Alpha-fetoprotein Promotes the Malignant Phenotype of Hepatocellular Carcinoma Cells: the Drugs Resistance Origination

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Abstract: Hepatocellular carcinoma (HCC) has a high increase, recurrence and mortality rate, but the signs of pathogenesis are not clear. At the time of diagnosis, most patients were middle-advanced stage and missed the optimal period of surgical resection. Pharmacotherapy is the primary choice in this period, but the efficacy of anticancer drugs is poor and the patient survival rate is low. Drugs resistance is the primary cause of the poor efficacy of anticancer drugs. HCC cells have innate drugs resistance and acquired drugs resistance, and understanding the drugs resistance mechanism of HCC cells has an important help in the treatment of HCC. Alpha fetoprotein (AFP) is a tumor-associated protein, about 70% high expression of AFP in HCC patients, and AFP has a trait to induce malignant phenotype, stimulate cancer cells with a highly aggressive, antiapoptotic, proliferated phenotype, etc. AFP can also promote HCC drugs resistance through regulating the transduction of signaling pathways and ultimately lead to drugs resistance. This review analyze AFP influences the origination of cancer stem cells, promotes malignant phenotype of cancer cells, inhibits the immune response of immune cells, and the mechanism that promote HCC cells drugs resistance.

Keywords: Hepatocellular carcinoma; Alpha fetoprotein (AFP); Drugs resistance; Cancer stem cells; Malignant phenotype

1. Introduction

Hepatocellular carcinoma (HCC) is divided into primary and secondary liver cancer, and HCC is the most common primary liver cancer. As the sixth most common

cancer in the world, HCC is the third malignant tumor of cancer-related cause of death^[1,2]. HCC has a high mortality rate, easy to relapse and metastasis, which has become a major health problem in world. The pathogenesis of HCC

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involve in genetic, as well as the prolonged consumption of foods containing aflatoxin and nitrosamines, hepatitis virus, cirrhosis, alcoholic liver, *etc.* HCC origins is associated with multiple dysregulated signaling pathways, such as growth factor-mediated angiogenic signals: vascular endothelial growth factor (VEGF), platelet-derived growth factor (PDGF), epidermal growth factor (EGF), insulin-like growth factor (IGF), hepatocellular growth factor (HGF/c-MET), mitogen-activated protein kinase (MAPK), phosphatidylinositol 3 kinase (PI3K)/AKT/rapamycin mammalian target (mTOR), and the Wnt/ β -catenin pathway^[3]. Nearly one million newly diagnosed HCC patients every year, and about three-quarters of the million cases die, with China accounting for over 50 percent of all liver cancers^[4]. The treatment strategies of liver cancer include surgical resection, liver transplantation, radiotherapy and chemotherapy. Surgical resection and liver transplantation have a great effect in early and middle stage patients. However, due to the hidden incidence of liver cancer, most of the patients have progressed in advanced stage when diagnosed^[5], and the best optional time for surgical resection and liver transplantation is lost. Drugs therapeutic is the first choice for the treatment of these patients.

The mainly therapeutic drugs in patients loading liver cancer are sorafenib, doxorubicin, 5-fluorouracil and platinum drugs. Sorafenib is a drug for inhibiting tyrosine kinase, including the signaling pathway mediated by RAF, VEGFR and PDGFR, this drug has a role in inhibiting the proliferation of tumor cells, to promote apoptosis of cancer cells. However, sorafenib is not highly effective, due to drugs resistance of HCC cells, and sorafenib can only extend the average survival time of patients for three months^[2,6]. To reveal the mechanisms of cancer drugs resistance can greatly improve the therapeutic efficacy of drugs and improve the prognosis of HCC. Therefore, this review details the reasons of HCC cells drugs resistance.

