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Roles of Mitochondria in Liver Cancer Stem Cells

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Abstract

Primary liver cancer (PLC) is heterogeneous and it is an aggressive malignancy with a poor prognostic outcome. Current evidence suggests that PLC tumorigenesis is driven by rare subpopulations of cancer stem cells (CSCs), which contribute to tumor initiation, progression, and therapy resistance through particular molecular mechanisms. Energy metabolism and mitochondrial function play an important role in the regulation of cancer stemness and stem cell specifications. Since the role of mitochondrial function as central hubs in cell growth and survival, studies on the critical physiological mechanisms of the liver underlying their therapy-resistant phenotype is important. In this review, we focus on liver CSC-related mitochondrial metabolism that contributes to the liver CSC features, in terms of enhanced drug-resistance and increased tumorigenicity, and to discuss their roles on potential therapies windows for PLC therapies.

Introduction

Primary liver cancer (PLC) is the sixth most common malignant cancer worldwide [57]. Moreover, liver cancer is among the most aggressive and difficult-to-treat malignancies, with a 5-year relative survival rate of less than 21% in the United States [34]. PLC mainly consists of two histologic types, i.e., hepatocellular carcinoma (HCC) and intrahepatic cholangiocarcinoma (iCCA). HCC is the most common type of PLC, accounting for 90% of all liver cancer cases, followed by iCCA [71]. Potentially curative treatments, such as surgical resection, radiofrequency ablation, and liver transplantation, can only be applied in 30-40 percent of patients in the West, and even a smaller proportion of patients in Asia [19]. In addition, recurrence is quite frequent even after curative treatment; therefore, the long-term outcome for PLC treatment is still unsatisfactory [71]. The main challenge to overcome this issue is that PLC is clinically and biologically heterogeneous [82]. Due to the high recurrence, high mortality and resistance to conventional therapies, the development of new chemopreventive agents for precision management of PLC is an important research priority.

Emerging evidence supports the hierarchical model of cancer stem cells (CSCs) as the main driver of tumor progression, cancer recurrence and metastasis [4]. CSCs exhibit features of normal stem cells, e.g., self-renewal and multilineage differentiation capacity but also is responsible for tumor initiation. Therefore, eradicating CSCs may be a critical approach to cancer therapy. Previous studies have shown the existence of CSCs in human liver cancers [61, 70, 90]. Indeed, accumulating evidence supports that liver CSCs are histologically heterogeneous and contain a small fraction of cells with stem cell properties (e.g., self-renewal and differentiation) in PLC such as expressions of a variety of CSC markers. Currently, a number of cell surface markers have been identified as liver CSC markers including epithelial cell adhesion molecule (EpCAM), CD44, CD24, CD133, CD90, and CD13 (Table 1). In addition, other markers including oval cell marker OV6, Hoechst dye efflux, detoxifying enzymes aldehyde dehydrogenases (ALDH) are also frequently used to identify liver CSCs [67]. Indeed, our group has identified a novel HCC subtype defined by the liver CSC markers EpCAM and alpha-fetoprotein (AFP), which is associated with poor prognosis [89]. As cells expressing these markers may be functionally linked to CSC properties, studies on targeting CSC markers may help understanding therapeutic resistance of PLC.

The mitochondria of the liver, compared to other tissue types, have unique features since the liver plays a central role in a variety of critical biological metabolism functions including the homeostasis of carbohydrate, lipid, amino acids and protein synthesis [50]. In addition, the liver is one of the abundant tissues in terms of density and count of mitochondria [3]. The density of mitochondria is distinct depending on the demands of mitochondrial oxidative phosphorylation (OXPHOS) in different organs. Accumulation of damaged mitochondria is a crucial factor in chronic liver diseases [3]. Consequently, mitochondrial dysfunctions are frequently described in PLC [64], which have been reported to be associated with decreased ROS production, impaired apoptosis, increased anabolism rate, and proliferative potential, reduced autophagic degradation [78]. Interestingly, mitochondria have been demonstrated specifically affecting stem cell faith and differentiation potential, suggesting that modulation of mitochondrial activities contribute to the stem cell phenotype. However, there is no simple concept for the role of mitochondria in liver CSCs. Given the central role of mitochondria of the liver and stem cells in cell function and death decisions, we will focus on mitochondrial metabolism in liver CSC biology. This review will summarize functions of mitochondria, including mitochondrial metabolism, mitochondrial biogenesis, mitochondrial dynamics and mitophagy, cell death, oxidative stress, and mitochondrial bioenergetics, in the context of functional regulations of liver CSCs (Figure 1).

