Targeting autophagy in liver cancer

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Abstract: Autophagy is a catabolic cellular process conserved in animals. It is characterized by the main role of recycling all the non-functional products of the cells. Once, autophagy players detect non-functioning sub-cellular organelles and proteins, they start the so-called nucleation process. The organelles will be surrounded by a double membrane vesicle mainly constituted by endoplasmic reticulum (ER) membrane and autophagy proteins, e.g., MAP1LC3B, Beclin-1, VPS34, Unc-51 like autophagy activating kinase (ULK1) and ubiquitination-related proteins. Then the autophagic membrane will go through an elongation phase involving additional autophagy players. Once the autophagic vesicle is complete, the sub-cellular organelles will be isolated from the rest of the cytosol and driven to the final fusion with lysosomes. Here, the digestion process will end. Alteration and or impairment of autophagy have been shown to be correlated with development of diseases affecting the central nervous system, e.g., Alzheimer and other neurodegenerative diseases. Nonetheless, autophagy defect is responsible for tumorigenesis in blood and solid malignancies, in particular liver cancer. Malignancies of the liver are determined by several genetics and epigenetics mechanisms triggering the up-regulation of survival mechanisms and resistance to cell death. Furthermore, liver cancer could result from pathologic conditions like cirrhosis and fibrosis related to virus infection, aflatoxin, alcohol consumption and high fat diet together with insulin resistance. The role exerted by autophagy in the pathogenesis of the liver and tumor development has been evidenced in recent years. The alteration of autophagy assumes a fundamental role for liver tumorigenesis determining an accumulation of non-functional proteins and organelles that trigger oxidative stress leading to genotoxic stress and gene alterations. Furthermore, the absence of this degradation mechanism could prompt the cells to alter their metabolic status and turn into malignant cells. Interestingly, the heterozygous loss of function of Beclin-1 is able to trigger liver tumorigenesis or even the simple accumulation of proteins caused by the block of the final autolysosome fusion and degradation process is responsible for liver cancer development. This review highlights the importance of targeting the autophagy process in liver cancer in order to restore its function and to promote autophagy-mediated cell demise.

Keywords: Autophagy; liver cancer; deacetylase inhibitors; epigenetics; metabolism; cell death

Introduction

Since the discovery of autophagy mechanism in 1956 (1), several studies have further identified autophagy as an intriguing mechanism responsible for catabolizing all the subcellular organelles and particles that could be recycled by the cell for its survival. Thus, autophagy vacuoles need the fusion with the lysosome to execute the degradation process (2).

Alteration and/or impairment of autophagy process have been shown to be implicated in the development of neurodegenerative diseases of central nervous system (3,4) and cancer (5,6).

Additionally, autophagy has been implicated in liver steatosis, especially nonalcoholic steatohepatitis (NASH), and exerts a key role during hepatitis B virus (HBV) and hepatitis C virus (HCV) hepatocytes infection. Furthermore, the alteration of autophagy process is responsible for the development of solid malignancies, e.g., breast cancer and liver cancer.

Interestingly, several key players of autophagy have been implicated in liver tumorigenesis. BECN1 (ATG6, Beclin-1) represents, for example, a haploinsufficient tumor suppressor; its heterozygous deletion causes the development of liver tumors in mice (6). ATG5, ATG7 and p62 (SQSTM1; sequestosome 1) have shown to exert tumorigenic role and their suppression reduces liver tumorigenesis (7,8).

In the last years, several studies have shown that some compounds are able to target autophagy and modulate the function of this metabolic process thus leading to block of cell proliferation and cell death in solid malignancies, e.g., liver cancer. For example, valproic acid and panobinostat, pan-deacetylase inhibitors (9,10), and the inhibitors of Cdc2-like kinase 1 (CLK1) (11) have shown the ability to induce autophagy. Still controversial is the role of sorafenib, a well-known tyrosine kinase inhibitor approved for the treatment of liver malignancies and other solid tumors, as modulator of autophagy in hepatocellular carcinoma (HCC) (12,13). Furthermore, unpublished data obtained from our group have shown the involvement of CUX1 (CUTL1, homeobox protein cut-like 1) in the autophagy process in liver cancer.

