Mitochondrial dysfunction-related genes in hepatocellular carcinoma

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1. ABSTRACT

Mitochondria dysfunction is associated with apoptotic resistance and metabolism of tumor cells. Hepatocellular carcinoma (HCC) is a complex and heterogeneous tumor with multiple genetic aberrations. Certain gene mutations in mitochondria may lead to mitochondrial dysfunction. The p53 family plays a key role in the mitochondrial apoptosis pathway and acts as part of the molecular mechanisms underlying mitochondrial dysfunction in HCC. The novel genes found in the mitochondrial apoptosis signaling pathways, such as mfn2, play a role in the p53 network. In mitochondrial metabolism, expression of certain genes may be associated with apoptosis when they are involved in hepatocarcinogenesis. MicroRNAs have also been found to play a role in this process. Some genes may even exhibit multiple functions in the mitochondrial dysfunction of HCC. In HCC therapy, genes have also been found to influence the chemotherapeutic treatment by killing cells via the apoptosis pathway or autophagy. Investigation of mitochondrial dysfunction-related genes could potentially provide evidence for novel therapies that target HCC.

2. INTRODUCTION

Hepatocellular carcinoma (HCC) is the fifth most frequently diagnosed cancer and the third leading cause of cancer-related death worldwide, causing over one half million mortalities each year (1). HCC accounts for approximately 75% to 85% of primary liver cancer cases in the United States (2) and over 90% in high-risk areas. It predominantly affects people in developing countries, such as sub-Saharan Africa, China, Korea, or Vietnam (3,4). The occurrence of HCC is strongly associated with several risk factors, including chronic hepatitis B virus (HBV), chronic hepatitis C virus (HCV) infection, alcohol abuse, and so on (5). In addition, multiple factors, including DNA diploidy, vascular invasion, a disease-free interval of more than 1 year after primary hepatectomy, single HCC at primary resection, and negative portal invasion at repeated resection, have been identified as outcome predictors for HCC (6-8). However, the exact mechanisms underlying HCC remain unclear.

In human cancer cells, resistance to cell death and reprogramming of metabolic pathways comprise major

hallmarks, and also cause inefficacy of chemotherapy (9). Mitochondria, which are crucial for cellular energy production and cell survival, are major regulators in the intrinsic apoptotic pathway (10). Thus, mitochondrial dysfunction promotes HCC progression. Cancer cells exhibit defects in the regulatory genes that govern normal cell proliferation and homeostasis due to progressive accumulation of mutations. Certain gene mutations in mitochondria potentially lead to mitochondrial dysfunction, which evolves into hepatocarcinogenesis. Freisinger and colleagues reported in patients with deoxyguanosine kinase (DGUOK) -related mitochondrial DNA (mtDNA) depletion syndrome, HCC was identified pathologically, suggesting the possible link between mtDNA mutation and HCC (11). In human HCC samples, 13 somatic mutations in the coding region of mtDNAs have been identified (12). Among these 13 mtDNA mutations, the T1659C transition in tRNA(Val) gene and G5650A in tRNA(Ala) gene were reported to be clinically associated with mitochondrial disorders. In addition, the T6787C (cytochrome c oxidase subunit I, COI), G7976A (COII), G9267A (COIII), and A11708G (ND4) mutations may result in amino acid substitutions in the highly conserved regions of the affected mitochondrial genes. These mtDNA mutations (10/13, 76.9%) have the potential to cause mitochondrial dysfunction in HCC (12). Thus, gene polymorphisms may participate in mitochondrial dysfunction in HCC. In this review, we discuss the role of mitochondrial dysfunction-related genes in HCC.

3. Mechanisms underlying gene polymorphism in mitochondrial dysfunction of HCC

The molecular mechanisms underlying hepatocarcinogenesis are very complex. Hepatocarcinogenesis is considered a multistep process involving subsequent gene mutations which control proliferation and/or apoptosis in the hepatocytes. Mitochondrial dysfunction-related genes are involved in the mechanisms underlying HCC. For instance, mitochondrial genes have been found to be differentially expressed in HBV X protein (HBx)-positive hepatoma cells (13, 14). Furthermore, three clones were shown to be human mitochondrial ATPase subunit 6 (mtATPase 6) (14). The HBx is a ubiquitous transactivator in HBV, which is essential for HBV replication *in vivo*. Mice expressing HBx in the liver may either develop HCC spontaneously (15) or display increased susceptibility to hepatocarcinogens (16). The introduction of HBx into HCC cell line Hep3B cells may induce apoptosis and sensitize Hep3B cells to TNFalpha-mediated cell killing; these processes may be accomplished via inhibition of Bcl-xL expression and subsequent promotion of cytochrome c release from mitochondria (17). In addition, the evidence gathered from epidemiological studies has demonstrated the connection between chronic HCV infection and the development of HCC (18,19). Additionally, in a mouse model of HCV-associated HCC, a deletion in mtDNA was reported (20). Taken together, these reports suggest the involvement of mitochondrial dysfunction-related genes in the pathogenesis of HCC. The function of mitochondria is associated with apoptosis, mitochondrial carriers, and metabolism; thus, we discuss the mechanisms underlying gene polymorphism in the mitochondrial dysfunction of HCC.