Mitochondrial metabolism in liver CSCs

One of the most striking characteristics of CSCs is their ability to form a specialized niche to adapt to changing microenvironmental conditions for their own benefit. This specialized niche is termed the CSC microenvironment [65]. This specific microenvironment maintains the principal properties of CSCs, protects them from multiple drug transporters and immune surveillance, and acquires resistance to DNA damage and mitochondria-mediated cell death mechanisms to facilitate tumor progression. The CSC microenvironment is dependent on activation of certain signaling pathways including altered tumor’s metabolic activities,

which is conducive to CSC resistance to anticancer treatments. Due to distinct microenvironmental conditions they survive in, CSCs are considered highly heterogeneous and exhibit a distinct metabolic phenotype in different tumor types in terms of stemness features [86]. Indeed, a number of studies suggest that CSCs preferentially rely on glycolytic pathways, which presents low or absent rates of OXPHOS and high lactate production [10, 86]. In fact, there are several metabolic benefits to use “Warburg effect” for CSC, including increased ATP production rates while glycolytic signaling produces intermediates for biosynthesis, reduced ROS production in response to stressful environmental conditions characterized by low oxygen (hypoxia) [10, 86]. Interestingly, some studies indicate that mitochondrial oxidative metabolism may be a prevalent source of energy for CSC, suggesting a possible function for metabolic plasticity in CSCs [18].

Immune escape plays an important role in the initiation and progression of a malignant tumor. CSCs have the ability to evade immune surveillance as well as promote immunosuppression to maintain stem-like features and resistance to therapy through a variety of niche-specific mechanisms [66]. Thus, disruption of the interactions between tumor cells and infiltrating immune cells that drives a CSC microenvironment will be crucial for effective treatment. In addition, a number of studies suggest that the “Warburg effect” also is critical for instigating immunosuppressive response [1, 24]. On the one hand, glycolysis of cancer cells has been implicated in the inhibition of the function of anticancer immune cells [24]. Glucose utilization is required for the functional activation of T cells; however, rapidly dividing tumor cells may compete with T-cells for limited resources thereby disrupting their activity. Specifically, cancer cells may increase lactate production via activated glycolytic metabolism to maintain an acidic, low-pH tumor microenvironment, which is a product of tumor glycolysis to suppress antitumor immune cells, such as T effector cells and NK cells [24]. On the other hand, mitochondria, as the master regulators of many stress-induced signals, may trigger signaling mechanisms that are critical for the activation of antitumor immune responses [22]. Collectively, existing data convincingly demonstrate that metabolic reprogramming from mitochondrial respiration to glycolysis is a key mechanism to block immune surveillance during tumorigenesis.

The liver is an exquisitely dynamic organ, being able to change metabolic shift in response to body is conditions during fasting and feeding. Recent studies demonstrate the importance of metabolic reprograming in liver CSCs (Table 1). The CSC metabolism in PLC can be functionally identified by the expression of liver CSC markers, including CD133, CD44, and Nanog, and in general, liver CSCs have been found to favor glycolysis and suppress OXPHOS to promote stemness and resistance to treatment [12, 32, 72, 74]. Indeed, $CD133^+$ tumors display higher expression of glycolytic enzymes and glycolytic capacity, coupled with a decrease in oxygen consumption rate (OCR) in order to promote glycolysis in liver CSCs [72]. Further, knockdown of glycolytic enzymes, LDHA and PDK4, in $CD133^+$ PLC displays increased protein levels of several stemness markers (NANOG, OCT4, and SOX4), suggesting that a metabolic shift is important factor in sustaining stemness [72]. Similarly, global metabolic analysis demonstrates that $CD133^+$ HCC exhibits an increased proliferation through enhancing glycolytic metabolism compared with $CD133^-$ HCC [32]. Furthermore, Thanee et al. found that $CD44^+$ iCCA is advantageous for maintaining low ROS levels through promoting glutathione synthesis, resulting in increasing chemotherapy

resistance [74]. In addition, knockdown of NANOG in HCC impaires glycolytic activity as demonstrated by the glycolytic flux (extracellular acidification rate or ECAR) [12]. Importantly, NANOG directly represses OXPHOS and maintains low intracellular ROS levels of CSCs required for the maintenance of CSCs properties [12]. Restoration of OXPHOS activity renders liver CSCs more susceptible to chemotherapy drugs [12]. Overall, these studies show that liver CSCs have specific metabolism pathways to maintain stemness properties of CSCs and resistance to chemotherapeutic drugs.

Mitochondrial biogenesis in liver CSCs

Mitochondrial dysfunction is often detected as an early alteration of liver diseases such as insulin resistance, non-alcoholic fatty liver disease (NAFLD), alcoholic liver disease (ALD) and HCC, suggesting a causative effect [3, 20]. Cells have developed different mechanisms, including mitochondrial biogenesis, mitophagy, fusion and fission, to maintain mitochondrial functions or to block the effects of mitochondrial damage in response to metabolic demands and stressors. Mitochondrial biogenesis plays an important role in keeping mitochondrial mass to balance energy homeostasis during energy deprivation or to adapt mitochondrial insults. Interestingly, stem cells with increased mitochondrial biogenesis are associated with various stem cell differentiation, suggesting that loss of mitochondrial function is required to maintain the stemness properties [13, 88]. In addition, the hepatogenic differentiation of stem cells is accompanied by an increase in the mitochondrial biogenesis [79, 93].

