Chapter 8

Interaction of Mitochondrial and Epigenetic Regulation in Hepatocellular Carcinoma

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Abstract

Hepatocellular carcinoma (HCC) is a pathology preceded mainly by cirrhosis of diverse etiology and is associated with uncontrolled dedifferentiation and cell proliferation processes. Many cellular functions are dependent on mitochondrial function, among which we can mention the enzymatic activity of PARP-1 and sirtuin 1, epigenetic regulation of gene expression, apoptosis, and so on. Mitochondrial dysfunction is related to liver diseases including cirrhosis and HCC; the energetic demand is not properly supplied and mitochondrial morphologic changes have been observed, resulting in an altered metabolism. There is a strong relationship between epigenetics and mitochondrion since the first one is dependent on the correct function of the last one. There is an interest to improve or to maintain mitochondrial integrity in order to prevent or reverse HCC; such is the case of IFC-305 that has a beneficial effect on mitochondrial function in a sequential model of cirrhosis-HCC. In this model, IFC-305 downregulates the expression of PCNA, thymidylate synthase, HGF and its receptor c-Met and upregulates the cell cycle inhibitor p27, thereby decreasing cell proliferation. Both effects, improvement of mitochondria function and reduction of tumor proliferation, suggest its use as HCC chemoprevention or as an adjuvant in chemotherapy.

Keywords: hepatocellular carcinoma, cell cycle, cell proliferation, mitochondria, epigenetics, IFC-305

1. Introduction

Hepatocellular carcinoma (HCC) represents 80% of the primary liver cancer and, in minor proportion, bile duct cancer and angiosarcoma of the blood vessels in the liver, but all of them have a poor prognosis. HCC is a major cause of cancer-related deaths globally. The incidence of HCC is increasing and has been rising in the last few decades [1]. The HCC is a complex pathology associated in 80–90% with chronic liver diseases like cirrhosis of diverse etiologies. Cirrhosis is a chronic degenerative disease of the hepatic parenchyma characterized by an inflammation process that leads to liver fibrogenesis. This process induces the loss of liver architecture and a diminution of functional parenchyma, which over time changes the environment of the cells resulting in chromosomal instability. The cause of cirrhosis transformation into HCC is not well known, but chromosomal instability could be an important factor for HCC generation in cirrhotic patients. The main problem of this pathology is the lack of early detection, recurrence of tumors following resection [2], and there are no effective therapies. To understand this complex pathology, it is convenient to have some knowledge of the structure and functions of the liver. Therapeutic options for HCC are very limited, and the incidence is very similar to the death rate per year. Only in the early stage of the disease, there are some approved therapies such as tumor ablation, surgical resection, and liver transplantation, but in advanced stages, when most patients are diagnosed, these treatments are not recommended. There is an average of 5-year survival below 20% with these therapies [3]. In intermediate and advanced stage-HCC, the approved options are transcatheter arterial chemoembolization (TACE) and the multi-kinase inhibitor, sorafenib. TACE therapy could extend survival to 2 years [3]. Sorafenib extends survival of patients with advanced stage disease for only 3 months, and this medication causes considerable adverse effects and offers no symptom palliation [4]. There are other several clinical trial efforts focused on therapies involving multiple signaling pathways, most commonly related to tyrosine-kinase growth factor receptors, but they have inferior survival benefits and several adverse effects. Immunotherapy has demonstrated some efficacy, but, in general, molecular characterization to find effective treatments of HCC is needed.

The liver is the largest internal and heterogeneous organ in the body constituted by different kinds of cells like hepatocytes, endothelial cells, cells of the bile duct, Kupffer cells, hepatic stellate cells (HSC), oval cells and pit cells [5]. The liver is an organ highly irrigated by the portal venous system and blood is distributed by the hepatic sinusoids and the hepatic artery [6]. About 80% of the liver cells are hepatocytes, and are epithelial cells that form cords with high metabolic activity and contain a complete set of organelles: mitochondria, peroxisomes, lysosomes, Golgi complex and a well-organized cytoskeleton [7]. The space between cords of hepatocytes and the endothelium is called the space of Disse. Endothelial cells constitute the wall of the hepatic sinusoids and are separated from the parenchymal cells by the space of Disse. They possess pores or fenestrae that permit the exchange of fluids [8]. These cells show endocytic activity and secrete several mediators such as interleukin-1 (IL-1), interleukin-6 (IL-6), interferon, and nitric oxide as paracrine modulators. Kupffer cells are the fixed macrophages of the liver that can migrate along sinusoids. Their main function is an immunomodulatory one [9]. Pit cells are intrahepatic leucocytes with natural killer cell activity [10] and exert a cytotoxic activity toward tumor and virus-infected cells [11]. HSC, also known as lipocytes, fat storing cells, perisinusoidal cells, and vitamin A storing cells, are quiescent in normal conditions.