3.1. Mitochondrial apoptotic pathways

Mitochondrial dysfunction is one of the crucial events involved in apoptotic cell death. Accumulating evidence suggests that apoptotic pathways converge at the mitochondria; signaling is initiated via a series of molecular events that culminate in the release of death factors (21). Gene polymorphisms in mitochondrial apoptotic pathways usually play a role in the apoptosis of hepatoma cells.

Heat shock protein 70 (HSP70), abundantly expressed in human tumors, protects cells from a wide range of apoptotic stimuli. HSP70 potentially modulates mitochondrial protein apoptosis-inducing factor (AIF)-induced cell death. Furthermore, HSP70 silencing has been shown to dramatically increase AIFDelta1-120 mutant (an active form of AIF after deletion of the mitochondria localization domain)-induced apoptosis (22). On the other hand, the potential pro-apoptotic molecule in HCC cells exhibits a gene polymorphism associated with mitochondrion. Fragile histidine triad (FHIT) is a confirmed tumor suppressor gene in HCC. Yu and colleagues (23) found that transactivator of transcription (TAT)-FHIT robustly inhibits growth and induces apoptosis of HCC cells *in vitro*; furthermore, they demonstrated that both exogenous and intrinsic apoptotic pathways are involved in TAT-FHIT-mediated apoptosis, and that this effect may be partially attenuated by mitochondrial protector TAT-BH4. These findings indicate that mitochondria play a critical role in TAT-FHIT-mediated pro-apoptotic effects in cancer cells. TAT-FHIT is a potential pro-apoptotic molecule in HCC cells. The tumor suppressive mechanism of the tyrosine aminotransferase gene has also been shown to play a pro-apoptotic role in a mitochondrial-dependent manner by promoting cytochrome-c release and activating caspase-9 and PARP (24). Moreover, a novel gene, homo sapiens longevity assurance homologue 2 (LASS2), has been isolated from a human liver cDNA library, a human homologue of the yeast longevity assurance gene LAG1 (Saccharomyces cerevisiae longevity assurance gene 1) (25). The LASS2 gene may increase intracellular H(+) of HCC cells via interaction with V-ATPase, thereby inducing cell apoptosis through the mitochondrial pathway.