There is a difference in the metabolic stage from the development and progression of PLC (Figure 2. In a healthy liver, the metabolic energy consumption relies primarily on mitochondrial OXPHOS, which is efficient to generate ATP than glycolysis pathway [94]. Once the liver starts to accumulate lipids, it enhances the liver susceptibility to subsequent damage induced by inflammation, oxidative stress, mitochondrial dysfunction, endoplasmic reticulum stress and fibrinogenesis, which may lead to nonalcoholic fatty liver disease (NAFLD) [7, 56]. Liver tissues from patients with early-stage NAFLD have increased mitochondrial respiratory rates and mitochondrial biogenesis to mitigate disease progression. However, mitochondrial adaptations are lost in the early stages of nonalcoholic steatohepatitis (NASH), which has a low mitochondrial respiratory function despite an increased mitochondrial mass [39]. In PLC, there is a metabolic shift from mitochondrial OXPHOS to glycolysis accompanied by a decrease in mitochondrial mass, which makes PLC more reliant on the glycolysis pathway than on mitochondrial metabolism [53, 77]. These findings indicate that liver adapts to stress conditions by acquiring a mitochondrial flexibility at early stages of NAFLD, which is subsequently lost as a liver tumor progresses. Therefore, increasing mitochondrial biogenesis while preserving intact antioxidant defenses would benefit future treatment for PLC.

Mitochondrial biogenesis is a highly regulated multi-step process involving the coordinated transcription and translation of both mitochondria and transcripts of nuclear origin [60]. Mitochondrial biogenesis is known to be regulated by peroxisome proliferator-activated receptor-γ coactivators (PGC1s), adenosine monophosphate (AMP)-activated protein kinase (AMPK), eNOS, NRFs, SIRT1, SIRT3, and mitochondrial transcription factor A (TFAM).

The PGC1a, including PGC1a and PGC1p, is a family of PGC-1 transcriptional coactivators that are master regulators of mitochondrial biogenesis as well as involve different liver cellular energy metabolic processes including OXPHOS and fatty acid β-oxidation and gluconeogenesis [64]. PGC1s co-activate a variety of transcription factors and nuclear receptors, such as the peroxisome proliferator-activated receptors (PPARs), estrogen-related receptors (ERR), NRF1 and NRF2, to activate expression of genes implicated in mitochondrial metabolism [56, 64]. PGC1a and PGC1p have distinct roles in the regulation of liver metabolism depending on the different environmental conditions. During energy starvation, PGC-1α could upregulate gluconeogenesis, which contributes to de novo glucose synthesis in the liver, to adapt to the fasting environment. Conversely, PGC1p could upregulate the hepatic de novo lipogenesis by co-activation of LXRα and the sterol response element binding protein (SREBP1) due to the reduction of saturated fatty acid consumption [48, 60]. During PLC, cancer cells may upregulate glycolysis by negative regulation of gluconeogenesis, thereby favoring cell survival in the hypoxic and nutrient-deprived tumor microenvironment that characterizes early stages of tumorigenesis [26, 85]. Indeed, the expression levels of PGC1a and its target genes involved in mitochondrial metabolism and gluconeogenesis are significantly decreased in late stages of PLC and in a mouse model of HCC [37, 80]. Interestingly, liver CSCs tend to have lower levels of mitochondrial metabolism compared with non-Liver CSCs through increased expression of acetylated PGC-1α [32], which leads to its inactivation and decreases mitochondrial biogenesis [2]. However, other studies demonstrate that cancer cells shift to more efficient energy production to support their migrating phenotype by PGC1a-mediated promotion of mitochondrial biogenesis and respiration, during the metastatic cascade in PLC [42, 47]. Thus, given that the role of PGC1a in regulating mitochondrial biogenesis and gluconeogenesis, it can be hypothesized that altered mitochondrial biogenesis could be related to the liver CSC microenvironment.

