Mitochondrial Dysfunction in Hepatocellular Carcinoma: Insights into the Diagnostic and Therapeutic Implications

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Abstract

Hepatocellular carcinoma (HCC) presents a significant problem that necessitates a greater understanding of its underlying molecular complexity in order to improve diagnostics and therapies. Recent studies have shed light on the critical role that mitochondrial dysfunction plays in the development and course of HCC. Once thought to be primarily involved in the synthesis of cellular energy, mitochondria are now known to be key participants in the regulation of a variety of cellular functions that go beyond bioenergetics. The purpose of this review is to elucidate the diagnostic and therapeutic implications of mitochondrial dysfunction in HCC by synthesising ideas from conventional and contemporary scientific literature. Within diagnostics, the discovery of mitochondrial biomarkers such as mutations in mitochondrial DNA and abnormalities in respiratory chain complex activities provides a state-of-the-art viewpoint for the timely identification and accurate description of HCC. In addition, non-invasive imaging methods that focus on mitochondrial function offer prospective means of improving clinical diagnostic accuracy. Moving on to treatment, a growing area of study investigates how mitochondria-targeted therapies might be used to treat HCC. Novel substances and repurposed medications with the potential to induce selective apoptosis in cancer cells, reduce oxidative stress, and restore mitochondrial homeostasis are highly promising. In the fight against hepatocellular carcinoma, an understanding of the complex interactions between mitochondrial dysfunction and HCC opens new diagnostic opportunities and paves the way for individualised treatment plans.

Keywords: Cancer, Diagnostics, Hepatocellular carcinoma, Homeostasis, Liver, Mitochondrial

dysfunction, Oxidative stress, Treatment.

1. Introduction

About 90% of instances of primary liver cancer are hepatocellular carcinoma (HCC), which is the second most common cause of cancer-related deaths globally. There are 850,000 new cases worldwide each year. In 2018, there were 8.5 liver cancer fatalities per 100,000 people, or 9.3 liver cancer cases per 100,000 people. The third leading cause of cancer-related fatalities worldwide is HCC. Globally, the highest incidence rates of HCC are found in Asia and Africa. Regretfully, proper prediction is still difficult to come by everywhere, which contributes to similar incidence and fatality rates because fewer cases receive adequate treatment [1]. Hepatitis B and C virus (HBV and HCV) infections, alcohol misuse, metabolic syndrome, and cirrhosis—chronic liver damage from fibrosis are among the well-established risk factors that lead to the development of HCC. The most common mutations lack effective treatment options, despite advancements in our knowledge of the molecular pathophysiology of HCC and the identification of important driver mutations [2].

The Barcelona-Clinic-Liver Cancer Classification is the predominant clinical algorithm for patient stratification based on prognosis and treatment allocation, however the molecular classification of HCC is still unclear [3]. Inflammation, which causes liver necrosis and end-stage liver disease, is a hallmark of HCC [2]. There are substantial gaps in the treatment of HCC, which may be filled by finding new treatments and combinations of them that work effectively as adjuvants for both intermediate and advanced stages of the illness. Biomarkers that aid in therapeutic stratification, tailored approaches aimed at driver mutations and/or signalling cascade activation, and validated quality of life measures are required. Multiple biological mechanisms contribute to the progression of HCC. These encompass epithelial-mesenchymal transition (EMT), tumour-stromal interactions, the tumour microenvironment, cancer stem cells (CSCs), and dysregulation of microRNAs and established signalling pathways [4]. These mechanisms have been depicted in *Figure 1*.

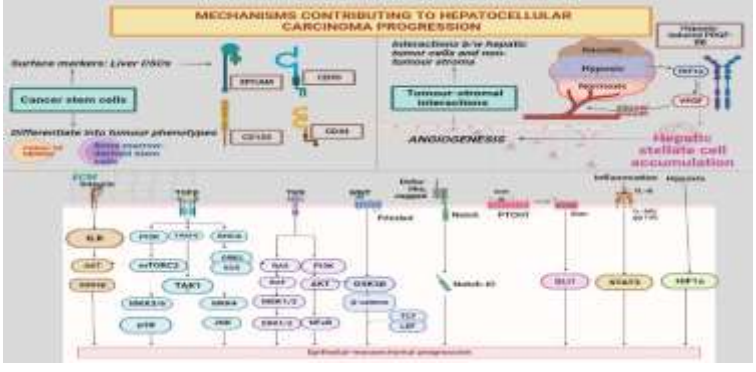

Figure 1. A schematic representation of some of the primary processes involved in the progression of HCC.

Liver cells directly involved in hepatocarcinogenesis may give rise to CSCs, with intrinsic and extrinsic factors influencing this transformation. Bone marrow-derived stem cells and canal of Hering (oval cells) are implicated in HCC progression, with specific biomarkers associated with each. Hepatic tumour cells and non-tumour stroma communicate with one another through tumor-stromal interactions, which affect angiogenesis. HSC accumulation is triggered by hypoxia-induced PDGF-BB, which results in angiogenesis. Activated HSCs influence HCC proliferation and metastasis through paracrine interactions with hepatocytes, and HSCs help the liver produce collagen. Identification of tumour-specific biomarkers is crucial for effective therapeutic and early detection strategies. Surface markers for liver CSCs include EpCAM, CD90, CD133, CD44, and CD13. The EMT is a process allowing epithelial cells to adopt a mesenchymal phenotype through biochemical changes, increasing migratory capacity, invasiveness, and resistance to apoptosis. Notably, TGF-beta, Wnt, Notch, and Hedgehog signalling pathways critically regulate EMT; these roles span embryonic development, wound healing, and in disease contexts, cancer progression and fibrosis. *CSCs: cancer stem cells, HCC: hepatocellular carcinoma, PDGF: platelet-derived growth factor, HSCs: hepatic stellate cells, EpCAM: epithelial cellular adhesion molecule, CD: cluster of differentiation, TGF: transforming growth factor, Wnt: Wingless/Integrated, EMT: epithelial-mesenchymal transition.*

Mitochondria, present in all nucleated cells, serve as the primary producers of cellular ATP through oxidative phosphorylation (OXPHOS), involving the electron-transferring respiratory chain (complexes I–IV) and ATP synthase (complex V). They house extra-chromosomal DNA, controlled by both nuclear DNA and the mitochondrial genome [5]. The mitochondrial genome comprises a circular dsDNA molecule (16.6 kb in humans), encoding essential polypeptides for OXPHOS and the necessary RNA machinery (2 rRNAs and 22 tRNAs) for intramitochondrial translation. Remaining protein subunits for respiratory-chain complexes and mitochondrial DNA (mtDNA) maintenance are nuclear-encoded, synthesized on cytoplasmic ribosomes, and specifically targeted to the organelle [6]. Despite its small size compared to the nuclear genome, the mitochondrial genome poses unique clinical and experimental challenges. Mutations in mtDNA are a significant cause of inherited diseases, and recent advancements have improved understanding of mitochondrial genetics, the link between inherited mutations and disease phenotypes, and the identification of acquired mtDNA mutations in aging and cancer. However, challenges persist, particularly in preventing and treating these diseases [7].