When they are activated, they play an essential role in the synthesis and degradation of the extracellular matrix (ECM) proteins and fibrogenic cytokines, like hepatocyte growth factor (HGF), insulin growth factor (IGR), transforming growth factor-β (TGF-β), and, consequently, induce cirrhosis. Biliary epithelial cells participate in the formation of bile; they are transported to the bile ducts or Canals of Hering. These cells have the potential to become oval cells [7]. The cell-free hepatic tissue represents 20% of the liver volume and constitutes the ECM located in the Disse space. The ECM contains structural proteins like collagen of different types, glycoproteins, fibronectin, tenascin, laminin, entactin, and perlecan. Their function is to maintain the hepatic architecture and the organization of the entire organ. Hepatocytes contribute with 80–90% of the synthesis of liver collagen, which is degraded by metalloproteinases (MMPs) [12]. The liver has multiple functions needed for its own metabolism and for other organs; it participates intensely in the intermediary metabolism that occurs mainly in hepatocytes and is connected with the nutrients of the diet, reaching from the portal circulation, that is, in carbohydrates, proteins, and lipid metabolism. The liver also generates purines and pyrimidines for its own use and their distribution to other tissues in the form of adenosine, inosine, and hypoxanthine [13]. It also synthetizes and secretes plasma proteins and participates in the biotransformation of endogenous and exogenous compounds.

Previously, we have demonstrated that adenosine is a metabolic modulator of glucose and lipids in the liver and adipose tissue [14]. This molecule also modulates in vivo the energy charge in the liver [15]. The nucleoside adenosine is a substance with multiphysiological effects in different tissues, the central nervous system, and cardiovascular system; it is responsible for the modulation of the immune response and acts as metabolic regulator. Its action could be autocrine, paracrine, and endocrine; its metabolism is very active with a high turnover and a very short half-live. Adenosine presents circadian variations in the rat, which correlated with energetic homeostasis of the cell, modulation of membrane structure and function, cell proliferation, and genetic expression by regulating physiological methylation [16]. Exogenous adenosine administration to normal rats showed some pharmacological effects, like increased ATP levels simultaneous to a decrease in ADP and AMP, resulting in an increase of the energy charge of the liver [14]. Also, in the liver of fasted rats, adenosine induces an enhancement of glycogen synthesis [16] and an inhibition of fatty acid oxidation by inhibiting the extramitochondrial acyl CoA synthase and decreasing the plasma ketone bodies [17] These findings allowed us to demonstrate in vivo the Atkinson hypothesis of metabolism regulation by energy charge [18].

The redox state of the cell in different compartments, calculated by the NAD^+/NADH (NAD^+ and NADH nicotinamide adenine dinucleotide, oxidized and reduced) system, has been shown to be a key point in the control of metabolism [19]. Adenosine administration induces mitochondrial oxidation and promotes the oxidized state in the cytosol and mitochondria in the presence of fatty acid oxidation inhibition, which is induced by the nucleoside. It has been reported that adenosine modulates vasodilatation and vasoconstriction in the hepatic vessels controlling blood flow from the hepatic artery [20]. All these results observed in normal animals led us to test the effects of the nucleoside in several models of acute hepatotoxicity: one induced with ethanol [21], the second with cycloheximide, and the third with carbon tetrachloride (CCl_4). Although the toxic mechanism of each one is different, they yielded a similar response generating a fatty liver that was prevented by adenosine [21–23]. In this way, the nucleoside, through different mechanisms, protects the liver against acute toxicity.