Mitochondrial mitophagy in liver CSCs

Mitophagy is a selective autophagic process to eliminatie dysfunctional mitochondria. This process is important for the maintenance of overall mitochondrial integrity to defective mitochondria following damage or stress [6]. Moreover, mitophagy/autophagy regulates cellular homeostasis and prevents cell death by keeping mitochondrial bioenergetics and decreasing oxidative stress and its alteration has been linked to various liver diseases including PLC [11, 45, 84]. Increasing evidence supports that dysregulation of mitophagy could be an etiological key factor in tumorigenesis of PLC [11]. In addition, an imbalance in the dysregulation of mitophagy could induce mitochondrial degradation process, which in turn results in alteration of cellular homeostasis and promotes tumor initiation and progression. Mitophagy/autophagy acts as a double-edged sword in the tumorigenesis, depending on the different environmental conditions [76, 91]. Whereas tumorigenesis of PLC relies on suppression of mitophagy, tumor progression possiblly relies on the bearing of functional mitophagy. Parkin RBR E3 Ubiquitin Protein Ligas (PARK2) is an E3 ubiquitin ligase that is responsible for ubiquitination of damaged mitochondria [87]. Homozygous mutations in PARK2 are commonly found in various tumors, which lead to repression of mitophagy, as well as an accumulation of dysfunctional mitochondria [11]. In addition, knockout of the PARK2 gene in mice develops spontaneous liver tumors [21]. Moreover,

hepatocyte-specific knockout of Becn1, a gene essential for autophagy, can develop spontaneous tumors, while knockout of other important autophagic genes such as Atg5 or Atg7 can develop only benign hepatic tumors [75]. Collectively, these results suggest that an ability to increase mitochondrial mitophagy may be an effective strategy for treatment to prevent PLC.

Interestingly, tumors cells isolated from atg5-knockout mice have a significantly reduced population of CD133 and ITGA6/CD49f double-positive cells [44]. Recent studies found that mitophagy/autophagy plays an important role in the maintenance of stemness properties in liver CSCs. Liu et al. found that when mitophagy is inhibited, serine-392 of p53 is phosphorylated to modulate p53 mitochondrial translocation, which may lead to its activation and translocation into the nucleus, thereby binding to the NANOG promoter to inhibit the expression of NANOG, which results in the decrease of stemness and self-renewal ability of liver CSCs [49]. However, when mitophagy/autophagy is enhanced, p53 is recruited to mitochondria and subsequently is removed through mitophagy/autophagy mechanism [49]. Thus, mitophagy plays a critical role in the quality control of mitochondria, it may be possible to target tumor suppressor to mediate the stemness of liver CSCs [44]. Together, it may be possible to target mitophagy to deplete liver CSCs, which is dependent on wild-type p53 status.

Mitochondrial induced cell death in liver CSC homeostasis

CSCs is resistance to treatment and is associated with other mitochondria-related function, such as impaired cell death [17, 25]. In fact, several studies suggest that CSC-resistant features can be impaired by targeting components of the anti-cell death machinery [68]. Therefore, unique metabolism in liver CSCs can be associated with abnormalities in mitochondrial function, which affect cell death programs.

Apoptotic death is an energy-dependent cell death program whose regulatory pathways are important in CSCs. Apoptotic processes are regulated via two signaling pathways: extrinsic pathways (death receptor pathway) and the intrinsic pathway (mitochondrial pathway) [29]. Moreover, apoptosis is also regulated by the inhibitors of apoptosis proteins (IAPs), such as survivin, which can inhibit the initial activation of caspases-8 and caspases-10. Extracellular stimuli, including cytokines, growth factors, nitric oxide or toxins, may trigger the extrinsic apoptotic pathway through the binding of extracellular death receptors to cell surface receptors, such as Fas ligand (FasL), nerve growth factor receptor (NGFR), TNF-α and TNF-related apoptosis-inducing ligand (TRAIL) receptors. The intrinsic pathway of apoptosis, also known as the mitochondria-mediated death pathway, refers to cell death by a variety of mitochondrial stress signals, which lead to the activation of BH3-only proapoptotic B-cell leukemia/lymphoma 2 (Bcl-2) family protein. The BCL-2 family proteins could inhibit (anti-apoptotic members) or induce (pro-apoptotic members) mitochondrial outer membrane permeabilization (MOMP) that releases the apoptosis-triggering factors, such as cytochrome c and Smac, from the mitochondrial intermembrane space into the cytoplasm, resulting in activated caspase-induced apoptosis. Mitochondrial dysfunction could trigger intrinsic apoptotic pathways, which is associated with overexpression of BCL-2 family protein in cancer [5]. Both intrinsic and extrinsic apoptosis

pathways may be linked to therapy evasion of liver CSCs. For instance, the Bcl-2 family proteins consist of anti-apoptotic proteins (Bcl-2, Bcl-XL, and Mcl-1) and pro-apoptotic proteins (Bax, Bak, Bid et. al), which regulate the intrinsic pathway of apoptosis in liver CSCs. Recent studies show that antiapoptotic genes (BCL-2, and BCL-xl), as well as IAP family of proteins (survivin) are up-regulated in liver CSCs [36], which is correlated with enhanced chemotherapy resistance to sorafenib [23]. Furthermore, TRAIL is a promising anticancer agent, which preferentially kills tumor cells without significant cytotoxicity toward normal cells [58]. It can bind to the death receptors TRAIL-RI (DR4) and/or TRAIL-RII (DR5) and activate caspase-8 to promote extrinsic apoptotic pathway. In addition, liver CSCs show up-regulation of DR4 and DR5 [46], which may lead to cancer cells and CSCs to have a differential sensitivity to TRAIL apoptosis induction. Moreover, liver CSCs treated with a recombinant human soluble TRAIL show increased cell death significantly [46]. These data suggest that one of the reasons for the failure of existing therapies is that CSCs may have the ability to evade cell death through anti-apoptotic mechanisms.