A key factor in carcinogenesis and a contributor to the intricate terrain of cancer progression is mitochondrial dysfunction. A growing body of research highlights the role that mitochondrial changes play in many facets of tumour formation. As the energy factories of the cell, mitochondria play a key role in OXPHOS, which produces energy [8]. Any problems with this process might have a significant effect on cancerous cells. Studies have demonstrated that common characteristics of cancer cells include mutations in the mtDNA, changes in respiratory chain complexes, and compromised mitochondrial quality control mechanisms [9]. These anomalies may result in heightened generation of reactive oxygen species (ROS), modified cellular metabolism, and resistance to apoptosis, all of which can foster the development and survival of tumours. Furthermore, the crosstalk between mitochondrial dysfunction and crucial signalling pathways linked to angiogenesis, immunological evasion, and cell proliferation highlights its influence on tumour biology [10]. Novel treatment approaches that target the

mitochondria have been made possible by our growing understanding of the molecular processes connecting cancer and mitochondrial dysfunction. Mitochondria-targeted medicines provide novel ways to treat cancer by taking advantage of the weaknesses caused by mitochondrial malfunction [11]. Many of these agents are already in use for other indications and have been tested in several preclinical HCC models for beneficial effects.

In this review, we intend to synthesise concepts from recent scientific literature to better understand the diagnostic and therapeutic implications of mitochondrial dysfunction in HCC. This paper highlights the key features of mitochondrial dysfunction in HCC, main types of somatic mitochondrial DNA alterations and their links with HCC pathogenesis. We further provide insights into the diagnostic significance of mitochondrial biomarkers in HCC, in addition to a focus on the therapeutic implications.

Progression of NASH to HCC

Triglyceride buildup in hepatocytes, or hepatic steatosis, is a hallmark of the early stages of nonalcoholic steatosis. An imbalance between lipid disposal (by fatty acid oxidation and export as very low-density lipoproteins) and lipid acquisition (via increased fatty acid intake and de novo lipogenesis) leads to the creation of lipid droplets. Although hepatic steatosis is frequently asymptomatic, in those who are vulnerable, it can worsen and lead to NASH, especially when other metabolic insults including insulin resistance and oxidative stress are present. The infiltration of immune cells into the liver parenchyma, primarily T lymphocytes and macrophages, is a step towards NASH progression. A hallmark of NASH is chronic inflammation, which also plays a role in hepatocellular damage and apoptosis. Tumour necrosis factor-alpha (TNF-α) and interleukin-6 (IL-6) are examples of pro-inflammatory cytokines that promote inflammation and trigger signalling pathways that are involved in fibrogenesis and carcinogenesis.

Fibrosis, which is defined by the excessive deposition of extracellular matrix proteins, mostly collagen, is the reparative reaction to chronic liver injury and inflammation. A continuum characterises the course of fibrosis in NASH, starting from moderate perisinusoidal fibrosis (stage 1), progressing to bridging fibrosis (stage 3), and finally ending in cirrhosis (stage 4). In addition to impairing hepatic function and upsetting liver architecture, fibrosis puts a person at risk for consequences such portal hypertension and liver failure. Notably, extensive fibrosis plays a significant role in predicting the morbidity and mortality associated with NASH, including the emergence of HCC. HCC represents the malignant transformation of hepatocytes and is the most severe consequence of chronic liver disease, including NASH. Multifactorial and including intricate interactions between genetic, environmental, and metabolic factors is the pathophysiology of HCC connected to NASH. In the context of NASH, hepatocarcinogenesis is influenced by oxidative stress, dysregulated lipid metabolism, persistent inflammation, and activation of pro-carcinogenic signalling pathways, such as TGF-β, PI3K/Akt, and Wnt/β-catenin. Notably, people who have underlying cirrhosis are more likely to develop HCC, which emphasises the crucial role that fibrosis progression plays in the carcinogenesis of NASH [12-14].

2. Mitochondrial Dysfunction in HCC: A Molecular Overview

Mitochondria are central organelles crucial for energy production, apoptosis regulation, and cellular homeostasis. In HCC, the dysregulation of mitochondrial function is multifaceted, involving genetic mutations and epigenetic modifications. Understanding these molecular mechanisms is imperative for advancing diagnostic and therapeutic strategies.

2.1 Mitochondrial Functioning in Hepatocytes

Many models for researching mitochondrial function have been investigated in order to delve into the complex relationship that exists between toxic chemicals, oxidative stress, and the liver mitochondria, particularly in hepatocytes with their high energy demands. It is essential to comprehend these pathways in order to evaluate toxicity and possible treatment strategies.

Oxidative stress and mitochondrial changes

A powerful peroxidizing chemical called tert-butylhydroperoxide (tBHP), which is frequently used to create oxidative stress in experimental models, was applied to cultured hepatocytes in a groundbreaking study on the impact of oxidative stress on mitochondrial dynamics. The research employed a comprehensive strategy that combined multiple techniques to clarify the complex relationship between oxidative stress and mitochondrial activity in hepatocytes. The main goal was to evaluate the effect of tBHP exposure on mitochondrial membrane potential (MMP), a vital sign of the health and function of mitochondria. For the creation of ATP and other metabolic processes, the electrochemical gradient across the inner mitochondrial membrane must be maintained, and MMP plays a critical role in this process. Significantly, exposed hepatocytes to tBHP resulted in a marked decrease in MMP, a sign of impaired cellular energetics and mitochondrial dysfunction [15]. The researchers used advanced molecular probes, such as the Rhodamine 123 accumulation test and the JC-1 fluorescence assay, to validate these results. Fluorescent dye JC-1 is frequently used to measure MMP variations since it shows different emission spectra when mitochondrial polarisation varies.