Continuous acute hepatotoxicity results in chronic liver injury with subsequent cirrhosis, with accumulation of ECM proteins, mainly collagen type I [24], accompanied by a deficient degradation of deposited collagen [25]. These conditions will induce a change in liver architecture with loss of its function. This is a complex process, for which no effective treatment has been developed yet. To study the effects of adenosine in this process, a model of cirrhosis induced in rats with CCl_4 was developed, in which two conditions were tested: prevention during cirrhosis development and reversion once it is already established [26, 27]. The simultaneous administration of adenosine partially blocked the stimulated collagen synthesis induced by the hepatotoxin, maintained high levels of hepatic collagenase activity, resulting in 50% diminution of fibrosis [26]. The effect of the nucleoside was clearly observed also in the reversion model; it was tested in well-established cirrhosis after 10 weeks of CCl_4 administration. Five weeks after suspension of the toxin, animals were treated with saline or adenosine, the saline group increased the cirrhotic characteristics but the group of animals treated with the nucleoside revealed blocked fibrogenesis, increased collagen degradation and normalized collagen types ratio, promoted hepatocyte proliferation, accelerated normalization of liver function, and decreased oxidative stress. These results suggest adenosine as a potential therapeutic agent in the treatment of chronic hepatic disease.

The transfer of an interesting research finding to a clinical setting is complicated, but in collaboration with Dr. Francisco Hernández Luis from the National Autonomous University of Mexico's School of Chemistry, we prepared several adenosine derivatives that were tested in the CCl_4 induced cirrhosis. The aspartate of adenosine, named IFC-305, showed interesting results [28]; beneficial effects in structure and functional recovery were obtained with a fourfold lower dose of this adenosine derivative because it has a longer half-life. The hepatoprotective mechanism of IFC-305 on fibrogenesis was investigated by means of DNA microarrays analysis [29], showing that the expression of 413 differential genes deregulated in cirrhosis tended to be normalized by IFC-305 treatment. Fibrogenic genes, such as TGF-β, collagen type I, fibronectin I, increased their expression in cirrhotic groups, and IFC-305 diminished their expression supporting the antifibrogenic action of the compound. These results highly suggest a diminution of chromosomal instability. With the increased understanding in chromatin organization of the eukaryote genome at genetic and epigenetic levels and remembering the previously commented role of adenosine on physiological methylations, a possible epigenetic mechanism of the IFC-305 could participate in the obtained results. Global changes in DNA methylation, 5-hydroxymethylation and histone H4 acetylation were decreased in cirrhosis and after the IFC-305 treatment the normal values were recuperated. In contrast, the promoter of *Col1a1* gene is hypomethylated in cirrhosis but gains DNA methylation upon treatment with IFC-305, correlating with a decrease of Col1a1 transcript and protein level, showing that the treatment restores globally and specifically epigenetic modifications [30]. The microarray analysis also showed modification of immunity genes which where explored in the CCl_4 model; it was found that the IFC-305 compound reduced inflammatory cytokines and increased the anti-inflammatory ones like IL-10, supporting the modulation of the macrophage phenotypes M1 and M2 [31].

2. Hepatocytes proliferation in cirrhosis and cancer, modulation by IFC-305

The liver is an organ with regenerative capacity. Partial hepatectomy or diverse stimuli promote proliferation of parenchymal and non-parenchymal cells in order to recover the liver

mass and architecture. This process is regulated by cell cycle proteins, cytokines, growth factors, and matrix remodeling [32].

In acute liver injury, there is a classic wound healing process in which inflammation triggers scar formation that is subsequently resolved to enable regeneration of the damaged hepatic parenchyma. However, when there is a chronic liver injury, the normal regenerative process is impaired, and instead a net deposition of fibrillar collagen is predominant [33].

Cirrhosis is characterized by a decrease in hepatocyte proliferation, in part, because liver cells have a limited regenerative capacity restricted by telomere length. After several rounds of replication, telomeres reach a critically short length that induces cell cycle arrest, senescence, and apoptosis of hepatocytes. Telomere shortening also activates DNA repair pathways leading to chromosomal fusions and instability [34]. During cirrhosis-activated HSC, inflammatory cells secrete proliferative and angiogenic cytokines that contribute to a proliferative condition milieu, including: HGF, vascular endothelial growth factor (VEGF), and IL-6 [33]. This proliferative milieu could stimulate the proliferation of altered hepatocytes carrying mutations of cell cycle checkpoint genes or could select genetically altered clones, promoting HCC [34].