Recently, the discovery of PD-1/PD-L1 as druggable target in solid tumor with aggressive and metastatic potential has shown of being able to modulate autophagy (14). The potential of targeting autophagy via PD-1 in liver cancer could be a future promising therapy for this severe malignancy that need to be further investigated.

Autophagy discovery

The investigation of autophagy was determined by the initial discovery of the lysosome in 1950s (1).

At that time, Novikoff *et al.* firstly observed in 1956, by electron microscopy, the presence of lysosome-rich fractions in rat liver, which they called "dense bodies". Also, they observed that these phosphatase acid-positive double membrane structures contain mitochondria and parts of the ER. But, they couldn't verify the correlation between dense bodies and lysosomes (15).

In 1960 Essner and Novikoff proved that the dense bodies of human hepatic cells, so called hepatocellular pigments, are lysosome vesicles (16).

Ashford and Porter firstly described in rat liver cells the engulfment and lysosome-mediated degradation of various cytoplasmic components after treatment with glucagon. In addition, they hypothesized that lysosomes were such a kind of portions of cytoplasm including other organelles (17). Later de Duve introduced the term autophagosome to describe a double membrane organelle containing cytoplasmic components and the term Autophagy ("self-eating", Greek) to describe the lysosomal digestion of own-cytoplasmic cellular components (18,19).

Arstila and Trump (2) firstly described that the autophagosome originate from the cisternae of ER. It represents an acid hydrolase-free double membrane vacuole. The transfer of hydrolytic enzymes into the autophagosome requires fusion of the autophagosome and lysosome. The new formed autolysosome enables the degradation of cytoplasmic elements by lysosomal enzymes and transforms from a double membrane to a single membrane structure depending on the maturation stage (2). Thereby, they characterized in principle the morphological development of autophagic flux. Despite of the catalytic role exerted by glucagon and starvation during autophagy, Pfeifer and Strauss demonstrated that food intake and insulin inhibit autophagy; thus, highlighting the link between autophagic activity and metabolism (20-22). For a long time, autophagy research was only based on morphological studies. No characteristic genes and proteins of autophagic machinery have been identified until Ohsumi discovered extensive autophagy induction through starvation in yeast cells (*Saccharomyces cerevisiae*) and started to use yeast as a model to study the molecular mechanisms and genes involved in autophagy (23,24). Up to now, 30 autophagy related (atg/ATG) genes of yeast (atg) and their mammalian homologs (ATG) were discovered (25). Interestingly, in 1999 a

connection between autophagic activity and tumorigenesis was observed (26).

Autophagy in mammalians and humans

Cellular stocks of organelles, proteins and other sub-cellular components are continuously synthesized and degraded. One way to enable energy dependent processes such as protein synthesis is the intake of nutrients from the external environment through endocytosis (27). During nutrient starvation, cells rely on autophagy to provide energy and sustain cell homeostasis through degradation and recycling of cytosolic compartments (28).

Autophagy is differentiated into three types (I) chaperone-mediated autophagy, (II) micro-autophagy and (III) macro-autophagy, which all lead to lysosomal degradation of cytosolic components. Chaperone-mediated autophagy enables the selective recognition of cytosolic proteins by a chaperone and their delivery to the lysosomal membrane, where the proteins are unfolded and translocated into the lysosome (29). Micro-autophagy describes the direct engulfment of cytosolic proteins and organelles by the lysosome (29).

In this review we focus on macro-autophagy (in the following referred to as "autophagy") whereby the formation of a double membrane vesicle, the autophagosome, leads to the engulfment of intracellular components such as proteins and organelles. Subsequently, the autophagosome cargo is degraded and recycled after fusion of the autophagosome with the lysosome (28).

The autophagy process is divided into the several steps: Induction of autophagy, initiation of isolation membrane, vesicle nucleation, elongation and finally fusion.

Regulation of autophagy/induction of autophagy

Autophagy is a cellular response to various stresses, especially starvation. The serine/threonine protein kinase mammalian target of rapamycin (mTOR) is the sensor of nutrition status and a key regulator of cellular metabolism, its inhibition in absence of nutrition, especially lack of amino acids, leads to inhibition of cell growth and induction of autophagy (30,31).