Interestingly, increased cell death occurs in a majority of human liver diseases, which could serve as a sensitive parameter for the detection of chronic and acute liver diseases due to toxic, viral, metabolic, or autoimmune origin-related insults [51]. Clinical data and animal models suggest that mitochondria is a crucial organelle in the trigger of liver disease progression characterized by increasing hepatocyte death, which is correlated with the subsequent development of inflammation, fibrosis, cirrhosis and PLC [20, 55]. Distinct modes of cell death including apoptosis (programmed cell death), necrosis (unprogrammed cell death, in response to injury) and necroptosis (programmed form of necrotic cell death) trigger specific cell death responses and the development of liver disease, such as I/ R injury, NASH, PLC [51]. Hepatocyte necrosis is a largely unregulated consequence of environmental stress, characterized by mitochondrial dysfunction and consequent rapid ATP depletion. This consequence results in rapid swelling of cells and ultimately cellular rupture, which then elicits significant inflammatory responses. Sakurai et al. found hepatocyte necrosis acts as a crucial mediator of carcinogen-induced HCC development [69]. The tumor-promoting effects of apoptosis of hepatocyte have been demonstrated by the antiapoptotic proteins Mcl-1 or Bcl-xl liver knockout model, which shows an increased rate of apoptosis of hepatocyte and an increased spontaneous development of HCC [83]. Interestingly, tumor development could be inhibited by hepatocyte-specific deletion of pro-apoptotic proteins Bak, which provides a direct connection between apoptosis and tumor development of PLC [30]. In contrast to the role of tumor-promoting effects of cell death in normal liver tissue, cell death in liver cancer represents the role of tumor suppressing effects. Accordingly, liver tumor, especially CSCs, may have undergone a selection process, such as mitochondrial dysfunction, which enables cells to evade apoptosis. Therefore, one needs to carefully distinguish the role of cell death between normal liver tissue and liver tumor for treatment of liver diseases.

Mitochondrial control of redox balance in liver CSC homeostasis

Mitochondria are the major contributors to the production of reactive oxygen species (ROS) [31]. ROS are chemically reactive molecules that have been implicated as a major contributor to stress and diseases, including cancer [33]. It is evident that intracellular ROS

in redox homeostasis also play prominent roles in normal stem cells and CSCs including maintenance of stem cell self-renewal, differentiation and survival. To maintain the steady state of cellular conditions, ROS can be scavenged by antioxidant enzymes, such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (Gpx) or peroxiredoxins (Prx). The superoxide dismutase converts superoxide anion radicals into hydrogen peroxide, then be further detoxified into the water by catalase [81]. Indeed, both stem cells and CSCs possess critical mechanisms by which to cope with the mitochondrial ROS accumulation through elevation of antioxidant defenses. Thus these defense mechanisms act as an important redox regulator on self-renewal and stemness. For instance, hematopoietic stem cells maintain a low intracellular level of ROS to acquire a quiescent state, whereas a high level of ROS confers potent capacity for cell proliferation and differentiation that abolishes self-renewal of stem cell [35]. Proliferative neural stem cells (NSCs) display a high ROS status, which could act as the second messengers [41]. In addition, ROS can cause mitochondrial DNA damage, which is associated with the development of PLC [62]. The accumulation of mitochondrial DNA mutations in PLC suggests its contribution to tumorigenesis [62]. Further, ROS production and ChREBP activation trigger advanced glycation end products (AGEs)–mediated cell proliferation in liver cancers [14]. Despite this, it was suggested that maintaining a low level of intracellular ROS within CSCs may be a crucial property of the self-renewal process. Haraguchi et al. have reported that $CD13^+$ liver CSCs are relatively resistant to chemo/radiation therapy, perhaps due to the low intracellular ROS, including lower levels of mitochondrial ROS (MitoSOX) [27, 38]. The enriched $CD13^+$ liver CSCs might actually be a “G0-like” subpopulation by keeping a lower level of ROS to survive from chemotherapy [27]. Pharmacologic depletion of CD13 expression in liver CSCs by ubenimex treatment inhibits stemness properties and reduces tumorigenicity [27]. Interestingly, CD13 inhibition exhibits high ROS level and proliferation status [27]. On the other hand, $CD13^+$/N-cadherin$^+$ cells display high ROS level in liver cancer, suggesting that the EMT process may be associated with an increase of ROS level [38]. Similarly, $CD133^+$ liver CSCs are also more radioresistance that is associated with low ROS levels [63]. In addition, $EpCAM^+$ liver CSCs have been shown to contain lower ROS levels to maintain long-term self-renewal and survival. However, Disulfiram (DSF), a CSC marker aldehyde dehydrogenase inhibitor, suppresses self-renewal capability of liver CSCs mainly through increases in mitochondrial ROS production rather than reducing the scavenging of ROS [16]. Further, NANOG decreases OXPHOS function to prevent mitochondrial ROS production, which maintains stemness characteristics of liver CSCs [12]. Liver CSC treated with the mitochondrial ROS inducer, Paraquat, represses the self-renewal capacity, indicating that induction of ROS inhibits self-renewal ability of liver CSCs [12, 54]. Additionally, we recently report that, in solid tumor, cancer cells harboring low ROS display enhanced stemness whereas cancer cells containing higher ROS are more proliferative [9]. Given these findings, CSCs may be partially differentiated due to the imbalance of redox homeostasis in the light of tumor microenvironment changes, which leads to heterogeneous tumor cell population including CSCs and non-CSCs.