Further highlighting the detrimental effects of oxidative stress on mitochondrial integrity, JC-1 fluorescence signals showed substantial mitochondrial depolarization after tBHP treatment, which was consistent with the reported reduction in MMP. The Rhodamine 123 accumulation assay, a well-established method for assessing MMP, also offered supporting proof of tBHP-induced mitochondrial malfunction. Membrane potential determines how specifically the fluorescent dye rhodamine 123 accumulates inside mitochondria. As a result, variations in the fluorescence intensity of Rhodamine 123 are a trustworthy marker of shifts in MMP. In line with the results of JC-1, treatment to tBHP reduced the accumulation of Rhodamine 123 in the mitochondria of hepatocytes, a sign of depolarization and poor function of the mitochondria.

The study also explored the molecular mechanisms behind mitochondrial dysfunction caused by tBHP, concentrating on respiratory Complex I activity.

The researchers investigated the vulnerability of respiratory Complex I to tBHP-mediated oxidative damage using permeabilized hepatocytes. Interestingly, the findings showed that Complex I was more sensitive to tBHP exposure, suggesting that oxidative stress is a major cause of mitochondrial dysfunction in hepatocytes. Increased vulnerability of the Complex I to

oxidative damage highlights how important oxidative stress is in upsetting mitochondrial calcium homeostasis and altering MMP, which are essential for preserving cellular bioenergetics and redox equilibrium. The thorough analysis revealed a series of processes that lead to MMP breakdown and altered calcium homeostasis, underscoring the deleterious effects of oxidative stress on mitochondrial activity in hepatocytes. The combination of various methodological approaches yielded strong evidence explaining the complex relationship between mitochondrial dynamics and oxidative stress, providing important new information about possible treatment approaches to reduce mitochondrial dysfunction caused by oxidative stress in liver pathologies.

Mitochondrial dynamics and ethanol exposure

The kinetics and structure of mitochondria in hepatocytes have been investigated in relation to exposure to ethanol. The dynamics of normal hepatocytes were found to be modest, but exposure to ethanol markedly reduced the dynamics of the mitochondria. This information, which offers a clearer picture of how ethanol affects mitochondrial morphology, was discovered by using electron imaging and fluorescent proteins targeted to specific organelles [16]. The evaluation of mitochondrial dynamics, which includes the fission, fusion, and motility processes that together control the shape and location of mitochondria in cells, was at the centre of the study. By using fluorescent proteins that are specific to organelles in conjunction with state-of-the-art electron imaging techniques, the researchers were able to closely examine the kinetics and structural integrity of mitochondria in both normal and ethanol-exposed hepatocytes.

Early studies on the dynamics of the mitochondria in healthy hepatocytes found an equilibrium with moderate rates of fission and fusion events, which is suggestive of normal mitochondrial turnover and the preservation of cellular homeostasis. But when exposed to ethanol, there was a clear difference in the dynamics of the mitochondria, as evidenced by a significant decrease in the number and size of fission and fusion events. This marked decrease in mitochondrial dynamics highlights the perturbing effect of ethanol on the morphology and function of mitochondria, providing insight into the molecular mechanisms behind ethanol-induced hepatotoxicity.

High-resolution visualisation of the mitochondrial ultrastructure was made possible using electron imaging techniques, which made it possible to examine in detail the morphological changes brought on by exposure to ethanol. Surprisingly, hepatocytes treated with ethanol showed abnormal mitochondrial morphology, which included cristae remodelling, swelling, and fragmentation. These abnormalities were suggestive of structural disruption and mitochondrial dysfunction. The observed decrease in mitochondrial dynamics was supported by these morphological abnormalities, which also shed light on the pathophysiological effects of ethanol-induced mitochondrial damage. In addition, the researchers used fluorescent proteins that were particular to organelles to draw a picture of the spatial arrangement of mitochondria in hepatocytes exposed to ethanol.

Confocal microscopy made it possible to precisely localise mitochondria in relation to other cellular structures, revealing changes brought about by ethanol in the distribution and clustering patterns of mitochondria. A major mediator of ethanol-induced hepatotoxicity, mitochondrial dysfunction is implicated by the disrupted spatial organisation of mitochondria, which highlights

the disruptive effects of ethanol on mitochondrial dynamics and cellular architecture. The thorough analysis revealed the significant effects of ethanol exposure on hepatocyte mitochondrial morphology and kinetics, clarifying a series of actions that lead to structural disruption and mitochondrial malfunction. Utilising cutting-edge imaging methods revealed hitherto unseen insights into the molecular mechanisms behind ethanol-induced hepatotoxicity, highlighting the critical role mitochondrial dynamics play in preserving cellular homeostasis and liver function. The development of focused treatment strategies aiming at alleviating mitochondrial dysfunction in affected individuals may be influenced by our findings, which have important significance for understanding the pathophysiology of ethanol-related liver disorders.

Miz1, ALR and mitochondrial dysfunction

The function of Miz1 in mitophagy has become a major area of interest in recent investigative studies, providing insight into its role in the pathophysiology of non-alcoholic steatohepatitis (NASH). Mitophagy is a type of selective autophagy that is essential for removing damaged or defective mitochondria. It is also involved in cellular homeostasis and mitochondrial quality control. Notably, severe mitochondrial dysfunction has been linked to the advancement of certain liver illnesses, including NASH. Dysregulation of mitophagy has been implicated in these diseases [17]. Research on Miz1, a transcription factor involved in many different biological functions, has revealed that it is essential for controlling hepatocyte mitophagy. Miz1 regulates the removal of damaged mitochondria by coordinating the transcriptional activation of important genes involved in the mitophagy pathway. Interestingly, it has been found that dysregulation of Miz1 expression exacerbates the pathophysiology of NASH by impairing mitophagy and causing an accumulation of defective mitochondria in hepatocytes. Additionally, the discovery of the augmenter of liver regeneration (ALR) protein has shed light on the molecular pathways that underlie the pathogenesis of NASH.