Among the principal cell cycle checkpoints that are generally altered in HCC are the tumor suppressor p53 and Rb proteins. p53 is implicated in cell cycle control, DNA repair, apoptosis, and regulates different metabolic pathways [35, 36]. p53 is frequently mutated in HCC (28–50%) and core proteins from hepatitis B and C viruses can repress p53 activity [36]. The pRB protein is implicated in the progression from G1 into S phase. The Rb pathway is disrupted in more than 80% of human HCC [34]. Gankyrin binds Mdm2 promoting proteasomal degradation of p53 and pRb. Both gankyrin and Mdm2 proteins are frequently overexpressed in human HCC [34, 35]. p53 is also implicated in the stimulation of ATP production by oxidative phosphorylation (OXPHOS). p53 also decreases glycolysis and cellular reactive oxygen species (ROS) production by inducing a protein called TP53-induced glycolysis and apoptosis regulator (TIGAR). TIGAR blocks glycolysis by degrading fructose-2,6-bisphosphate. This inhibition redirects glucose-6-phosphate into the pentose phosphate pathway, which increases NADPH production increasing the antioxidant defenses. The inactivation of p53 should decrease OXPHOS and increase glycolysis and ROS production in cancer cells [37].

It has been demonstrated that IFC-305 is able to stimulate hepatocytes proliferation in CCl_4-induced cirrhotic liver through the upregulation of proliferating cell nuclear antigen (PCNA), HGF, and p53, with an increase in energy and preservation of mitochondrial function [38].

On the other hand, in a sequential model of cirrhosis-HCC induced by diethylnirosamine (DEN), IFC-305 caused a tumor reduction, and this protective effect was associated with decreased cell proliferation in the HCC stage. This effect was associated with a decreased expression of PCNA, thymidylate synthase, HGF and its receptor c-Met, and the induction of the cell cycle inhibitor p27. IFC-305 also induced a diminution of gankyrin expression contributing to restoring p53 protein expression to control levels [39].

How could the same compound IFC-305 have opposing effects on proliferation in normal versus transformed hepatocytes? These could be mediated partly by a differential expression of the HGF-c-Met pathway driven by IFC-305 treatment, and the dual role of HGF/c-Met in cirrhosis and liver tumorigenesis. HGF expression is restricted to cells of mesenchymal origin, whereas the receptor c-Met is expressed in epithelial and endothelial cells. HGF is implicated

in cell proliferation, survival, morphogenesis, cell motility, and metastasis. This pathway plays a critical role in tissue protection and regeneration. It has been used as a therapeutic agent in fibrosis of different organs. The protective actions of HGF are associated with promotion of cell proliferation, migration, and morphogenesis that would help tissues reorganization [40]. Its protective role is also related to its anti-inflammatory action and its regulation of the cellular redox state, driven by upregulation of the antioxidant enzymes and glutathione reduced (GSH), as well as by repression of two major pro-oxidant systems: NADPH oxidase and/or Cyp2E1 [41]. Nevertheless, the HGF/c-Met pathway in HCC contributes to tumor development by stimulating cell proliferation, invasion, and metastasis [40]. We observed that, in the cirrhotic liver induced by CCl_4, the hepatoprotector IFC-305 incremented HGF expression [38], which could have a protective role in the regenerative capacity of the liver. On the other hand, in DEN-induced HCC, the IFC305 treatment downregulated HGF and c-Met expression, which contribute to liver tumorigenesis reduction [39]. HGF and c-Met can be potentiated by ROS in hepatoma cells [41, 42]. It was described that, in the sequential model of cirrhosis-HCC with DEN, there are dysfunctional mitochondria and the administration of IFC-305 restored the mitochondrial function and regulated parameters implicated in metabolism, as well as the mitochondrial dynamics modified by DEN intoxication [43]. Therefore, the IFC-305 could be suppressing expression of HGF via the improvement of mitochondrial redox in DEN carcinogenesis. On the other hand, the restoration by IFC-305 treatment of the p53 protein expression in CCl_4-induced cirrhosis and in DEN-induced carcinogenesis, among other effects, could contribute to the restoration of ATP production by OXPHOS and to the decrease of ROS production. However, the exact molecular mechanism by which IFC-305 causes different effects on hepatocytes proliferation in cirrhosis and HCC requires further clarification.