Conclusions and future perspectives

Most of the anticancer drugs such as cisplatin and 5-fluorouracil preferentially kill proliferating non-CSC tumor cells that, initially, causes the shrinkage of tumor size. However, due to mostly unharmed CSC populations, prolonged treatment with these drugs results in enriched CSCs, consequently contributing to therapy resistance. In this view, it is crucial to understand mitochondrial metabolism in the context of chemoresistance contributed by liver CSCs, with the purpose of improving the development of novel therapeutic strategies for targeting liver CSCs.

Accumulating evidence supports that liver CSCs preferentially relying on the glycolytic pathway and presents low or absent rates of OXPHOS. Due to the presence of unique metabolic activities of CSCs, drugs that suppress glycolysis have been studied as potential anticancer agents. Consistently, 2-deoxy-D-glucose (2-DG), a glucose analog by competitively inhibits glucose-uptake, has been found to induce apoptosis of liver CSCs in combination with Sorafenib [73]. ADI-PEG20 have been found to inhibit the Warburg Effect, which upregulates OXPHOS and targeting glutamine and glycolysis metabolism [40]. A randomized phase II study shows a beneficial effect of ADI-PEG20 in stabilizing the progression of pretreated advanced HCC in an Asian population [92]. Furthermore, the use of ADI-PEG20, in combination with other molecularly targeted or cytotoxic agents, should be investigated, which may improve the success of the therapeutic effect of PLC [28]. On the other hand, Metformin, which interferes with OXPHOS by repressing NADH-coenzyme Q oxidoreductase (complex I), has been shown to enhance tumor aggressiveness and resistance to Sorafenib treatment in diabetic patients with advanced HCC [8]. In addition, studies suggest that Sorafenib enhances glycolysis of liver CSCs [73]. Thus, co-treatment with glycolytic inhibitors or upregulation of OXPHOS to target CSCs in combination with chemotherapy might be more effective in the future treatment of PLC. Interestingly, Griffin et al. conducted a study with a large cohort of patients with diabetes [59]. They found a strong association between the use of metformin and a reduction of liver cancer. This study may support the hypothesis that enhancing mitochondrial function may be an effective strategy for treatment to prevent PLC.

Moreover, since mitochondrial function and liver CSC features seem to be closely linked, drugs that regulate mitochondrial function may be worth exploring as novel therapies. XIAP is the most effective inhibitor of caspases and has been identified as a major repressor of mitochondrial-mediated apoptosis [15]. AEG35156 is an antisense oligonucleotide to promote apoptosis by inhibiting the apoptosis protein XIAP. A randomized phase II study show that AEG35156 in combination with Sorafenib has a better effect in progression-free survival (PFS) of advanced HCC compared to sorafenib alone [43]. Furthermore, Vitamin C increased intracellular ROS in liver CSCs, leading to cell cycle arrest and apoptosis. In addition, intravenous Vitamin C use is associated with improved disease-free survival in HCC patients [52]. Together, the vision of PLC as a metabolic disease strengthens the clinical significance of mitochondria function, particularly the relevance of liver CSCs for cancer initiation, progression, recurrence, and therapy. Although the Warburg effect is linked to enhanced glycolysis, mitochondrial dysfunction, as the concept of cancer metabolism for the benefit survival of PLC, argues the importance of mitochondrial functions in promoting

tumor progression. Furthermore, revealation of mitochondrial metabolism in chemoresistance of liver CSCs may have important therapeutic implications in the future.