Hepatopoietin, or ALR, is a multifunctional protein involved in various cellular functions such as cell survival, oxidative stress response, and mitochondrial biogenesis. Research findings indicate that the depletion or malfunction of ALR leads to notable abnormalities in the mitochondria of hepatocytes. These abnormalities include reduced respiratory chain activity, faulty mitochondrial dynamics, and oxidative stress. Beyond mitochondrial failure, the effects of ALR deficiency include disruption of lipid homeostasis and the development of hepatic steatosis, which are important aspects of NASH pathogenesis. The hallmark of NASH, steatosis, is developed when lipid metabolism pathways are disrupted by ALR deficiency, resulting in abnormal lipid accumulation. Additionally, ALR has been linked to the regulation of inflammatory signalling pathways and the activation of hepatic stellate cells, which exacerbates the development of NASH into advanced liver cirrhosis and fibrosis.

Understanding the roles of Miz1 and ALR in hepatocyte mitochondria has been crucial in understanding the pathophysiology of nonalcoholic steatohepatitis. In NASH, dysregulation of mitophagy and mitochondrial dynamics, controlled by Miz1, leads to hepatocyte damage and mitochondrial malfunction. Similarly, ALR deficiency exacerbates the metabolic disturbances typical of NASH by compromising mitochondrial activity and lipid balance. It is possible to create tailored therapeutic strategies to mitigate mitochondrial dysfunction and stop the

progression of NASH to severe liver disease by understanding the complex interactions between various cellular pathways.

MATα1, Alcohol-Associated Liver Disease, and Hypoxia-Related NAFLD Progression Critical insights into the pathophysiology of alcoholic liver disease (ALD) have been provided by a fundamental work by Chen J. and colleagues, which examined the role of methionine adenosyltransferase alpha 1 (MATα1) in mitochondrial regulation and alcohol-related liver damage. The enzyme MATα1, which is involved in the synthesis of S-adenosylmethionine (SAMe), is essential for controlling methylation processes inside cells and preserving the homeostasis of lipids in the liver. The development of liver illnesses, such as ALD, which is characterised by hepatic steatosis, inflammation, and fibrosis, is significantly increased by alcohol consumption. According to the researchers, drinking alcohol causes a decrease in mitochondrial MATα1 levels, which interferes with the synthesis of SAMe and affects mitochondrial function [18].

Additionally, the investigation explored the complex interactions between peroxisome proliferator-activated receptor alpha (PPARα) and hypoxia-inducible factor 2-alpha (HIF-2α) in relation to the pathophysiology of NAFLD. Nephrotic Acid Fever is a rapidly emerging worldwide health issue that is typified by the accumulation of hepatic lipids without substantial alcohol consumption. Hepatic steatosis and metabolic dysfunction have been linked to hypoxia, a prevalent characteristic of NAFLD. Chen J. and associates clarified the mechanism via which HIF-2α exacerbates NAFLD by suppressing PPARα, a key modulator of hepatic lipid metabolism. HIF-2α activation inhibits PPARα transcriptional activity in hypoxic environments, which prevents fatty acid oxidation and encourages lipid buildup in hepatocytes. This disruption of lipid metabolism emphasises the complex interaction between metabolic dysfunction and hypoxia signalling in liver pathology, prolonging hepatic steatosis and aggravating the course of NAFLD.

These various investigations add to our understanding of the complex interplay among oxidative stress, toxic chemicals, and hepatocyte mitochondrial activity. Every aspect of liver disorders, including tBHP-induced oxidative stress and the roles of Miz1, ALR, MATα1, and HIF-2α in mitochondrial dynamics and dysfunction, provides insight into possible targets for therapeutic approaches. A diagrammatic depiction of the harmful effects of mitochondrial dysfunction in NASH, eventually progressing to HCC has been shown in *Figure 2*.

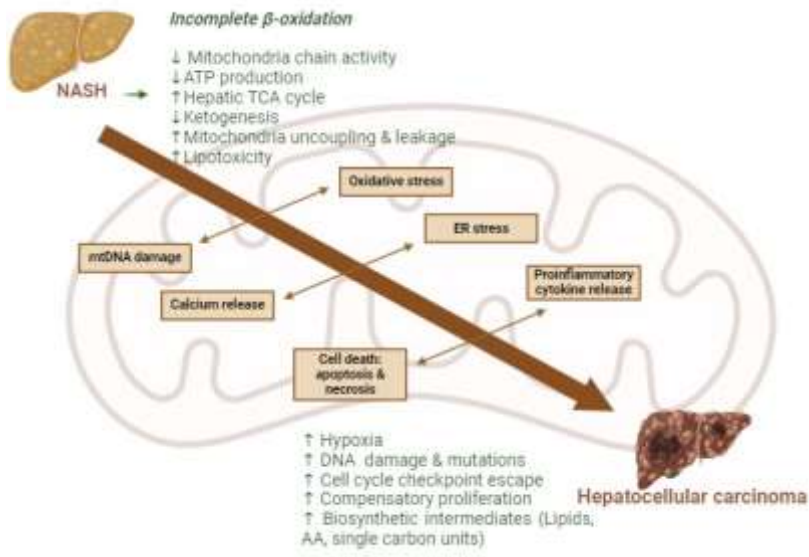

Figure 2. A representation depicting the harmful effects of mitochondrial dysfunction leading the progression from NASH to hepatocellular carcinoma.

2.2 Mitochondrial Dysfunction in HCC: Genetic and Epigenetic Alterations

Genetic Alterations

Somatic mutations in mtDNA have been linked to HCC in numerous studies. The dysfunction of the electron transport chain (ETC) is caused by point mutations, insertions, and deletions that occur within the mitochondrial genome [19]. Notably, alterations in mtDNA copy number and gene expression have been detected in the D-loop region, which is linked to transcription regulation and replication. A common finding in HCC is a reduction in the number of copies of mtDNA, which is associated with genetic abnormalities and promotes mitochondrial dysfunction. A lower number of mtDNA copies is associated with a worse prognosis and more aggressive tumours. In HCC, genetic changes in nuclear-encoded mitochondrial genes are frequently observed. Mutations that impact components of the ETC, such as Complex I and IV subunits, impair oxidative phosphorylation, resulting in an energy crisis and elevated generation of ROS [20-22].