Under nutrient-rich conditions and growth factor signaling, mTOR is activated leading to autophagy inhibition via PI3K-/AKT-mTOR pathway (30,32). mTOR is also part of mTORC1 and mTORC2 complexes (33).

mTORC1 serves as one of the main amino-acid sensors in cell metabolism (30) and is indirectly activated by AKT-mediated inhibition of the mTOR negative regulator tuberous sclerosis complex (TSC) (32). The inhibition of TSC complex enables the small GTPase Rheb (Ras homolog enriched in brain) to activate mTORC1, after the small Rag GTPase recruited mTORC1 to the surface of the lysosome (34).

mTORC1 is thereby able to promote cell growth and inhibit the catabolic activity of autophagy by inhibition of Unc-51 like autophagy activating kinase (ULK1), a serine/threonine protein kinase responsible for autophagy initiation, and by inhibition of the transcription factor transcription factor EB (TFEB) (31,35,36). TFEB transcribes for lysosomal genes and autophagy genes and is involved in induction of autophagosome formation and autophagosome-lysosome fusion (37,38).

Under nutrient-rich conditions Rag recruits, besides mTORC1, cytosolic TFEB to the lysosomal membrane, where mTORC1 can then phosphorylate TFEB and prevent its nuclear translocation (32,34).

mTORC2 is not directly involved in autophagy regulation, but can influence autophagy inhibition through phosphorylation and activation of AKT (39). Therefore, mTORC2 enables the activation of mTORC1 by inhibition of TSC complex through AKT activation (32).

AMP-activated protein kinase (AMPK), a kinase responsible for the activation of autophagy, is the counterpart of mTOR in autophagy regulation. AMPK functions as an energy sensor and is stimulated by elevated AMP level to promote catabolic pathways for generation of energy (40).

AMPK and AKT interact with each other through mutual phosphorylation (41). AKT reduces AMPK phosphorylation at Thr-172, which is required for AMPK activity, by phosphorylation of AMPK at Ser-487/491 or by direct blocking of AMPK phosphorylation (41). Also AMPK is able to indirectly inhibit AKT activity (41).

Prolonged starvation induces AMPK, which then activates TSC2 by phosphorylation, leading to the inhibition of mTORC1 (42) and the activation of ULK1 (43,44).

Another important regulator of autophagic activity is the calcium-mediated activation of Ca^{2+}/Calmodulin-dependent kinase kinase-β (CaMKK-β) (45). Reduction of amino acids content increases free cytosolic Ca^{2+} and promotes formation of Ca^{2+}/Calmodulin complexes by inducing calcium efflux from intracellular stores such as the ER and the lysosomes into the cytosol (46). Ca^{2+}/

calmodulin activated CaMKK-β stimulates the autophagy inducer AMPK, leading to mTORC1 inhibition and ULK1 stimulation (45). In Addition, CaMKK-β can further induce the phosphatase calcineurin, which dephosphorylates TFEB and favors its nuclear translocation (47).

The ULK1 will complex with the autophagy genes transcribed from TFEB and then start the autophagy initiation (38,48).

Autophagosome formation

The ULK1 complex, the VPS34 complex and the ubiquitin-like conjugation systems ATG5-ATG12 and MAP1LC3-PE (microtubule associated protein light chain 3-phosphatydilethanolammine) will be the functional units of autophagosome formation (49).

Initiation and nucleation of the isolation membrane

The ULK1 complex and VPS34 (vacuolar protein sorting 34) complex, an autophagy specific class III PI3K complex, form a membranous cistern called isolation membrane (initially called "phagophore") (50,51).

Under starvation, ULK1 is localized on the isolation membrane and forms, additionally, a complex with ATG13, FIP200 (focal adhesion kinase family interacting protein of 200 kDa) and ATG101 (50,52,53). The ULK1 complex recruits the VPS34 complex to the autophagy machinery and increases VPS34 activity resulting in enhanced phosphatidylinositol-3-phosphate (PI3P) production (48). PI3P accumulates at the ER membrane and promotes nucleation and growth of the autophagosome isolation membrane (54).

The VPS34 complex is formed by the autophagy-specific class III PI3K (VPS34), Beclin-1, ATG14L and VPS15 (51); its formation is disrupted by the inhibitory effect of the anti-apoptotic protein B-cell lymphoma-2 (Bcl-2), which blocks calcium channels efflux out of the ER, and inhibits the ULK1-dependent phosphorylation of Beclin-1 (55-57).