During the normal individual development, AFP is synthesized from the fetal yolk sac endoderm and liver, and can spread into the mother through the blood circulation. After birth for two years, the concentration of maternal serum AFP decreased rapidly, and high levels can be maintained at 3-4 weeks in the newborn (shown in **Figure 1**). AFP has an immunosuppressive function

that reduces maternal rejection to fetal^[7]. AFP can carry important fatty acids involved in the fetal neuronal development^[8]. AFP consists of a polypeptide chain with a molecular weight of about 68-70Kd, half-life of about 5 days. The gene encoding AFP is located in 4q11-13 with 15 exons and 14 introns. AFP is a molecule morphologically similar to type V, with three domains I, II and III, and three domains containing four, five and six disulfide bonds, respectively. This morphological features contribute to AFP maintain its high stability. In HCC patients, high concentration of AFP in serum can be detected in 70% cases, as a tumor-associated protein, AFP can be used as a screening protein for diagnosis of HCC, but Cao, et al, reported that apyrimidinic endodeoxyribonuclease 1 (APEX1) is a better diagnostic and prognostic biomarker than alpha-fetoprotein in HCC. These results indicated that APEX1 was a high specific and sensitive index in the diagnosis for liver cancer^[9]. The AFP produced by HCC is associated with the tumor growth rate, the size of the tumor, and the composition of the chromosome. Slow-growth and well-differentiated HCC is generally not associated with elevated serum AFP concentration. Higher serum AFP concentration often appears in fast-growing and poorly differentiated HCC patients. It has been shown that AFP can promote HCC cells proliferation, invasion, metastasis and reduce apoptosis of cancer cells. AFP can also promote the malignant progression of HCC and lead to a poor prognosis. Many studies showed that AFP was able to induce HCC cells drugs resistance and reduce the efficacy of drug therapy. The mechanism of AFP stimulates drugs resistance of cancer cells has not been defined. Drugs resistance is caused by multiple factors, in this review, it details the role mechanism that AFP may induce drugs resistance of HCC cells, to clarify the effect of AFP on drugs resistance and novel strategy that AFP is applied as a novel bio-target for the therapeutic of liver cancer.

Figure 1. Serum concentration of (AFP) at the stage during growth.

2. AFP Stimulates the Generation of HCC Stem Cells

Cancer stem cells (CSC) have similar functions to normal stem cells, including self-renewal and differentiation. CSC plays an important role in the genesis and progression of HCC; and CSC in tumors may originate from the malignant transformation of normal hepatocytes stem cells in the liver. CSC has stemness, the trait of stem cell has the ability to maintain cell self-renewal and differentiate to maintain a balance between cell quiescence, proliferation, and regeneration. CSC is tumorigenic, pluripotent, and immunogenicity. AFP functions to stimulate origination of CSC, promotes the proliferation and invasion of HCC cells by regulating the cancer stem cell surface markers CD 133, CD44, and CD90. It has been found that liver cells that produce AFP to exhibit the function of directed progenitors, thus suggesting that cancer cells can produce AFP are branching from CSC, and that cancer cells producing AFP have more tumorigenic functional than cancer cells that do not produce AFP^[10]. AFP can stimulate the PI3K/ATK signaling pathway, which can promote the expression of hypoxia induced factor-1 α and 2 α (HIF-1 α , HIF-2 α), these factors induce genesis of CSC and enhance cancer progression. These results suggest that AFP can speed up the progress of liver cancer. CSC can enter a stationary state during radiotherapy or drugs treatment, lead to the serum concentration of AFP is reduced. When systemic drugs treatment stops or ends, CSC is activated from quiescence, and re-secretion of AFP, AFP can positive feedback to stimulate CSC, promotes CSC proliferation and division, and promotes malignant phenotypic transformation of cancer cells, new neoplasenesis leads to patient relapse. The interaction of AFP with CSC, forming a linkage reaction to promote the formation of a poor prognosis. These evidences verified that AFP induces the genesis of CSC is a pivotal factor for drugs resistance of HCC cells.

3. Effect of AFP on the Malignant Phenotype of HCC Cells

3.1 AFP Effect on the Proliferation of HCC Cells

The most critical link of carcinogenesis and development is the malignant proliferation of cancer cells. The infinite proliferation of cancer cells is a problem for refractory of cancer. AFP can promote the