Transforming growth factor-beta (TGF-beta) is a potent inhibitor of hepatocyte growth (26), and is known to induce apoptosis in both hepatocytes and HCC cell lines (27,28). One of the mediators of the TGF-beta pathway is the Smad family of genes, and candidate tumor suppressor genes in Smad2 and Smad4 gene mutations have been identified in HCC; these mutations were suspected to result from oxidative stress, as observed in mtDNA (29). More recently, Zhang and colleagues (30) found that TGF-beta1 treatment of Huh7 cells resulted in increased PDCD4 expression and apoptosis, concomitant with mitochondrial events. The human HCC cell line Huh7 transfected with PDCD4 resulted in increases of cytosolic cytochrome c and mitochondrial Bax, which was accompanied with decreases
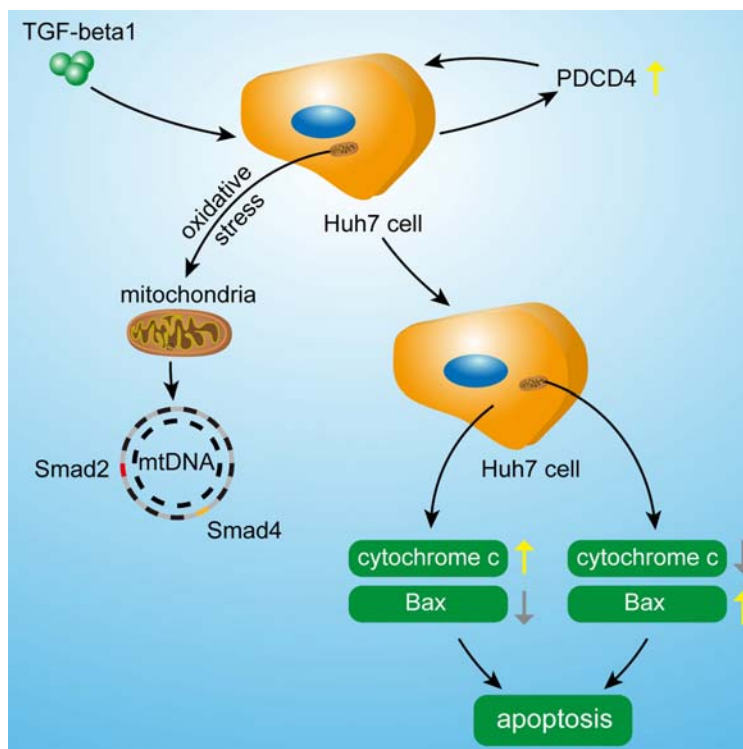
Figure 1. Genes in the TGF-beta pathway or downstream of TGF-beta promote apoptosis due to mitochondrial changes. One of the mediators of the TGF-beta pathway is the Smad family of genes, and candidate tumor suppressor genes in Smad2 and Smad4 gene mutations have been identified in HCC; these mutations were suspected to result from oxidative stress, as observed in mtDNA. The human HCC cell line Huh7 transfected with PDCD4 resulted in increases of cytosolic cytochrome c and mitochondrial Bax, which accompanied with decreases of mitochondrial cytochrome c and cytosolic Bax, then inducing apoptosis.

of mitochondrial cytochrome c and cytosolic Bax, then inducing apoptosis. Thus, genes in the TGF-beta pathway or downstream of TGF-beta could promote apoptosis due to mitochondrial changes (Figure 1).

The tumor suppressor p53 gene plays a major role in preventing tumorigenesis by responding to both cellular stress and DNA damage; p53 mutation is frequently associated with oncogenesis (31,32). In some HCC cell lines, its activation leads to the induction of pro-apoptotic Bax expression (33,34), whereas pro-apoptotic Bax also antagonizes anti-apoptotic Bcl-2 and other anti-apoptotic molecules. The mitochondria play a central role in apoptosis, which is regulated by members of the Bcl-2 family through an intrinsic apoptosis pathway (35-37). In p53-deficient Hep3B cells, apoptosis induced by pectenotoxin-2 (PTX-2) is associated with the down-regulation of anti-apoptotic Bcl-2 members (Bcl-2 and Bcl-xL) and IAP family proteins, and the up-regulation of pro-apoptotic Bax protein, tumor necrosis factor-related apoptosis-inducing ligand (TRAIL)-receptor 1/receptor 2 (DR4/DR5), and mitochondrial dysfunction (38).

In contrast, interference with the p53 family network contributes to a gain of oncogenic function of mutant p53 in HCC via mitochondrial apoptotic pathway. Mutant forms of p53, including p53R143A, p53R175D,