Epigenetic Alterations

Epigenetic modifications, particularly DNA methylation, play a crucial role in HCC-related mitochondrial dysfunction. ETC function may be hampered by aberrant hypermethylation of mtDNA CpG islands, particularly the D-loop region, which can suppress the production of mitochondrial genes [23]. Histone alterations, including methylation and deacetylation, are linked to mitochondrial dysfunction in head and neck cancers. Impaired energy metabolism results from dysregulated expression of histone-modifying enzymes, which impacts

mitochondrial biogenesis. MicroRNAs and long non-coding RNAs are examples of non-coding RNAs that take role in the epigenetic control of mitochondrial activity. By modifying important mitochondrial genes, the dysregulated expression of these compounds in HCC leads to mitochondrial dysfunction [24].

In HCC, there is a complicated interaction between genetic and epigenetic changes. Epigenetic alterations can be influenced by somatic mutations and vice versa. This complex interaction promotes a pro-tumorigenic microenvironment by intensifying mitochondrial dysfunction.

2.3 Potential Mitochondrial Biomarkers for HCC

Dysregulation of mitochondrial function is a hallmark of HCC, and the identification of specific mitochondrial biomarkers holds promise for diagnostic, prognostic, and therapeutic applications. mtDNA somatic mutations are emerging as key indicators for HCC. In mtDNA, point mutations, deletions, and insertions are frequently seen [25]. The 4977-bp deletion, often known as the "common deletion," is linked to HCC and could be used as a marker for diagnosis. Furthermore, a poor prognosis and the advancement of the disease are correlated with particular mutations in the D-loop area. In HCC, changes in the copy number of mtDNA are also suggestive of mitochondrial malfunction. A reliable biomarker linked to the severity and aggressiveness of the disease is reduced mtDNA copy number. Measuring the amount of mtDNA copy number may be a useful predictive and informational tool for the development of HCC [26]. Furthermore, another important characteristic of HCC is mitochondrial respiratory chain complex dysfunction. It is common to hear of aberrations in Complex I, III, and IV activities. Measurements of particular subunits or activity within these complexes show promise as HCC biomarkers since they indicate deficient oxidative phosphorylation. In HCC, a result of compromised electron transport chain performance is elevated production of ROS within the mitochondria. Mitochondrial ROS level measurement is a dynamic biomarker that reflects oxidative stress and advances our knowledge of how HCC progresses [27, 28].

Changes in MMP are suggestive of apoptotic resistance and mitochondrial dysfunction in HCC. Decreased MMP is linked to advanced HCC stages and unfavourable patient outcomes. Hence, measuring MMP is a non-invasive biomarker that can be used to evaluate mitochondrial health and forecast the course of disease [29-31]. Mitochondrial dysfunction is reflected in the altered expression of biomarkers associated with mitochondrial biogenesis in HCC, such as Nuclear Respiratory Factor 1 (NRF-1) and Peroxisome Proliferator-Activated Receptor-Gamma Coactivator 1-alpha (PGC-1α) [32]. The development of HCC is further linked to abnormalities in mitochondrial dynamics, including fission and fusion. Potential biomarkers that provide information on the alterations in mitochondrial morphology linked to HCC include the dysregulated expression of the proteins involved in mitochondrial fission (Drp1) and fusion (MFN1, MFN2, and OPA1) [33].

3. Types of somatic mitochondrial DNA alterations in HCC

3.1 Point mutations

Approximately 52% of patients with HCC have at least one point mutation in their mtDNA, whether it is homoplasmic or heteroplasmic, according to research done on HCC samples [34]. Of these mutations, rRNA genes account for 2%, tRNA genes for 3%, mRNA genes for 19%, and the D-loop area for 76%. This trend emphasises the D-loop region's vulnerability to mutations in HCC and other malignancies, and it is consistent with observations made in other cancer types [35]. The poly-C sequence at np 303–309 in particular, which is located in the D-loop area, is extremely vulnerable to oxidative stress, which can lead to mutations in the D-loop or instability of mono- or dinucleotide repeats in mtDNA [36]. Notably, HCC does not exhibit a distinct G-to-T transversion brought on by oxidative DNA damage. Approximately 59% of the mtDNA mutations found in HCC are transition mutations (G/A-to-A/G or C/T-to-T/C), and 38% involve mono- or di-nucleotide instability. These findings imply that oxidative damage is not the main cause of these mutations in HCC. Qualitative alterations in mtDNA may be influenced by individual or a combination of factors such as alcohol misuse, liver cirrhosis, and hepatitis B infection. The amount of mtDNA copies in HCC is affected by mutations in the D-loop region, especially those close to the replication origin [37]. Research has demonstrated a significant link between a low differentiation grade of HCC and the quantity of mtDNA mutations in the D-loop region.

This suggests that the mutations have an impact on mitochondrial function and may contribute to the progression of HCC. Certain mutations occur in evolutionarily conserved regions of impacted mitochondrial genes, producing amino acid substitutions in the coding sequence and premature stop codons in T6787C, G7976A, G9267A, and A11708G [38]. Furthermore, tRNA mutations (T1659C in tRNAVal and G5650A in tRNAAla) may change tRNA structure and cause frame-shift mutations, as well as base-pair deletions and insertions like 11032delA and 12418insA, which may be linked to mitochondrial illnesses. These mtDNA point mutations might be a factor in mitochondrial malfunction of HCC [39].

3.2 Insertions

Mutations in the D-loop region of mtDNA have been found in a number of human malignancies, including HCC. These insertions are made up of two little DNA pieces that are tandem duplications and tandem triplications, respectively, measuring about 260 bp and 520 bp [37, 40]. Interestingly, poly-cytosine (poly-C) sequences at np 303–309 and np 568–573 surround these insertions. About 4% of cases of HCC had this tandem duplication or triplication, which is tightly linked to length differences in the poly-C sequence at np 568. It is noteworthy that these insertions are not unique to cancer tissues; older people's somatic tissues have also been shown to contain them. Consequently, even though these insertions are linked to HCC, they are not unique markers of the disease because they can also be detected in tissues that are not malignant [39, 41].