proliferative capacity of HCC cells. Studies indicated that in HepG2 cells lines, which high expression of AFP, while silence the expression of AFP in this liver cancer cells, MTT experiments showed that the proliferation of these cells were significantly decrease, and the cells mostly stayed in G0/G1 phase^[11]. p53 functions as a tumor suppressor, it is able to inhibit growth of HCC cells^[12]. Studies have showed that p53 could inhibit the expression of AFP in HCC cells, led to the proliferation arrest of HCC cells^[13-15]. Transforming growth factor- β (TGF- β) affects the transcribed function of p53 in HCC, and TGF- β signaling can promote proliferation of HCC cells by controlling the cell cycle regulator p27^[16], showing that AFP can interact with TGF- β to promote the proliferation of HCC cells. Phosphatase and tensin homologs (PTEN) locates on chromosome 10, it is a tumor suppressor gene that inhibits the proliferation, migration and invasion of cancer cells, it also can promotes apoptosis of cancer cell of^[17,18]. AFP can inhibit the activity of PTEN, to activation of PI3K/AKT signaling pathway, lead to inhibit autophagy in HCC cells, promoting the proliferation of cancer cells^[19]. All trans-retinoic acid (ATRA) has the effect on inducing apoptosis in liver cancer cells. Previously, studies have displayed that AFP could agonist ATRA effect and played the anti-apoptotic effect of HCC cells^[20]. The mechanism maybe involve in AFP binding to retinoid acid receptor (RAR), inhibit the formation of RAR-RA complex, promote the upregulation of survivin gene, reduce apoptosis of liver cancer cells and increase tumor volume^[21]. Caspase-3 is a pivotal molecule to mediate tumor necrosis factor (TNF) signaling pathway to induce apoptosis of HCC cells, AFP can bind with caspase-3 to inhibit the activation of this pathway, to suppress apoptosis of HCC cells, and promote HCC cells survival^[22,23]. It has been shown that knockdown the expression of AFP by silencing AFP gene, led to apoptosis was significantly increased in HCC cell lines^[24,25]. These evidences indicated that AFP can stimulate the proliferation and inhibit apoptosis of HCC through promoting the proliferative phenotype of HCC cells.

3.2. Effect of AFP on Invasion and Metastasis of HCC Cells

In the progression of cancers, it aggress not only on normal cells and tissues in situ, but also on adjacent

and distant tissues. It was confirmed that the serum levels of AFP were positively correlated with HCC cells differentiation and tumorous size, and that a high level of serum AFP predicted a chance of higher for vascular invasion^[26]. Higher AFP levels are often accompanied by larger tumorous volume, and tumorous size growth is associated with increased AFP levels^[27], which reveals biological patterns in which AFP can induce invasiveness of HCC cells. AFP levels can be stabilized by acetylation, which is achieved by inhibition of ubiquitination and proteasome degradation, acetylation of AFP increases the interaction of caspase-3 and PTEN, thereby reduce apoptosis of tumor cells and activate PI3K/AKT signaling pathway, increase the chance of transformation of HCC cells to a highly aggressive phenotype, and promote cancer cells invasion and metastasis^[28-30] (showed in **Figure 2**). High levels of acetylated AFP showed an association

with a poor prognosis in patients with HCC^[31]. It has been confirmed in other cancers, such as gastric cancer that secreted AFP significantly increases by lymphovascular infiltration, peripheral nerve infiltration (PNI) compared with gastric cancer without AFP^[32]. Colorectal cancers with secreted AFP differ greatly from unsecretory pathological histological features and exhibit higher aggressive behaviors^[33]. PI3K/AKT signaling pathway not only has a role in promoting the proliferation of cancer cells, but also promotes metastasis of cancer cells^[34-36]. AFP can promote expression of matrix metalloproteinase(MMP) 2/9 (MMP2/9), CK19, EpCAM and CXCR4 by activating PI3K/AKT signaling pathway, and upregulation expression of these genes contributes to the metastasis and invasion of HCC cells^[37-39] (showed in **Figure 2**). These evidences indicated that AFP was able to stimulate the invasion and metastasis of HCC cells.

Figure 2. Effect of AFP on the malignant phenotype of HCC cells.

AFP can bind with AFP receptor (AFPR) on the cellular membrane, to regulate the transcribed activity of Ras and c-Jun by promoting release of calcium(Ca^{2+}), and activating cAMP and PKA, to induced HCC cells proliferation; AFP interacted with caspase-3 to inhibit cytochrome C release, and downregulation expression of Bax, but upregulation expression of Bcl-2, lead to the Bax/Bcl-2 ratio decreases; AFP stimulates transduction of PI3K/AKT signaling pathway to regulation of the downstream protein mTOR, to induces the expression of EpCAM, Sox2, CD133, CD44, etc; AFP can bind competitively to RAR, inhibit the formation of RAR-RA complex.