3. Mitochondrial alterations in the HCC: the effect of the IFC-305 compound

Mitochondria are responsible for energy metabolism in eukaryotic cells; they generate ATP through oxidative phosphorylation. In addition, an important part of the ATP synthesis is the donation of electrons by the tricarboxylic acids chain (TCA) to the electron transport chain (ETC), constituted by five complexes (I-V), NADH enters complex I and generates NAD^+, and complex V forms ATP. Mitochondria regulate the energetic state, the redox state, and the metabolism of the cells, being able to generate the epigenetic intermediaries becoming the main therapeutic target of many kinds of cancer [44].

As a response to stress, the cells acquire a metabolic adaptation, which is an important area of research due to its relationship with different illnesses [45]. In chronic liver diseases like cirrhosis, energetic deficiency and alterations in energy parameters have been demonstrated independently of their etiology [46]. Otto Warburg suggested that mitochondria from tumor cells supply energy through glycolytic flow due to lack of oxygen or genetic-epigenetic alterations that affect oxidative metabolism [47]. Mitochondrial dysfunction is implicated in metabolic reprogramming in HCC. The increased ROS production and the reduced ATP generation may contribute to the HCC malignancy [48]. Metabolic alterations may decrease the

levels of acetyl CoA, which also plays an important role as modulator of gene expression [49]. In experimental models, including the CCl_4-induced cirrhosis, mitochondrial dysfunction has been demonstrated because impaired mitochondrial respiration and ATP decreased levels have been observed [50, 51]. A metabolic adaptation in response to the ATP diminished levels is increased glycolysis [51]. A consequence of oxidative stress in chronic liver diseases is the decrease in metabolic flux, which includes alterations in the TCA enzymes, such as isocitrate dehydrogenase (IDH), which can produce oncometabolites when it undergoes mutations [52].

The redox state can be represented by the NAD^+/NADH ratio, which is regulated by the ETC. Several enzymes depend on NAD^+ like sirtuin-1 (Sirt-1), a member of deacetylases, and poly (ADP-ribose) polymerase-1 (PARP-1). A Sirt-1 substrate is the peroxisome proliferator-activated receptor gamma co-activator 1-alpha (PGC-1α), which is upregulated in HCC and is responsible for orchestrating mitochondrial biogenesis, favoring accumulation of defective mitochondria [44]. On the other hand, PARP-1 modulates the transcription and DNA repair; however, in HCC, it is upregulated and is considered a hallmark of cancer [53]. The over-regulation of both enzymes in HCC may deplete the NAD^+ that can be related to loss of mitochondrial membrane potential (ψm) and mitochondrial dysfunction [54]. Alterations in ψm induce the process of mitochondrial dynamics as a repair response to possible damage to this organelle. Mitochondrial dynamics depends on two mechanisms: fission and fusion; the first one is caused by various types of stress and requires protein activity such as Drp-1, on the other hand, fusion requires the recovery of ψm and proteins such as mitofusin 1 and 2 (MFN 1 and 2) [44]. Mitochondrial fusion promotes cristae formation and normal mitochondria phenotype [55]. Morphological alterations in mitochondria determined through electronic microscopy in various models of hepatic fibrosis have been described a long time ago [56, 57].

Previously, it has been discussed some of the effects of adenosine (base molecule of IFC-305), which include increase in energy parameters and regulation of the redox state. Considering this background and what has been described regarding the metabolic and mitochondrial changes in chronic liver damage, such as cirrhosis and HCC, it was decided to evaluate whether IFC-305 had any mitochondrial effect in the sequential model of cirrhosis-HCC.

In the sequential model of cirrhosis-HCC, decreased mitochondrial respiration, determined through oxygen consumption, and a decreased ψm were found, which reflected in a diminished ATP synthesis. In fact, the dimeric form (active form) of the F1F0 complex of ATPase is lost [43].

On the other hand, alterations in the mitochondrial redox state were observed, determined through the ratio of the levels of β-hydroxybutyrate/acetoacetate (NAD^+/NADH). The activity of NAD-dependent enzymes was also affected, such is the case of IDH and PARP-1; this alteration induced a metabolic adaptation because increased levels of lactate were observed suggesting an increase in aerobic glycolysis [43].