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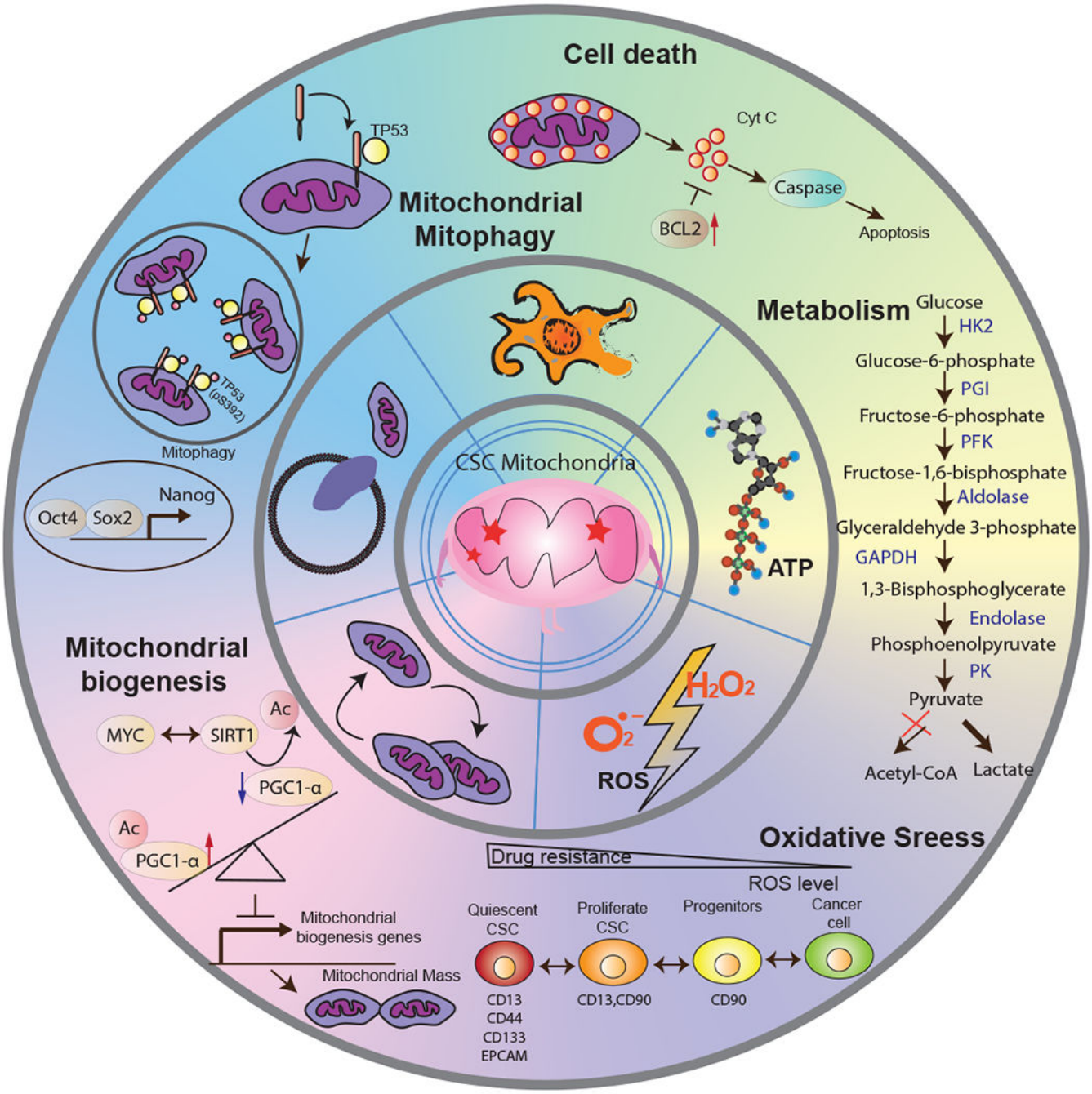

Figure 1. An integrative model of the roles of mitochondrial metabolism in liver cancer stem cells.

This illustration encompasses five key features of mitochondrial dysfunction needed for the maintenance of liver CSCs. In particular, Liver CSCs may preferentially (1) rely on glycolytic pathways to increase ATP production for biosynthesis; (2) reduce ROS levels to acquire a quiescent state in response to drug resistance; (3) decrease mitochondrial biogenesis through increased expression of acetylated PGC-1α to reduce mitochondrial respiration; (4) enhance mitophagy to block p53 mitochondrial translocation, which may bind to the NANOG promoter to inhibit the expression of NANOG, resulting in reduced

stemness and self-renewal ability of liver CSCs; (5) acquire the ability for evading mitochondria-mediated death pathway by overexpressing BCL-2 family proteins thereby resistance to anticancer treatments. Inner circle: a dysfunctional CSC mitochondrium. Middle circle: five features of mitochondrial dysfunction. Outer circle: molecular signaling pathways of mitochondria, including mitochondrial metabolism, oxidative stress, mitochondrial biogenesis, mitochondrial mitophagy, and cell death, in the context of functional regulations of liver CSCs. Abbreviation: CSC: cancer stem cell; Cyt C: Cytochrome C; ROS: Reactive Oxygen Species; HK2: Hexokinase 2; PGI: Phosphoglucoisomerase; PFK: Phosphofructokinase; PK: Pyruvate kinase.