p53R175H, p53R248W, and p53R273H, can acquire anti-apoptotic gain-of-function in HCC by repressing the activity of genes that regulate both the extrinsic apoptosis pathway initiated by ligation of death receptors and the intrinsic/mitochondrial apoptosis pathway. Furthermore, p53 gain-of-function mutants significantly decrease activation of pro-apoptotic target genes by wild-type p53, TAp63, and TAp73. This contributes to the ability of cancer cells to withstand DNA damage-induced apoptosis (39). TAp63 and TAp73 are the promoters of the Trp63 gene and Trp73 gene, respectively. In general, Trp63 gene and Trp73 gene have two respective promoters that drive the expression of two major p63 and p73 isoform subfamilies: TA and N-terminally truncated (DeltaN). TAp63 and TAp73 isoforms exhibit pro-apoptotic activities, whereas members of DeltaNp63 and DeltaNp73 subfamily exhibit antiapoptotic functions. Müller and colleagues (40) found that TAp73 beta could transactivate the CD95 gene via the p53-binding site in the first intron. Inhibition of CD95 gene transactivation was one mechanism by which DeltaNp73 functionally inactivated the tumor suppressor action of p53 and TAp73 beta. Concomitantly, DeltaNp73 inhibited the apoptosis emanating from mitochondria. Thus, DeltaNp73 expression in tumors selects against both the death receptor and the mitochondrial apoptosis activity of TAp73 beta. In HCC DeltaNp63alpha and DeltaNp73 beta expression interferes
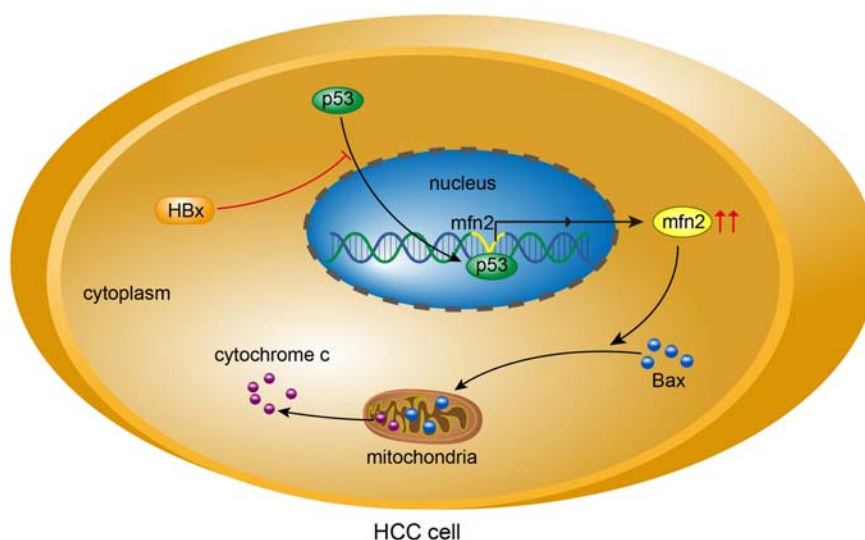
Figure 2. Mfn2 exerts apoptotic effects and inhibits proliferation in HCC cells by the mitochondrial apoptotic pathway. Overexpression of mfn2 also induced cytochrome c release to the cytoplasm by enhancing Bax translocation from the cytoplasm to the mitochondrial membrane. Furthermore, HBx was shown to inhibit p53-mediated up-regulation of mfn2 in HCC cells.

with both the death receptor and the mitochondrial apoptosis activity of the TA isoforms (41,42). DeltaNp63alpha could inhibit the activation of p53 family target genes (41), whereas DeltaNp73beta is oncogenic in HCC by blocking apoptosis signaling via death receptors and mitochondria (42). These findings suggest that DeltaNp63 and DeltaNp73 isoforms repress apoptosis-related genes of mitochondrial apoptosis signaling pathways.

The mitochondrial GTPase mitofusin-2 gene (mfn2), also named hyperplasia suppressor gene (HSG), located on chromosome 1p22.3, encodes a mitochondrial membrane protein that participates in mitochondrial fusion and contributes to the maintenance of the mitochondrial network (43,44). It exhibits a potential apoptotic effect mediated by the mitochondrial apoptotic pathway (45-47). Qu and colleagues (48) reported that heterozygosity (LOH) at mfn2 gene in HCC is associated with clinicopathological features of patients, including tumor size, age, capsule, differentiation, and t HBV infection. Mfn2 was identified as a novel direct target of p53 that could also exert apoptotic effects via Bax signaling and potentially inhibits proliferation in HCC cells (49,50). Overexpression of mfn2 also induced cytochrome c release to the cytoplasm by enhancing Bax translocation from the cytoplasm to the mitochondrial membrane (50). Furthermore, the relationship between mfn2 and HBx was examined. HBx was shown to inhibit p53-mediated up-regulation of mfn2 in HCC cells, though HBx exhibited little effect on the expression of mfn2 or P53. (51) (Figure 2).

Novel genes in the mitochondrial apoptosis signaling pathways have been identified; however, the mechanisms of the p53 family network remain unclear in HCC. The activation of p53 can cause the induction of pro-apoptotic Bax expression, which antagonizes anti-apoptotic Bcl-2. Afterwards, the Bcl-2-mediated apoptotic process is initiated by cytochrome c release from mitochondria and is executed by the caspase family (52,53). Mutant forms of p53 and mfn2 genes play a role in the network.