It is known that the mitochondrion is capable of responding to several insults of stress through the activity of various nuclear-encoded proteins like PGC-1α and Sirt-1. However, the over-regulation of these proteins has been associated with the accumulation of dysfunctional mitochondria, as described above. In the model previously described, these proteins were found increased. Dysfunctional mitochondria have been related to their morphology,

and we know that morphology is closely linked to dynamism. The ratio of Drp-1/MFN-2, proteins that regulate the mitochondrial dynamics, was increased favoring the fragmented form of mitochondria as verified through electron microscopy [43].

Important findings were observed with the IFC-305 treatment as described in **Table 1** [43].

Uncoupled mitochondria depicted lower ATP synthesis due to the altered ψm and complex I activity. Previously, it has been demonstrated that complex I is sensitive to DEN toxicity, as NAD^+ linked respiration is inhibited [58]. Recovery of these parameters with IFC-305 treatment was observed, including the activity of NAD^+-dependent IDH. The PARP-1 activity inhibition probably favored the NAD^+ availability and contributed to the maintenance of the redox state. Mitochondrial function preservation and restoration allowed the normalization of the metabolism observed by lactate levels diminution.

On the other hand, the decreased Sirt-1 and PGC-1α in the groups treated with IFC-305 suggested that abnormal mitochondrial accumulation was inhibited. In fact, mitochondrial dynamics regulation was induced by IFC-305. These results demonstrated mitochondrial impairment through functional, metabolic, and dynamic alterations in HCC, and the hepatoprotector IFC-305 helps to repair them, being a tumor suppressive mechanism.

These findings support the mitochondrial role in the establishment of HCC and the interplay with the nuclear genome as targets in the design of new therapeutic strategies for the HCC treatment. In this regard, the IFC-305 supports that idea and emerges as a new possible HCC therapy through mitochondrial regulation.

According to the above, there is a growing interest to find pharmacological strategies to block the effects of mitochondrial dysfunction in HCC. Regarding this, in the model of HCC induced with DEN, a study was conducted to determine the mitochondrial effects of ginkgolide B in

Mitochondrial parameter	Effect
Function	Maintained and recovered: • mitochondrial respiration • ATP synthesis • mitochondrial membrane potential • dimeric form of the F1F0 ATPase subunit • normal cellular redox state
Metabolic	• Recovered the normal mitochondrial redox state • recovered the IDH activity • reduced lactate production • diminished increased PARP-1 activity
Dynamics	Avoided the accumulation of dysfunctional mitochondria through: • down-regulation of PGC-1α and Sirt-1 • diminution of DRP-1/MFN-2 ratio • Sirt-3 increment

Table 1. Effects of IFC-305 administration in mitochondria in the sequential model of cirrhosis-HCC.

two different pharmaceutical formulations, finding a decrease in the mitochondrial generation of ROS and a decrease in the dissipation of the mitochondrial membrane potential [59]. Moreover, two of the most studied hepatoprotective compounds until now are resveratrol and N-acetylcysteine (NAC) [60]. On the one hand, resveratrol inhibits the formation of hepatocyte nodules in the DEN-induced HCC model plus phenobarbital administration; moreover, it is capable of modulating mitochondrial biogenesis [61]. On the other hand, NAC blocked phosphorylation of β-catenin, JNK, and c-jun activation, avoiding the development of liver damage in HCC transaldolase-deficient mice, a limiting enzyme for the non-oxidative branch of the pentose phosphate pathway, which is, at least in part, responsible for HCC generation [62]; furthermore, NAC stabilizes the mitochondrial membrane potential regulating mitochondrial dynamics [61].