3.3 Deletions

Numerous cancer types have been found to have large-scale deletions in their mtDNA, with the 4977-bp deletion being the most prevalent in tumors [42]. Interestingly, in HCC, malignant tissues had a lower incidence and accumulation level of the 4977-bp deletion than non-tumor tissues in HCC patients, which is consistent with findings in other cancers [43]. Long-term

alcohol usage and gender are two possible factors that may have an impact on the accumulation of this deletion in HCC. Although the precise function of mtDNA deletion in HCC is still unknown, it has been proposed that tumour cells may have adapted to a novel milieu during hepatocarcinogenesis by decreasing their mtDNA levels [41, 44]. Additionally, a 50-bp deletion that is located in the mtDNA's D-loop region and is bordered by a 9-bp direct repeat has been documented in a single HCC patient [38]. This loss is homoplasmic in the HCC tissue but absent in the equivalent non-tumor liver tissue, in contrast to the 4977-bp deletion. The accumulation of the 50-bp deletion in tumours is different from the pattern seen in tumours with the 4977-bp loss. The mtDNA copy number in HCC tissue with this deletion is much lower than in liver tissue without a tumour because it truncates the regulatory region of mtDNA [45]. Due to mtDNA depletion and/or impaired mitochondrial gene transcription, this specific mtDNA loss may be a factor in mitochondrial dysfunction [46].

3.4 Copy number changes

Table 1. A summary of the somatic mutations of mitochondrial DNA in hepatocellular carcinoma [34, 50, 52-56].

Nucleotide position at mtDNA	Mutation	Gene	Amino acid change	Associated function
3842	G→A/G	ND1	Trp (TGA)→Stop (TAA)	Potential to cause mitochondrial Complex I dysfunction (truncated ND1)
9545	A/G→G	COIII	Gly (GGA)→Gly (GGG)	-
1659	T→C/T	$tRNA^{Val}$	Unknown	Unknown 20
11032	A7→A6/7	ND4	Frame shift	Loss of Complex I activity
956	Poly-C	12S rRNA	Unknown	Unknown 20
3894–3960/3901–3967	66 bp del	ND1	Frame shift	Potential to cause mitochondrial Complex I dysfunction (truncated ND1)
5650	G→A/G	$tRNA^{Ala}$	-	Decreased Complex I and IV activity
6787	T→C	COI	Val (GTA)→Ala (GCA)	Potential to cause mitochondrial Complex IV dysfunction (mutation in highly conserved residue)
9263	A→G	COIII	Thr (ACA)→Thr (ACG)	Potential to cause mitochondrial Complex IV dysfunction (mutation in highly conserved residue)
9267	G→A	COIII	Ala (GCC)→Thr (ACC)	Potential to cause mitochondrial Complex IV dysfunction (mutation in highly conserved residue)
7976	G→A	COII	Gly (GGC)→Ser (AGC)	Potential to cause mitochondrial Complex IV dysfunction (mutation in highly conserved residue)
12418	A8→A8/9	ND5	Frame shift	Defective mitochondrial respiratory function, higher lactate production and increased tumorigenesis

11708	A→G	ND4	Ile (ATC)→Val (GTC)	-

A reduction in the number of mtDNA copies is a frequent observation in hepatocellular carcinoma (HCC) [47-49]. When compared to similar non-tumor liver tissues, the mtDNA copy number in tumours is lower in almost 60% of HCC patients. In the D-loop region of mtDNA, point mutations close to the replication origin are especially linked to this reduction [48]. The reduction in the number of mtDNA copies in HCC may be attributed, in part, to the changed expression of genes involved in mitochondrial biogenesis, such as mitochondrial single-stranded DNA binding protein (mtSSB) and peroxisome proliferator-activated recept activator-1 (PGC-1) [50].

It interesting to note that female HCC patients experience the mtDNA copy number reduction more commonly than male HCC patients [47]. The disparity in gender could have an impact on mortality rates, illness progression, and/or clinical symptoms. Research has revealed a substantial correlation between a big tumour size and the prevalence of liver cirrhosis in HCC patients with low mtDNA copy numbers. Additionally, compared to patients with higher mtDNA copy numbers in their tumours, those with lower mtDNA copy numbers in their tumours have a worse 5-year survival. It has been proposed that variables such alcohol misuse, liver cirrhosis, and hepatitis B infection may affect the quantitative alterations in mtDNA in HCC. Recent studies have also suggested that the mtDNA copy number in peripheral blood leukocytes may be used as a predictor of the likelihood of HCC in cases of hepatitis B virus-related HCC [51]. *Table 1* provides a list of various somatic mutations of mitochondrial DNA in HCC.

4. Links between Mitochondrial Dysfunction and HCC Pathogenesis

Numerous clinical disorders, including cancer, have been linked to mitochondrial dysfunction; HCC is not an exception [57]. The most prevalent kind of primary liver cancer, HCC, is frequently linked to several genetic and cellular changes. New research suggests that mitochondrial dysfunction plays a critical role in the development and spread of HCC. There are several connections between the pathophysiology of HCC and mitochondrial dysfunction. The development and advancement of HCC are caused by a combination of mtDNA mutations, increased ROS generation, and altered mitochondrial metabolism. Comprehending the molecular pathways involved in the interplay between mitochondrial biology and hepatocarcinogenesis not only offers valuable understanding of the aetiology of the illness but also identifies possible therapeutic targets for the creation of innovative and focused anti-cancer approaches. Clarifying the complex relationships between mitochondrial dysfunction and HCC will probably open the door for novel therapeutic and diagnostic strategies as this field of study develops [58].

A. Altered mitochondrial metabolism. In order for cells to produce energy through oxidative phosphorylation, mitochondria are essential. Disturbances in energy homeostasis result from the altered metabolic activities of dysfunctional mitochondria in HCC. The Warburg effect is frequently seen in cancer cells, particularly HCC. It is typified by a switch from oxidative phosphorylation to glycolysis even in the presence of oxygen. Rapid cell survival and proliferation are supported by this metabolic reprogramming. Deviations from the normal course

of mitochondrial metabolism give cancer cells the energy and ingredients for biosynthesis they need to continue growing [59].

B. ROS production. ROS generation is closely correlated with mitochondrial dysfunction. The formation of ROS and antioxidant defence mechanisms are out of balance in HCC, which leads to oxidative stress, which in turn promotes genomic instability and aids in the development of cancer. ROS have the ability to alter the tumour microenvironment, cause damage to DNA, and activate signalling pathways that support cell survival. Elevated ROS levels in HCC are largely caused by mitochondrial malfunction, which is frequently accompanied by anomalies in the electron transport chain and creates an environment that is favourable to carcinogenesis [60].

