disease therapy. R Soc Open Sci 2018;5(11):181457. doi:10.1098/rsos.181457, PMID:30564426.
[77] Wang N, Feng Y, Zhu M, Tsang CM, Man K, Tong Y, *et al*. Berberine induces autophagic cell death and mitochondrial apoptosis in liver cancer cells: the cellular mechanism. J Cell Biochem 2010;111(6):1426–1436. doi:10.1002/jcb.22869, PMID:20830746.
[78] Yu M, Alimujiang M, Hu L, Liu F, Bao Y, Yin J. Berberine alleviates lipid metabolism disorders via inhibition of mitochondrial complex I in gut and liver. Int J Biol Sci 2021;17(7):1693–1707. doi:10.7150/ijbs.54604, PMID:33994854.
[79] Huang CH, Tsai SJ, Wang YJ, Pan MH, Kao JY, Way TD. EGCG inhibits protein synthesis, lipogenesis, and cell cycle progression through activation of AMPK in p53 positive and negative human hepatoma cells. Mol Nutr Food Res 2009;53(9):1156–1165. doi:10.1002/mnfr.200800592, PMID:19662644.
[80] Liang G, Tang A, Lin X, Li L, Zhang S, Huang Z, *et al*. Green tea catechins augment the antitumor activity of doxorubicin in an in vivo mouse model for chemoresistant liver cancer. Int J Oncol 2010;37(1):111–123. doi:10.3892/ijo_00000659.
[81] Shang RZ, Qu SB, Wang DS. Reprogramming of glucose metabolism in hepatocellular carcinoma: Progress and prospects. World J Gastroenterol 2016;22(45):9933–9943. doi:10.3748/wjg.v22.i45.9933, PMID:28018100.
[82] Hassan S, Peluso J, Chalhoub S, Idoux Gillet Y, Benkirane-Jessel N, Rochel N, *et al*. Quercetin potentializes the respective cytotoxic activity of gemcitabine or doxorubicin on 3D culture of AsPC-1 or HepG2 cells, through the inhibition of HIF-1α and MDR1. PLoS One 2020;15(10):e0240676. doi:10.1371/journal.pone.0240676, PMID:33052979.
[83] Liu T, Li Z, Tian F. Quercetin inhibited the proliferation and invasion of hepatoblastoma cells through facilitating SIRT6-medicated FZD4 silence. Hum Exp Toxicol 2021;40(12_suppl):S96–S107. doi:10.1177/09603271211030558, PMID:34219513.
[84] Huang WW, Tsai SC, Peng SF, Lin MW, Chiang JH, Chiu YJ, *et al*. Kaempferol induces autophagy through AMPK and AKT signaling molecules and causes G2/M arrest via downregulation of CDK1/cyclin B in SK-HEP-1 human hepatic cancer cells. Int J Oncol 2013;42(6):2069–2077. doi:10.3892/ijo.2013.1909, PMID:23591552.
[85] Han B, Yu YQ, Yang QL, Shen CY, Wang XJ. Kaempferol induces autophagic cell death of hepatocellular carcinoma cells via activating AMPK signaling. Oncotarget 2017;8(49):86227–86239. doi:10.18632/oncotarget.21043, PMID:29156790.
[86] Hsu JY, Lin HH, Chyau CC, Wang ZH, Chen JH. Aqueous Extract of Pepino Leaves Ameliorates Palmitic Acid-Induced Hepatocellular Lipotoxicity via Inhibition of Endoplasmic Reticulum Stress and Apoptosis. Antioxidants (Basel) 2021;10(6):903. doi:10.3390/antiox10060903, PMID:34204987.
[87] Li K, Li M, Luo Z, Mao Y, Yu Y, He Y, *et al*. Overcoming the hypoxia-induced drug resistance in liver tumor by the concurrent use of apigenin and paclitaxel. Biochem Biophys Res Commun 2020;526(2):321–327. doi:10.1016/j.bbrc.2020.03.010, PMID:32220496.
[88] Ye M, Fan S, Li X, Yang S, Ji C, Ji F, *et al*. Four flavonoids from propolis ameliorate free fatty acids-induced non-alcoholic steatohepatitis in HepG2 cells: Involvement of enhanced AMPK activation, mTOR-NF-κBp65 interaction, and PTEN expression. J Funct Foods

2023;102:105460. doi:10.1016/j.jff.2023.105460.