4. Interaction of mitochondria and epigenetics in HCC: An overview

The epigenome can be altered not only by environmental factors, such as exposure to exogenous chemicals [63] but also by changes in the levels of endogenous cofactors and metabolites [64, 65]. The exact correlation between nucleus and mitochondrion allows for the maintenance of mitochondrial structure and function. On the one hand, the nuclear gene expression is regulated by mitochondrial intermediates, like acetyl-CoA, ATP, NAD^+, and s-adenosylmethionine, which are the link between the epigenome and calorie availability [47, 66]. In addition to the production of epigenetic substrates, mitochondria may be modified in their DNA (mtDNA). Some mitochondrial genes have been reported as hypermethylated in HCC; for example, mitochondrial ribosomal protein S12 (*Mrps12*), mitochondria-localized glutamic acid-rich protein (*Mgrap*), and transmembrane protein 70 (*Tmem70*) genes [67, 68]. On the other hand, the disruption of the step in the methylation of 5-mC to 5-hmC in the mitochondrial genome leads to the alteration of several OXPHOS genes, such as: NADH dehydrogenase (ubiquinone) 1 subunit C2 (NDUFC2), NADH dehydrogenase (ubiquinone) flavoprotein 1 (NDUFV1), NADH: ubiquinone oxidoreductase subunit S6 (NDUFS6) from complex 1. These modifications, added to the mitochondrial damage by oxidative stress, can favor the loss of ETC function. In addition to that, it has been reported that the mitochondrial genome damage can affect the expression of nuclear genes [69–71]. Moreover, there is a deregulation of hepatic one carbon, and TCA cycle, therefore it driving the aberrant epigenetics changes [72–74]. The main consequence of depressing the TCA cycle is the reduced availability of α-ketoglutarate, leading to a decrease in the activity of α-ketoglutarate-dependent proteins, which are responsible for the hydroxylation of many substrates in the cell that are important in epigenomic control [74].

Tumor cell metabolism can be linked to epigenetic changes during carcinogenesis; recent research has focused on epigenetic studies in relation to metabolic pathways [75, 76]. HCC is a heterogeneous disease affected by various lifestyles and environmental factors. Epigenetic alterations are frequently caused by these factors and contribute to hepatocarcinogenesis. During HCC development, different alterations in global DNA methylation have been described; for example, global hypomethylation leads to aberrant overexpression of oncogenes and large chromosomal instability [77, 78].

In cirrhosis and HCC, distinct patterns of aberrant DNA methylation associated with cirrhosis and HCC have been confirmed [79, 80].

5. Conclusion

The pathophysiology of HCC is multifactorial and involves mitochondrial dysfunction. Mitochondria usually generate relevant modulators of gene expression controlled by epigenetic mechanisms. These alterations induce chromosomic instability that could give advantages to subclones of cells to their outgrowth (**Figure 1**). Further studies are needed to find