Elongation of the isolation membrane

The process of elongation and closure of the autophagosome isolation membrane depend on the two ubiquitin-like conjugation systems MAP1LC3-PE (LC3-II) and ATG5-ATG12 (58).

The cytosolic form of the ubiquitin like protein LC3 is cleaved at its carboxyl-terminus by the proteases ATG4 to form cytosolic LC3-I with an exposed c-terminal glycine (59,60). Thereby LC3-I can be conjugated at its c-terminal glycine with phosphatidylethanolamine, which is located on the isolation membrane, to form membrane-bound LC3-II (LC3-phosphatidylethanolamine/LC3-PE) (60). This process is mediated by the conjugation system ATG5-ATG12 (61).

The E1 enzyme ATG7 and the E2 enzyme ATG10 enable the conjugation system ATG5-ATG12, which is located in the isolation membrane and facilitates LC3-I to be bound on the membrane of the developing autophagosome (49,58).

The engulfment of cytosolic components principally acts not only as unspecific degradation mechanism but also as a selective up-take of intracellular cargo such as protein aggregates through a link between LC3-II and p62/SQSTM1 (62-64).

The ubiquitinated protein aggregates, and/or parts of the cytoplasm containing damaged organelles are surrounded by the isolation membrane that will form a double membrane-bound autophagosome by fusion of its terminal membrane parts.

Autophagosome-lysosome fusion

The autophagosome moves along microtubules to reach the lysosome (65) and delivers its cargo into the lysosomal lumen by fusion of the outer autophagosome membrane with lysosomal membrane.

The ability of the autophagosome to fuse with the lysosome depends on the small GTPases Rab7 (66) and is mediated by tethering factors and SNAREs (67,68).

At this point, the ATG conjugation systems are not necessary for autophagosome-lysosome fusion, but they are required for the degradation of the inner autophagosome membrane by lysosomal enzymes in the new formed autolysosome (69). The newly formed autolysosome can finally degrade the inner membrane and the cargo of the autophagosome. Thereby autophagy fulfills its physiological function of supplying energy and substrates to sustain cell homeostasis under starving conditions.

Autophagy in pathogenesis

As shown here before, several factors are enrolled in the regulation of autophagy process. The several steps of autophagosome formation and fusion with lysosome could

be target of various impairments that could alter autophagy activity determining adverse effects in the intracellular environment. Additionally, impairment of autophagy could not rescue the cells during starvation.

Thus, it has been shown that dysregulations of autophagy are implicated in the development of many different diseases affecting the central nervous system and causing neurodegenerative disorders like Alzheimer and Parkinson, lysosomal storage diseases and tumorigenesis (3,4,26). Deletion of the essential ATG Beclin-1 promotes tumorigenesis in various cancer like breast cancer (5,6). In contrast to this, Beclin-1 overexpression and the consequently activation of autophagy inhibits tumorigenesis in breast carcinoma cells (26). Also the impairment of other genes involved in the positive regulation of autophagy such as PTEN (phosphatase and tensin homolog), which is an inhibitor of PI3K/Akt/mTOR pathway, can be crucial for cancer development (70).

Some cancer cells are able to build resistance against anti-tumor drugs by activating autophagy, resulting in cancer cell survival (71,72).

Autophagy in liver pathogenesis

Autophagy can act as a tumor suppressor by compensation of cellular distress or it can promote tumor growth by acting as a mechanism of cell survival (73).

Here, we report the involvement of autophagy in the development of pathologies correlated with liver.

It has been evidenced that autophagy is an important regulator of liver homeostasis. In particular, reticulophagy and mitophagy, even being autophagy processes, are acting independently and are highly increased in patients affected by severe steatosis (74). Furthermore, Fukushima *et al.* have shown that accumulation of p62 inclusion bodies in patients affected by non-alcoholic fatty liver disease (NAFLD) is correlated with macrophage infiltration, thus correlating the inflammatory response with ongoing autophagy process (75). Interestingly, Wang *et al.* discovered that the dietary intake of medium chain fatty acids in mice lower the lipotoxicity caused by high fat diet and mitigated type 2 diabetes and non-alcoholic steatohepatitis (NASH) via Rubicon-mediated autophagy (76). Our unpublished data showed an involvement of autophagy during NASH progression in mice and the correlation between Leptin and AMPK-mediated autophagy.