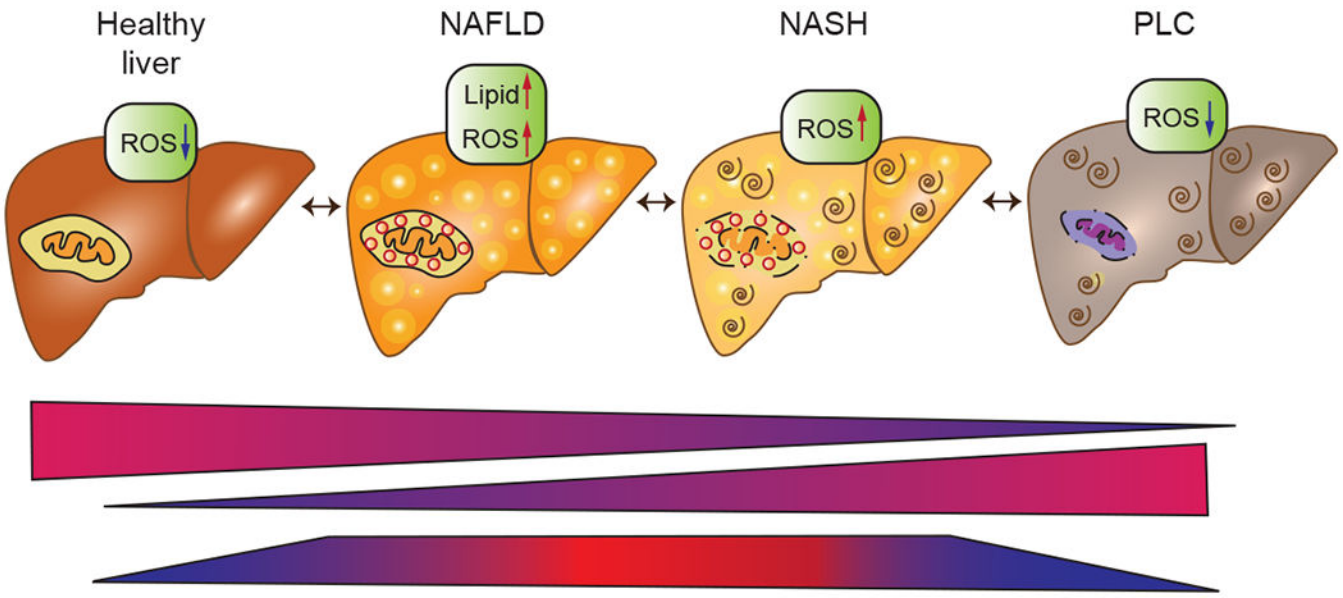

Figure 2. Principal metabolic alterations during hepatocarcinogenesis

The metabolic energy consumption relies primarily on mitochondrial OXPHOS in a healthy liver. Once the liver starts to subsequent damage induced by oxidative stress, and lipids accumulation, which may lead to NAFLD. In the early stages of NASH, mitochondrial adaptations are lost, which includes a low mitochondrial respiratory function. Finally, a metabolic shift from mitochondrial OXPHOS to glycolysis accompanied by a decrease in mitochondrial mass occurs during malignant transformation to PLC. Abbreviation: NAFLD, nonalcoholic fatty liver disease; NASH, nonalcoholic steatohepatitis; PLC, Primary liver cancer.

Table 1:

Known liver CSC markers and their role in cellular metabolism

Marker	Metabolic phenotype	Function in therapy	Reference
CD13	Maintain lower levels of mitochondrial ROS	Combining a CD13 inhibitor with a chemo/radiation therapy inhibit tumor progression	27,38
CD133	Decrease mitochondrial OXPHOS and promotes glycolysis	Targeting glycolytic enzymes represses stemness properties in CD133+ PLC cells	32,72
CD90	Higher levels of the mitochondrial ROS	Actively proliferate and are sensitive to 5-FU therapy	27
CD44	Maintaining low ROS levels through promoting glutathione synthesis	Combining a CD44 inhibitor with a Sulfasalazine inhibit tumor progression	74
EPCAM	Maintaining low ROS levels	Not reported in this paper	16
CD24	Not reported	Not reported	
DLK1	Not reported	Not reported	
OV6	Not reported	Not reported	