C. Mitochondrial DNA mutations. Mutations in mtDNA are commonly seen in HCC, and they play a significant part in the development of hepatocarcinogenesis. Mitochondrial DNA is more prone to damage than nuclear DNA because it lacks effective DNA repair mechanisms and protective histones. The accumulation of mtDNA mutations exacerbates the metabolic and bioenergetic changes seen in HCC by impairing mitochondrial function [61]. These alterations have the potential to affect the electron transport chain's functionality, which would increase the generation of ROS and encourage cell survival [62].

D. Integrated mechanisms. A feedback loop that accelerates the course of HCC is produced by the interaction of mtDNA mutations, increased ROS generation, and altered mitochondrial metabolism. The malignant phenotype is sustained by a self-perpetuating cycle involving mitochondrial malfunction and ROS-induced DNA damage, including mtDNA mutations. Furthermore, cancer cells have a selective advantage due to the dysregulated metabolic profile caused by mitochondrial malfunction, which allows them to flourish in the nutrient-starved tumour microenvironment [61, 63].

These points have been summarised in *Table 2*.

Table 2. Summary of the aspects leading to HCC pathogenesis with respect to mitochondrial dysfunction.

Sr. No.	Aspect	Description
1.	Mitochondrial metabolism alteration	Disturbances in energy homeostasis, characterized by a shift from oxidative phosphorylation to glycolysis (Warburg effect) - rapid cell survival and proliferation in cancer cells.
2.	ROS generation	Imbalanced ROS generation and antioxidant defence mechanisms - elevated ROS levels promote genomic instability, alter the tumor microenvironment, damage DNA, and activate survival pathways.
3.	mtDNA mutations	Exacerbates metabolic changes by impairing mitochondrial function. mtDNA vulnerability to damage - lack of repair mechanisms contribute to HCC development.
4.	Integrated mechanisms	The interplay between mtDNA mutations, increased ROS generation, and altered mitochondrial metabolism creates a feedback loop accelerating HCC progression. Dysfunctional mitochondria sustain the malignant phenotype and confer selective advantage to cancer cells.

5. Mitochondrial Quality Control in HCC

In order to adapt to a variety of external stressors, including oxidative stress, viral infections, hypoxia, nutritional deficiencies, and aberrant metabolite buildup, mitochondria are essential. They turn on an intricate mechanism known as mitochondrial quality control, which guarantees that there are enough healthy mitochondria to meet the demands of the cell even in the event of damage. Both nuclear and mitochondrial genes regulate the quality control mechanism found in mitochondria. Coordinated protein synthesis and the import of proteins with nuclear gene codes into mitochondria are two components of this complex process. The mechanism guarantees the preservation of mitochondrial integrity and function [64, 65].

HCC has a different microenvironment from other tumours and is more common in people with alcoholic liver disease, viral hepatitis, and non-alcoholic fatty liver disease (NAFLD). In HCC cells, this particular environment affects the state of the mitochondria and quality control mechanisms. Studies on HCC patients with viral infections such as HCV and HBV show distinct changes in mitochondrial quality control. When HCV infection occurs, MFN2 is down-regulated and DRP1 is recruited, which causes mitochondria to split and undergo mitophagy [66]. Similar to this, HBV infection causes Drp1 to translocate into the mitochondria, which increases the expression of Parkin, PINK1, and LC3B, causing mitophagy and enhancing the survival of HCC cells [67]. It can be seen that NAFLD circumstances can promote mitophagy by employing oleic acid to induce NAFLD in HCC cells experimentally. This behaviour suggests that HCC cells can adapt through mitophagy to changes in their environment, potentially affecting their survival and persistence [68, 69].

5.1 Diagnostic Significance of Mitochondrial Biomarkers in HCC

The potential value of mitochondrial biomarkers in the early diagnosis and detection of HCC has been brought to light by recent studies. These biomarkers include mutations in the mitochondrial DNA, modifications to the metabolism of the mitochondria, and adjustments to the dynamics of the mitochondria. Finding the alterations in the mitochondrial DNA linked to HCC is one intriguing line of research [70]. With the use of cutting-edge sequencing methods, these mutations can be found and may function as early markers of cancer. Furthermore, changes in metabolic pathways such oxidative phosphorylation and glycolysis have been linked to deregulation of mitochondrial metabolism in HCC. Using biomarkers to track these changes in metabolism may provide important information about how the disease develops and facilitate an early diagnosis. Moreover, the development of HCC has been linked to modifications in mitochondrial dynamics, such as shifts in the shape and location of mitochondria inside cells. Biomarkers that capture these patterns may offer important insights into the development and course of tumours.

The combination of mitochondrial biomarkers with currently used diagnostic techniques, like imaging and serum biomarker analysis, has the potential to improve HCC early detection and diagnosis. Furthermore, these biomarkers might be useful targets for the creation of cutting-edge treatment plans that alter mitochondrial activity in an effort to stop tumour growth and enhance patient outcomes [71]. It is well known that mitochondria may store calcium ions (Ca^{2+}) and that they are essential for both healthy cellular operations and Ca^{2+}-handling pathological

conditions. The regulation of multiple internal activities in mitochondria is contingent upon the presence of Ca2+. Among these, pyruvate dehydrogenase (PDH), α-ketoglutarate dehydrogenase, and isocitrate dehydrogenase are three important enzymes involved in the tricarboxylic acid (TCA) cycle that are activated, and this is seen to have a substantial role in modulating metabolic activity.

Moreover, the production of ROS, which are connected to processes including carcinogenesis and drug resistance in hepatocellular carcinoma (HCC), is influenced by the levels of Ca2+ in the mitochondria [72]. The mitochondrial Ca2+ uniporter (MCU) complex is a protein complex that is primarily responsible for controlling the levels of Ca2+ within mitochondria. Cyclic adenosine monophosphate response element-binding protein (CREB) is a transcription factor that binds to the promoter region of the MCU gene and increases its expression. This regulates the expression of MCU. It is well established that CREB activity affects the growth of both healthy and malignant liver cells. Additionally, the activation of CREB by the hepatitis B virus X protein suggests a further involvement for CREB in the development of HCC.