AFP plays a pivotal role in promoting HCC progression and the malignant phenotype of HCC cells.

4. The Regulatory Mechanism of AFP on the Malignant Phenotype of HCC Cells

4.1. AFP Regulates Transduction of Signaling Pathway in HCC Cells

Cell signaling transduction is a key link of cellular phenotype, and dysregulation of signaling is an important factor for the underlying malignant phenotype of cancer cells. Wnt/ β -catenin signaling is highly conserved in normal cells and is involved in tissue *in vivo* homeostatic^[40,41]. The classical Wnt/

β -catenin signaling pathway is crucial for HCC development^[42,43]. β -catenin is a functional protein that plays a dual role in cell adhesion and gene transcription, and has a role in intracellular signaling in the Wnt signaling pathway. The high activation of the Wnt/ β -catenin pathway in HCC patients leads to substantial accumulation of β -catenin in tissues and the nucleus, it is a hallmark of Wnt signaling. Activation of Wnt/ β -catenin signaling has been demonstrated to promote liver cancer stem cells (LCSC) self-renewal, differentiation, and invasive of HCC stem cells^[44,45]. Studies found that HCC stem cells were able to secrete AFP, and AFP could stimulate the Wnt/ β -catenin signaling pathway, thus affecting the cancer cell cycle^[46-48]. AFP inhibits apoptosis of tumor cells by blocking the signaling of apoptotic factors and affecting the expression of apoptotic genes in tumor cells. The caspase-3 signaling pathway is the main signaling pathway for inducing apoptosis of cells, and AFP can directly interact with the amino acid residues Glu-248, Asp-253 and His-257 of the caspase-3 molecule loop-4 in the cytoplasm^[49], blocking the biological activity of caspases, thereby inhibiting the role of tumor necrosis factor (TNF). Silencing of the AFP gene is able to reduce expression of p53, resulting in downregulation of Bcl-2 expression and upregulation of Bax expression. Increases of the Bax/Bcl-2 ratio, further triggered the release of mitochondrial cytochrome C, to activate caspase-3 to induce apoptosis of HCC cells, suggesting that AFP could inhibit apoptosis of tumor cells through activating the p53/Bax/caspase-3 signaling pathway^[22-25]. AFP also activates PI3K/AKT signaling pathway and reduces the killing effect of drugs on HCC cells. AFP can influence the life cycle of HCC cells through stimulating proliferation associated signaling pathway or inhibiting apoptosis-related signaling pathway, resulting in increased proliferation and inhibited apoptosis of cancer cells.

The altered phenotype of HCC cells is the important factor in HCC and an pivotal reason affecting the efficacy of the drugs. Altered cellular phenotype can increase the occurrence of drugs resistance. The important reason for altering cellular phenotype is abnormal signaling, and the important signaling pathways are Wnt/ β -catenin, Akt/mTOR, EGFR-RAS-MAPKK, c-MET, IGF, VEGF and PDGFR signaling

cascade^[50]. Sorafenib can inhibit the VEGFR1 and Ras/Raf/MEK/ERK signaling pathways^[51,52], and enhance the therapeutic efficacy of sorafenib. Effects on the transduction of major signaling pathways of the cellular phenotype may be responsible for drugs resistance. Increase the expression of epidermal growth factor (EGF) can be emerged in HCC cells, but EGF is not or poorly expressed in normal liver cells. EGF may bind to its receptor (EGFR), EGFR can play multiple biological functions, EGFR is able to alter the HCC cellular phenotype through activating some signaling pathways, such as PI3K/AKT and JAK, and variants of EGFR can promote tumor cell growth and inhibit apoptosis. EGFR reduces the effect of sorafenib in HCC cells^[53]. VEGF is an important signaling for tumor invasion and angiogenesis, and is highly expressed in HCC cells^[54], the alteration of these signaling pathway may be one of the mechanisms underlying HCC cells drugs resistance. AFP interacts in these proteins network, affects signaling transduction, and promotes the malignant phenotype of cells.