The autophagy process plays also a key role in alcohol-related liver disease. Injury of the hepatic tissue caused by alcohol in SNX10 knockout mice was determined by activation of autophagy. In particular, it was observed that the loss of SNX10 gene in mice determined the over-expression of autophagy markers LAMP-2A, Nrf2 and AMPK in alcohol-mediated liver steatosis (77). Furthermore, several studies noticed that alcohol consumption induce adipose tissue atrophy leading to autophagy impairment and block of tissue homeostasis, which lead to development of diseases correlated with development of pathologies affecting several tissue and organs, including liver (78).

HBV and HCV are responsible for chronic infection of hepatocytes. Tissue microarray analysis of human liver biopsy of patients infected by HBV and HCV revealed an up-regulation of MAP1LC3B expression in infected hepatocytes compared with non-infected liver cells (79). Interestingly, the up-regulation of autophagy process can be mediated by the expression of the HBV X protein, which binds to and activates phosphatidylinositol-3-kinase class 3 (PI3KC3). Thus, the viruses can use this catabolic process to enhance the duplication of viral DNA (80). Furthermore, this mechanism could be clarified by the ability of the HBV X protein to promote the nuclear translocation of high mobility group B1 (HMGB1) that could trigger the transcription of autophagy genes (81,82). Interestingly, an accumulation of p62 has been observed in patients with HCC involving chronic HBV infection and aflatoxin B1 (AFB1) exposure. These patients have shown a poor overall survival that could be correlated with the high expression of p62 (83). Once more, the HBV could use autophagy process to promote the degradation of tumor necrosis factor superfamily member 10 receptor (TNFSR10B/TRAILR1/DR5) in order to suppress the immune surveillance against virus-infected or transformed cells, thus inhibiting immune-mediated apoptosis (84). Interestingly, autophagy could still play a protective role against viral replication. The oxidative cellular stress caused by the HBV infection can induce the subunit of AMPK, PRKAA. Activation of AMPK promotes autolysosome-dependent degradation of HBV viral particles through stimulation of cellular ATP levels, which then leads to the depletion of autophagic vacuoles (85). Furthermore, our study has shown that HBV envelope proteins are responsible of ER stress induction in liver cancer cells in CB1-dependent manner (86). This mechanism could be responsible of promotion of autophagy as shown by Döring *et al.* and Lazar *et al.* (87,88), by the induction of the ER-associated degradation (ERAD) and the over-expression of Atg5-12/16L1 and Atg10/Atg3 complexes. Nonetheless,

miRNAs could exert a key role by regulating autophagy in NASH. Several miRNAs of the maternally imprinted region at the chromosome 14q32.2 have been shown of being modulated in a NAFLD mouse model and their expression could exert an inhibitory effect on the expression of several targets including autophagic markers (89,90).

Autophagy and liver cancer

The development of liver diseases caused by high fat diet together with insulin-related pathologies, alcohol abuse and viral infection are responsible for activating tumorigenesis process in the liver, thus involving autophagy process. The first evidence of autophagy involvement in tumorigenesis and in hepatocarcinogenesis also, was shown by the development of mice expressing a heterozygous mutant form of Beclin-1. Those mice showed an impaired autophagy process and a high incidence of spontaneous tumors including liver cancer (91). The mutant heterozygous Beclin-1 was also responsible for increasing the frequency of spontaneous malignancies and accelerates the development of hepatitis B virus-induced premalignant lesions. Thus, showing that Beclin-1 is a haploinsufficient tumor suppressor (6).

Furthermore, Takamura *et al.* (7) showed that the deletion of ATG5 and ATG7 is responsible for the development of benign liver adenomas in mice characterized by autophagy impairment and accumulation of p62. Simultaneous deletion of p62 reduced the size of the Atg7$^{-/-}$ liver tumors. Other studies evidenced the involvement of p62 during liver tumorigenesis. In particular, oxidative stress determined by defective autophagy in tumor cells is characterized by accumulation of p62, reactive oxygen species, damaged mitochondria and ER chaperones. Sustained p62 expression resulting from autophagy defects was sufficient to alter NF-κB regulation and gene expression and to promote tumorigenesis (8). Wu *et al.* (92) found that increased autophagic activity promotes the ubiquitination and p62-mediated degradation of the oncogenic cyclin D1, which is high expressed in patients affected by HCC.