Research has demonstrated the importance of mitochondrial Ca2+ homeostasis in the pathogenesis of tumours, with particular subtypes of breast cancer exhibiting increased MCU expression. It has been demonstrated that blocking the MCU gene inhibits the metabolism of cancer cells in HCC, resulting in a decrease in cell division and metastasis [73]. Nevertheless, there is still work to be done to fully understand the entire list of genes implicated in mitochondrial Ca2+ homeostasis in HCC and how these genes affect patient prognosis. Li and associates [74] carried out an extensive investigation using the Cancer Genome Atlas (TCGA) database to look at the relationships, expression patterns, and survival results of particular genes in liver cancer. They also examined genetic differences between normal liver cell lines and different types of liver cancer to confirm their findings. They then recruited 354 Asian patients with liver cancer for a comprehensive study of the mitochondrial genes CREB, MCU, MICU1, and MICU2, including patients with HBV and HCV infections. By utilising immunohistochemistry (IHC) and bioinformatics techniques, they assessed these genes' clinical features to predict the prognosis of HCC [74].

6. Therapeutic Implications: Targeting Mitochondrial Dysfunction

Currently, no clinical trials have been conducted to look into treatments for hepatocellular carcinoma (HCC) that particularly target mitochondria. Nonetheless, a number of research study conclusions point to the possibility of affecting mitochondrial function when treating HCC. A key component of systemic treatment for HCC is sorafenib, a multi-kinase inhibitor that provides patients with advanced illness with a valuable therapeutic option. The molecular insights into sorafenib's anticancer activity highlight the complex interactions between sorafenib and mitochondrial function, explaining its pleiotropic effects on cellular metabolism and signalling pathways, even if its effectiveness in extending overall survival is well-documented. Targeting many kinases implicated in carcinogenic signalling pathways, such as RAF kinases, vascular endothelial growth factor receptors (VEGFRs), and platelet-derived growth factor receptors (PDGFRs), sorafenib's anticancer effects are achieved at the molecular level.

Nevertheless, a growing body of research indicates that sorafenib also interferes with mitochondrial function, directly affecting the formation of ATP and mitochondrial respiratory complexes [75]. Sorafenib inhibits the function of mitochondrial respiratory complexes I and III, key components of the ETC responsible for generating ATP through oxidative phosphorylation. It reduces ATP generation and mitochondrial respiration by interfering with electron transfer within the ETC, jeopardising cellular bioenergetics and metabolic balance. A decrease in mitochondrial membrane potential ($\Delta\Psi m$), a crucial factor in determining both mitochondrial function and cellular viability, is caused by sorafenib-induced inhibition of mitochondrial complexes [76]. Increased proton leakage across the inner mitochondrial membrane causes $\Delta\Psi m$ to decrease because it affects the electrochemical gradient required for ATP generation and other mitochondrial processes. Treatment with sorafenib causes an increase in mtROS as a result of compromised respiratory chain electron transport. Increased mtROS levels cause oxidative stress and mitochondrial malfunction, which in turn cause mitochondria to enlarge, release cytochrome c, and activate apoptotic signalling pathways [77].

Likewise, a lower risk of HCC formation in diabetic people has been linked to metformin, an AMPK activator and complex I inhibitor [78].

Research employing xenograft models and HCC cell cultures has demonstrated that metformin enhances apoptosis and decreases tumour proliferation [79, 80]. Metformin-induced AMPK activation has pleiotropic effects on cell survival, proliferation, and metabolism—all of which are linked to the development of tumours. A major regulator of cell growth and proliferation, mTOR signalling is inhibited by AMPK activation, which has antiproliferative effects on cancer cells. Furthermore, by inhibiting anabolic pathways and promoting cellular catabolism, AMPK activation deprives cancer cells of the metabolic substrates necessary for their growth and survival. Moreover, metformin-induced AMPK activation increases cellular stress resistance and induces apoptosis in cancer cells, which inhibits the growth and spread of tumours [81]. The anticancer effects of metformin are mediated by suppression of mitochondrial complex I activity in addition to activation of AMPK. Metformin reduces mitochondrial ATP synthesis and raises the AMP/ATP ratio, which triggers AMPK activation and metabolic reprogramming by interfering with electron transfer within the respiratory chain. Furthermore, one of metformin's anticancer activities is the induction of mitochondrial oxidative stress and apoptotic cell death in cancer cells by inhibition of mitochondrial complex I [82].

Metabolic reprogramming induced by metformin involves changes to the tumour microenvironment in addition to cancer cells. Metformin alters the metabolism of fats and carbohydrates, which makes the metabolic environment unfavourable for the development and spread of tumours. Moreover, metformin has immunomodulatory effects in the tumour microenvironment, promoting antitumor immune responses and blocking pro-tumorigenic inflammatory pathways. Together, these immunomodulatory and metabolic actions support metformin's chemopreventive qualities against the development of HCC in diabetics [83].

Novel treatments aimed at the mitochondria are being researched. For example, it has been discovered that the peptide R-Tf-D-LP4 inhibits the binding of anti-apoptotic proteins, disrupts mitochondrial respiration, and induces apoptosis in HCC cell lines by targeting the voltage-dependent anion channel (VDAC) on mitochondria. This peptide lowered inflammation,

accelerated apoptosis, and decreased tumour growth in animal models of head cancer [84-86]. Another study looked at the effects of antioxidants in HCC models, emphasising the need of comprehending the role mitochondria play in carcinogenesis. It is interesting to note that while untargeted antioxidants had positive effects, antioxidants that target the mitochondria were found to worsen the development of HCC in some animals. This emphasises the need of fully comprehending the functions of ROS produced by mitochondria and mitochondrial signalling pathways in controlling cell division and death in HCC [87, 88].

7. Conclusion

In summary, research on mitochondrial dysfunction in hepatocellular carcinoma offers important new understandings for treatment and diagnosis strategies. Enhancing early identification and diagnosis of HCC may be possible with a better understanding of the function mitochondria play in the onset and course of the illness. Furthermore, focusing on mitochondrial activity shows potential as a therapeutic approach to HCC treatment. Through deciphering the complex pathways that underlie mitochondrial dysfunction in hepatocellular carcinoma, scientists might create more efficacious diagnostic instruments and treatment strategies. This information could eventually result in better outcomes for those suffering from this difficult illness. To improve clinical management techniques and advance our understanding of mitochondrial dysfunction in HCC, more studies are needed into its implications for diagnosis and treatment.

Conflict of Interest

The authors declare they don’t have any conflict of interest.

Author contributions

Corresponding author was drafted the original manuscript with significant contributions in form of literature search and data collection from all authors. All authors were contributed to revising the manuscript critically for approved the final version and agree to be accountable for all aspects of the work.

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Ethical Approval

Not Applicable

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