3.2. Mitochondrial metabolism

Enzymes produced from cytochrome P450 genes (CYP) are involved in the formation (synthesis) and breakdown (metabolism) of various molecules and chemicals within cells. Cytochrome P450 enzymes are primarily found in liver cells. Because the differential expression of CYP2E1 may play a pleiotropic role in the multistep process of liver carcinogenesis, Yang and colleagues (54) examined the effect of trichostatin A (TSA) on CYP2E1 expression and the role of CYP2E1 in inducing apoptosis of HepG2 cells; they found that TSA selectively induced CYP2E1 in human HCC cell lines (Huh7, PLC/PRF/5, Hep3B, and HepG2), but not in normal primary human hepatocytes. Moreover, TSA-mediated up-regulation of CYP2E1 expression was associated with histone H3 acetylation and recruitment of HNF-1 and HNF-3beta to the CYP2E1 promoter in HepG2 cells (54). These findings suggested that histone modification is involved in CYP2E1 gene expression and that CYP2E1-dependent mitochondrial oxidative stress plays a role in TSA-induced apoptosis. In addition, the adeno-associated virus (AAV)3-mediated transfer and expression of the pyruvate dehydrogenase E1 alpha subunit gene could cause metabolic remodeling and apoptosis of human liver cancer cells (55).

Mutated expression of microRNAs (miRNAs) may contribute to HCC. MiRNA-122 is a regulator of mitochondrial metabolic gene network in HCC. Increased expression of miR-122 seed-matched genes leads to a loss of mitochondrial metabolic function. *In vitro* miR-122 data further provide a direct link between induction of miR-122-controlled genes and impairment of mitochondrial metabolism (56). Because miR-122 regulates mitochondrial

metabolism, its loss may be detrimental to sustaining critical liver function and may contribute to morbidity and mortality of liver cancer patients. Further elucidation of the miRNAs regulating mitochondrial metabolism, or other pathways in mitochondria, could potentially help with the development of novel HCC therapies.

3.3. Mitochondrial carrier

Mitochondrial carriers (MC) form a highly conserved family involved in solute transport across the inner mitochondrial membrane in eukaryotes (57-59). Gene changes comprise the major source of MCF functional diversity (59, 60-63). In HCC, MC homolog 2 has been found to locate in the minimal liver tumor suppressor region within 11p11.2-p12 (64). In addition, high resolution melting analysis had been developed for SLC25A13 mutation scanning. SLC25A13 gene encodes citrin, which is a mitochondrial solute transporter with a crucial role in urea, nucleotide, and protein synthesis. However, researchers did not find that SLC25A13 mutations contributed to the pathogenesis of HCC in Taiwan (65).

In summary, gene-mutation-related mitochondrial dysfunction is involved in the underlying mechanisms of HCC. These genes may play a single role in this process or exhibit multiple functions. For instance, p28GANK-knockdown induces the generation of reactive oxygen species, which in turn activates p38. Because p38 signals Bax, its activation may lead to mitochondrial transmembrane potential loss, cytochrome c release from mitochondria to cytosol, and caspase-9 activation, eventually triggering the mitochondrial pathway of apoptosis (66). These genes that exhibit multiple functions may play a potential role in connecting the mitochondrial apoptosis pathway and other pathways.

4. HCC THERAPY IS ASSOCIATED WITH MITOCHONDRIAL DYSFUNCTION

4.1. Mitochondrial apoptosis pathway

The link between chemotherapy, the p53 family, and the mitochondrial apoptosis pathway in HCC has been previously demonstrated. Chemotherapeutic treatment may induce expression of proapoptotic Bcl-2 family members like Bax and BCL2L11 and the expression of Apaf1, BNIP1, Pdcd8, and RAD. Thus, following DNA damage, p53, p63, and p73 promote apoptosis via the extrinsic and intrinsic signaling pathway (67). In the mitochondrial apoptosis pathway, DeltaNp63alpha may inhibit activation of p53 family target genes and apoptosis. In a clinical setting, this process could be induced by chemotherapeutic drugs, thereby contributing to HCC chemoresistance. Moreover, chemotherapeutic treatment induces the expression of Bax, Bim, Noxa, Puma, and Perp, which is antagonized by DeltaNp63alpha (41).

In HCC, HBx leads to the activation of a series of transcription factors including NF-kapa B and STAT-3 via its association with mitochondria and oxidative stress (68). STAT-3 is a member of family of transcription factors that are activated upon tyrosine phosphorylation in response to extracellular signals such as cytokines and growth factors

(69,70). Combined treatments using rapamycin and STAT3 gene silencing have been found to increase apoptosis in human HCC Bel-7402 cells, displaying a more dramatic effect than any single treatment (71).