Aberrant autophagy is responsible for the over-expression of HGF in cirrhotic liver of rats. Thus, sustaining hepatocarcinogenesis via Met/JNK and Met/STAT3 signaling (93).

Liver non-parenchymal cells, including hepatic resident macrophage Kupffer cells, are also influenced by defects in the autophagy process. In autophagy-deficient macrophages, mitochondrial ROS mediated inflammation- and fibrosis-

promoting effects by increasing IL1α/β production via enhancing NF-κB-associated pathways (94).

Recently, a protective role of thyroid hormone (TH) has been discovered during liver tumorigenesis. In detail, this study has shown that administration of TH is responsible for autophagy activation by promoting DAPK2 expression that mediates the phosphorylation of p62. Autophagy-mediated clearance of protein aggregates, in diethylnitrosamine-induced HCC mouse model, attenuates the hepatotoxicity and liver tumorigenesis (95).

In contrast to the protective role of autophagy against hepatocarcinogenesis, other studies have shown the implication of this catabolic process as tumor promoting mechanism. The long non-coding RNAs (lncRNA) have been implicated for autophagy-mediated liver carcinogenesis. In particular, the lncRNA HOTAIR resulted over-expressed in HCC. Its over-expression was responsible for the up-regulation of ATG5 and ATG7 genes, thus promoting tumor cell proliferation (96). Once more, the study of Umemura *et al.* (97) showed that p62 activity is required for the activation of NRF2 and mTORC1, the induction of c-Myc and to protect the HCC cells from the oxidative stress.

To summarize, the role exerted by autophagy to protect from oxidative stress, starvation and insult coming from viral infection, high fat diet and alcohol represent an important mechanism to protect the cells from stress and injury but tumor cells are also able to use this catabolic process to protect from stress and further proliferate (*Figure 1*).

Targeting autophagy in liver cancer

As described here above, the autophagy process requires the expression of several ATG genes and lysosomal genes. The autophagosome vesicle formation needs the interaction with ER and the involvement of several kinases that inhibit the mTOR/AKT pathway and activate the pro-autophagy factors. The final fusion with the lysosome is a complex mechanism that requires the action of lysosomal proteins. Based on this concern, autophagy is characterized by several druggable targets that could modulate autophagy status and promote anti-tumorigenic effects.

Our previous studies have shown that up-regulation of autophagy leads to cell death of HCC cells. Specifically, the treatment with the pan-deacetylase inhibitor panobinostat promoted an increase of the number of autophagy vesicles in HCC cells, the formation of the Beclin-1/MAP1LC3B/Atg12/UVRAG complex, the involvement of p53 and
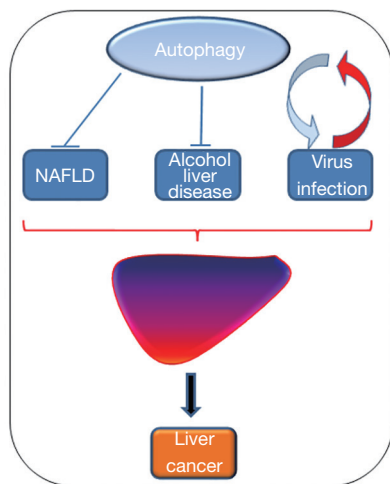
Figure 1 Involvement of autophagy in the pathogenesis of the liver. Autophagy inhibits NAFLD and alcoholic liver disease. It exerts a double role during viral infection. Impairment of autophagy causes liver carcinogenesis. NAFLD, non-alcoholic fatty liver disease.

DRAM1 (DNA damage regulated autophagy modulator 1) and the final maturation of those, culminating into cell demise (10). This process could also be supported by the previous studies showing that panobinostat is able to trigger ER stress in HCC cells, which is also responsible for sustaining the autophagy process (98). Nonetheless, we have also shown that panobinostat caused the down-regulation of oncogenic miRNAs leading to the re-expression of several markers including autophagy proteins, e.g., Beclin-1 (99).

Further unpublished data have shown that autophagy process, specifically the expression of autophagy and ER stress related genes, could be regulated by the expression of CUX1 after treatment with deacetylase inhibitors. These studies evidenced that histone deacetylase inhibitors are capable to induce autophagy in HCC cells, which terminally causes cell demise.