[89] Guo H, Liu G, Zhong R, Wang Y, Wang D, Xia M. Cyanidin-3-O-β-glucoside regulates fatty acid metabolism via an AMP-activated protein kinase-dependent signaling pathway in human HepG2 cells. Lipids Health Dis 2012;11:10. doi:10.1186/1476-511X-11-10, PMID:22243683.
[90] Soto-Domínguez A, Ballesteros-Elizondo RG, Santoyo-Pérez ME, Rodríguez-Rocha H, García-Garza R, Nava-Hernández MP, *et al*. Peroxisomicine A1 (toxin T-514) induces cell death of hepatocytes in vivo by triggering the intrinsic apoptotic pathway. Toxicon 2018;154:79–89. doi:10.1016/j.toxicon.2018.09.010, PMID:30273702.
[91] Nagappan A, Kim JH, Jung DY, Jung MH. Cryptotanshinone from the Salvia miltiorrhiza Bunge Attenuates Ethanol-Induced Liver Injury by Activation of AMPK/SIRT1 and Nrf2 Signaling Pathways. Int J Mol Sci 2019;21(1):265. doi:10.3390/ijms21010265, PMID:31906014.
[92] Lee SR, Kwon SW, Lee YH, Kaya P, Kim JM, Ahn C, *et al*. Dietary intake of genistein suppresses hepatocellular carcinoma through AMPK-mediated apoptosis and anti-inflammation. BMC Cancer 2019;19(1):6. doi:10.1186/s12885-018-5222-8, PMID:30606143.
[93] Kong S, Xiao W, Ma T, Chen Y, Shi H, Tu J, *et al*. Carnosol Inhibits the Proliferation, Migration, and Invasion of Hepatocellular Carcinoma Cells in vitro by Regulating the AMPK Signaling Pathway. Anticancer Agents Med Chem 2023;23:1. doi:10.2174/1871520623666230418093254, PMID:37073668.
[94] Dong T, Jiang J, Zhang H, Liu H, Zou X, Niu J, *et al*. PFP@PLGA/Cu(12)Sb(4)S(13)-mediated PTT ablates hepatocellular carcinoma by inhibiting the RAS/MAPK/MT-CO1 signaling pathway. Nano Converg 2021;8(1):29. doi:10.1186/s40580-021-00279-2, PMID:34606010.
[95] Lai HY, Tsai HH, Yen CJ, Hung LY, Yang CC, Ho CH, *et al*. Metformin Resensitizes Sorafenib-Resistant HCC Cells Through AMPK-Dependent Autophagy Activation. Front Cell Dev Biol 2020;8:596655. doi:10.3389/fcell.2020.596655, PMID:33681180.
[96] Park D, Lee S, Boo H. Metformin Induces Lipogenesis and Apoptosis in H4IIE Hepatocellular Carcinoma Cells. Dev Reprod 2023;27(2):77–89. doi:10.12717/DR.2023.27.2.77, PMID:37529015.
[97] Sun S, Liu X, Wei X, Zhang S, Wang W. Diallyl trisulfide induces pro-apoptotic autophagy via the AMPK/SIRT1 signalling pathway in human hepatocellular carcinoma HepG2 cell line. Food Nutr Res 2023;67:8981. doi:10.29219/fnr.v66.8981, PMID:37868628.
[98] Abdelhamid AM, Saber S, Youssef ME, Gaafar AGA, Eissa H, Abd-Eldayem MA, *et al*. Empagliflozin adjunct with metformin for the inhibition of hepatocellular carcinoma progression: Emerging approach for new application. Biomed Pharmacother 2022;145:112455. doi:10.1016/j.biopha.2021.112455, PMID:34844106.
[99] Sim KH, Shu MS, Kim S, Kim JY, Choi BH, Lee YJ. Cilostazol Induces Apoptosis and Inhibits Proliferation of Hepatocellular Carcinoma Cells by Activating AMPK. Biotechnol Bioproc E 2021;26:776–785. doi:10.1007/s12257-021-0002-8.
[100] Wang Y, Xu B, Zhou J, Wu X. Propofol activates AMPK to inhibit the growth of HepG2 cells in vitro and hepatocarcinogenesis in xenograft mouse tumor models by inducing autophagy. J Gastrointest Oncol 2020;11(6):1322–1332. doi:10.21037/jgo-20-472, PMID:33457004.