Growth arrest and DNA damage-inducible gene 153 (GADD153) is a member of the CCAAT/enhancer-binding protein family of transcription factors; it is expressed at low levels under normal conditions but is strongly induced upon growth arrest, DNA damage, and endoplasmic reticulum (ER) stress. Garcinol activates not only the death receptor and the mitochondrial apoptosis pathways but also the ER stress modulator GADD153. Garcinol treatment has been shown to lead to the accumulation of reactive oxygen species (ROS) and increased GADD153 expression (72). The overexpression of GADD153 could be observed in parthenolide-induced apoptosis of HCC cells (73).

In addition to gene changes in HCC therapy, a novel cancer targeting gene-viro-therapy strategy has been proposed. HCC suppressor 1 (HCCS1)-armed, quadruple-regulated oncolytic adenovirus specific for HCC could serve as a cancer targeting gene-viro-therapy strategy (74). HCCS1 is a novel tumor suppressor gene that is able to specifically induce apoptosis in cancer cells. The underlying mechanism of Ad.SPDD-HCCS1-induced liver cancer cell death was found to occur via the mitochondrial apoptosis pathway. Ad.SPDD-HCCS1 was able to elicit reduced toxicity and enhanced efficacy both *in vitro* and *in vivo* compared to a previously constructed oncolytic adenovirus.

4.2. Autophagy associated with mitochondrial dysfunction

Macroautophagy (herein called autophagy) is a cellular, catabolic process that breaks down and recycles macromolecules and organelles under conditions of starvation. Autophagy function is executed by a series of autophagy related genes (ATGs) that are evolutionarily conserved from yeast to mammals (75).

Tetrandrine-induced mitochondrial dysfunction may lead to ROS accumulation and autophagy. ROS generation activates the ERK MAP kinase, and the ERK signaling pathway at least partially contributes to tetrandrine-induced autophagy in HCC cells. Tetrandrine transcriptionally regulates the expression of ATG7, which promotes tetrandrine-induced autophagy (76). Moreover, by reducing expression of ATG7, miR-375 inhibits autophagy of HCC cells under hypoxic conditions in culture and in mice (77). In addition, berberine-induced cell death in human HCC cells is diminished by interference with the essential autophagy gene ATG5, whereas berberine induces autophagy in HCC cells (78).

5. CONCLUSION

Mitochondria are crucial for cellular energy production, and play a central role in the regulation of cell survival and death signaling in liver cells. Mitochondria dysfunction is associated with apoptotic resistance and

metabolism of tumor cells. HCC is a complex and heterogeneous tumor with multiple genetic aberrations. Several molecular pathways involved in the regulation of cell death are implicated in hepatocarcinogenesis. Certain gene mutations in mitochondria may lead to mitochondrial dysfunction, which is involved in hepatocarcinogenesis and HCC therapy. During the molecular mechanisms underlying mitochondrial dysfunction and genes in HCC, the p53 family is the key point of the mitochondrial apoptosis pathway. The novel genes found in the mitochondrial apoptosis signaling pathways, such as Mfn2, play their role in the p53 network. In mitochondrial metabolism, the expression of certain genes may be associated with apoptosis when they are involved in hepatocarcinogenesis. MiRNAs were also found to play a role in this process. Some genes exhibit multiple functions on the mitochondrial dysfunction in HCC, which could serve as a potential key point that connects the mitochondrial apoptosis pathway and other pathways and could serve as a potential therapy target. With regard to HCC therapy, some genes were found to influence chemotherapeutic treatment by killing cells via apoptosis or autophagy. Further investigation into mitochondrial dysfunction-related genes could provide evidence for novel targeting therapies of HCC.

6. ACKNOWLEDGMENTS

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Abbreviations: HCC: hepatocellular carcinoma; HBV: hepatitis B virus; HCV:chronic hepatitis C virus; DGUOK:deoxyguanosine kinase; mtDNA:mitochondrial DNA; HBx:HBV X protein; mtATPase 6:mitochondrial ATPase subunit 6; HSP70:heat shock protein 70; AIF:apoptosis-inducing factor; FHIT:fragile histidine triad; TAT:transactivator of transcription; LASS2:longevity assurance homologue 2; LAG1: (Saccharomyces cerevisiae longevity assurance gene 1); TGF-beta:transforming growth factor-beta; PTX-2:pectenotoxin-2; TRAIL:tumor necrosis factor-related apoptosis-inducing ligand; mfn2:mitofusin-2 gene; HSG:hyperplasia suppressor gene; CYP:cytochrome P450 gene; miRNAs:microRNAs; MC:mitochondrial carriers; GADD153:growth arrest and DNA damage-inducible gene 153; ER:endoplasmic reticulum; ROS:reactive oxygen species; HCCS1:HCC suppressor 1; ATG:autophagy related gene

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