The study of Zhang *et al.* (100) showed that transfection with a novel oncolytic adenovirus targeting Wnt signaling effectively inhibits cancer-stem like cell growth via metastasis, in HCC models. The transfection with the adenovirus sensitized the liver CSC-like to the treatment with classic substances like doxorubicin, which lead to autophagy related cell death. Valproic acid has shown to mediate the cellular internalization of doxorubicin in liver cancer HepG2 cells and to promote autophagy, reactive oxygen species and finally cell death. The activity was

blocked by autophagy inhibitors (9).

The potent and selective Inhibitors of CLK1 have shown the peculiar ability to induce autophagy in mice. The up-regulation of autophagy process could protect the murine hepatocytes from the cytotoxic effect of acetaminophen (11).

Recent studies reported that the down-regulation of SIRT1 (NAD-dependent deacetylase sirtuin-1) signaling, a deacetylase responsible for cellular epigenetic reprogramming, underlies hepatic autophagy impairment in glycogen storage disease type Ia (101). The impairment of glucose-6-phosphatase-α (G6PC) can alter the metabolic programming of liver cells leading to inhibition of autophagy process and liver tumorigenesis (102).

The modulation of autophagy by treatment of HCC with sorafenib is still controversial. Some studies showed that sorafenib is able to promote autophagy-mediated cell death by up-regulation of autophagy markers, e.g., Beclin-1, by induction of ER stress and by suppression of the autophagy inhibitor mTORC1 and Akt (12,103-105). In contrast, it has been shown that PSMD10, together with Atg7, are markers of poor prognosis for patients affected by HCC. Treatment with sorafenib promotes the nuclear translocation of PSMD10 that promotes the transcription of Atg7 and induces autophagy resistance to sorafenib (106). Additionally, the inhibition of autophagy mediated by ADRB2 favors the stabilization of HIF-1a thus promoting sorafenib resistance in HCC cells (13).

Another recent study showed that the analysis of combined autophagic biomarkers like ULK1 and MAP1LC3B and their correlation with patient prognosis would better represent the dynamic stage of autophagy and it might provide a potential therapeutic way to target autophagy in HCC (107).

Recently, the discovery of the cell death signaling PD1 as a potential druggable target in several solid and blood malignancies (108-110) has given new possibilities for the treatment of cancer via the interference with the immune system. The potential of driving the immune response to mediate cell death of malignant cells could offer new perspectives for those kinds of tumors showing an involvement of the immune cells during their development stage. Liver cancer is a solid malignancy with high aggressiveness and metastatic potential which results in a poor prognosis for the patients. Triggering autophagy not only in the liver cancer cells but also in the immune cells involved in the tumor environment, e.g., T cells and macrophages, could improve the treatment of this fatal solid malignancy (*Figure 2*).
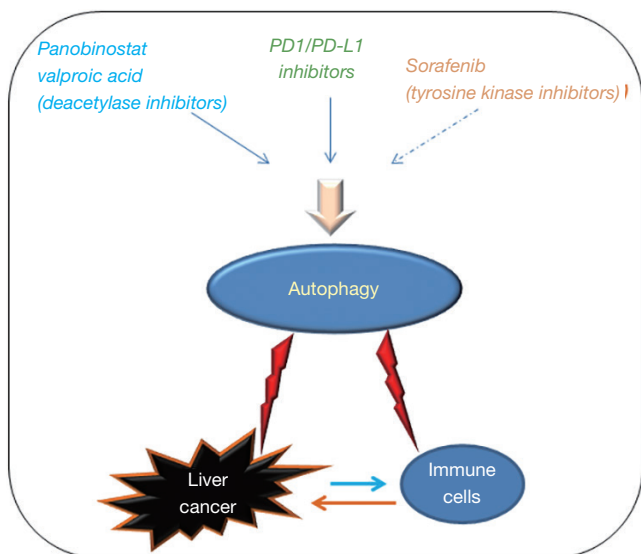
Figure 2 Possible strategy for the treatment of liver cancer. The combination of deacetylase inhibitors and/or tyrosine kinase inhibitors together with PD1/PD-L1 inhibitors could trigger autophagy leading to cell death in liver cancer cells and re-setting of immune cells of the tumor environment, which make them prone to mediate cell death in tumor cells.

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Footnote

Conflicts of Interest: The authors have no conflicts of interest to declare.

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