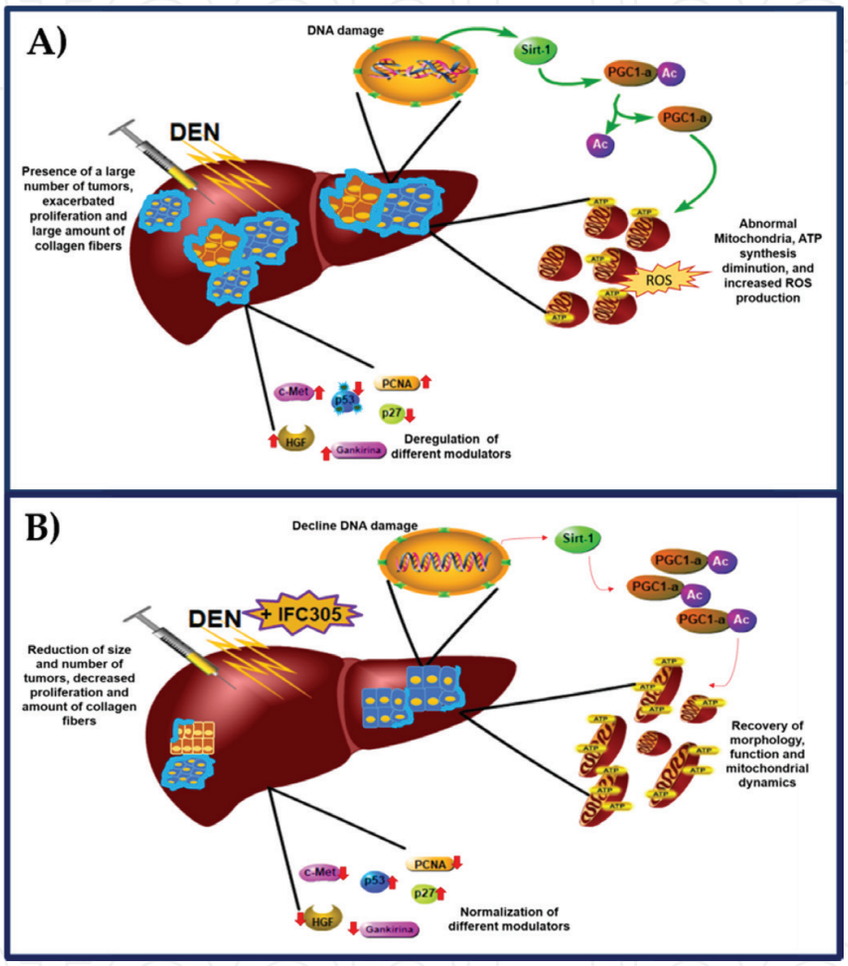

Figure 1. (**A)** In the model of liver injury induced by diethylnitrosamine (DEN), the architecture of the liver parenchyma is altered causing an exacerbated proliferation of various transformed clones, where the presence of a large number of tumors randomly distributed in each one is observed in the hepatic lobules. The preneoplastic nodules that form are surrounded by septa of collagen fibers; thus, favoring the evasion of the immune system and an ideal hypoxic microenvironment for the tumor cells. The genomic instability caused by the toxic as well as favoring mutations, for example in p53, and various alterations in different cellular modulators, among them HGF, c-Met, PCNA, gankyrin and p27. It also causes an increase of proteins, deacetylating PGC1-α, and, thus, modifies various nuclear genes exported to the mitochondria, causing accumulation of abnormal and dysfunctional mitochondria. (**B)** In the model of hepatocarcinoma induced by DEN, the administration of the adenosine derivative, IFC-305, has been shown to have various regulatory effects. The excessive accumulation of collagen fibers in preneoplastic nodules as well as the number and size of tumors are reduced. Also, cell morphology and DNA recover significantly. A decrease in the deacetylase Sirt-1, whose target is PCG1-α, has been observed, which allows the latter to remain acetylated and can be internalized to mitochondria, where it will promote its adequate morphology, dynamics and function. It has also been found that the compound IFC-305 acts on the levels of some important modulators in cancer (p53, HGF, C-Met…), maintaining or returning them to their concentrations under normal conditions. Overall, the aforementioned effects make this compound a possible therapeutic alternative.

therapeutic strategies capable of maintaining and improving the mitochondrial integrity to avoid alterations in the epigenetic regulation of nuclear- and mitochondrial-encoded genes. These effects could suppress failures in cell cycle checkpoints and the uncontrolled proliferation to prevent or reverse HCC as demonstrated for IFC-305.

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Conflict of interest

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Abbreviations

HCC	hepatocellular carcinoma
PARP-1	poly (ADP-ribose) polymerase-1
TACE	transcatheter arterial chemoembolization
HSC	hepatic stellate cells
IL-1	interleukin-1
IL-6	interleukin-6
ECM	extracellular matrix
HGF	hepatocyte growth factor
IGR	insulin growth factor
TGF-β	transforming growth factor-β
MMPs	metalloproteinases
NAD+	nicotinamide adenine dinucleotide oxidized
NADH	nicotinamide adenine dinucleotide reduced
CCl4	carbon tetrachloride
IFC-305	aspartate of adenosine

VEGF	Vascular Endothelial Growth Factor
OXPHOS	oxidative phosphorylation
SCO2	chaperone protein “synthesis of cytochrome c oxidase 2”
ROS	reactive oxygen species
TIGAR	TP53-induced glycolysis and apoptosis regulator
PCNA	proliferating cell nuclear antigen
DEN	diethylnitrosamine
GSH	glutathione reduced
TCA	tricarboxylic acids chain
ETC	electron transport chain
IDH	isocitrate dehydrogenase
Sirt-1	sirtuin-1
PGC-1α	peroxisome proliferator-activated receptor gamma coactivator 1-alpha
ψm	mitochondrial membrane potential
MFN 1	mitofusin 1
MFN 2	mitofusin 2
NAC	N-acetylcysteine
mtDNA	mitochondrial DNA
Mrps12	mitochondrial ribosomal protein S12 gene
Mgrap	mitochondria-localized glutamic acid-rich protein gene
Tmem70	transmembrane protein 70 gene
NDUFC2	NADH dehydrogenase (ubiquinone) 1 subunit C2
NDUFV1	NADH dehydrogenase (ubiquinone) flavoprotein 1
NDUFS6	NADH: ubiquinone oxidoreductase subunit S6

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