4.2. AFP Regulates Expression of Proliferation, Invasion and Metastasis related Genes

AFP, as a tumor-associated protein of HCC, has a regulatory effect on genes expressed in cancer cells. The IGF2 mRNA binding protein IMP2 is thought to be an autoantibody to HCC, and IMP2 is a highly expressed in HCC tissue, and high AFP expression is closely associated with IMP2, showing that HCC prefers to present an invasive phenotype^[55]. With maximum tumor diameter (MTD), the HCC tumor MTD was significantly associated with AFP, γ -glutamyl transpeptidase (GGTP) and platelet levels, and patients with high AFP expression in serum had higher tumor diameter and higher serum bilirubin levels, showing an invasive biological pattern of HCC^[56]. More studies have shown that high serum AFP is accompanied by large tumor diameter and in multiple tumors, and researchers considered that possible involve in AFP upregulating the expression of Wnt/ β -catenin gene. LASP-1 plays an important role in various tumorigenesis, cell experiments show that LASP-1 was able to promote proliferation and migration of cancer cells, induced cancer cells aggressive phenotype. High expression of LASP-1 is significantly positive associated with low overall survival, LASP-1 is closed

related to with serum AFP levels^[57]. Previously, we have found that AFP stimulated the expression of growth-related genes, such as Ras, Src and CXCR4^[58], the expression of these growth-related genes were able to promote hepatocarcinogenesis. The studies also found that cAMP concentration and activities of PKA were significantly elevated mediated by AFP receptor and induces the expression of K-ras^[59]. These evidences display that AFP regulates the expression of multifaceted genes to promote the phenotype of drugs resistance.

Studies showed that AFP stimulated cancer cells proliferation, invasion and metastasis and other malignant phenotype, is completed by signaling activation and regulation of multiple genes express, AFP also has a trait to simulate expression of Fn14, indicated that AFP plays a signal molecule-like role in stimulating growth of HCC cells^[60], AFP induces malignant behavior and liver tumor diameter, enhances anti-apoptosis ability. AFP also promote migration and invasion of HCC cells to other tissues. AFP induces HCC cells to obtain drugs resistance phenotype, leading to HCC cells resist the effect of sorafenib and doxorubicin, eventually cause low therapeutic effect, high recurrence rate and low survival.

5. The Role Mechanism of AFP Effect on the Drugs Resistance in HCC Cells

5.1 AFP Effect on the Resistance of Various Drugs

The onset of HCC is hidden, most patients are advanced stage at diagnosis, at this time, drugs treatment is the first clinical choice. However, due to individual differences, and HCC drugs resistance, the treatment of drugs in HCC patients is not effective. During the therapeutic, most patients develop drugs resistance, Cheng et al, reported that temperature-sensitive PEGylated supramolecular nanocarriers with good biocompatibility are effective in combating MDR-1 mediated drugs resistance in both in vitro and in vivo models, which is of significant importance for the advanced drug delivery platform designed to combat drug resistant of HCC^[61]. Ke, et al, found that excellent Nur77ΔDBD gene delivery can reverse Bcl-2 from the HCC cells protector to killer for inhibiting non-pump resistance, and the higher transfection efficacy, rapid doxorubicin cellular uptake, and significant synergetic

tumor cell viability inhibition were achieved in a pump and non-pump drugs resistance cell model^[62]. The combined strategy with dual drug resistance mechanisms holds great potential to combat drug-resistant cancer^[63]. But AFP as the primary associated protein of HCC, most studies have demonstrated the involvement of AFP in HCC cells drugs resistance. Revealing the mechanisms by which AFP regulates HCC cells drugs resistance is a breakthrough in the treatment of HCC. Sorafenib is the preferred drug for the treatment of advanced HCC without liver surgery, sorafenib suppresses tumor growth and angiogenesis by targeting RAF/MEK/ERK pathway and receptor tyrosine kinase^[64], most patients produce resistant after sorafenib treatment^[65,66]. The factors that HCC producing sorafenib resistance may be associated with abnormal signaling transduction pathways such as EGFR, VEGF, and AKT^[67-69]. AFP participates in angiogenesis and is currently thought to be involved in regulating the HCC cells growth^[70,71], binding to various signaling proteins, weakening the therapeutic efficacy of sorafenib in HCC cells^[70]. Higher expression of AFP is able to decrease survival during sorafenib treatment^[72, 73], due to high levels of serum AFP were negatively correlated with clinical treatment effects of sorafenib^[74] (shown in **Figure 3**). Doxorubicin is a DNA topoisomerase II inhibitor used to treat a variety of cancers. In HCC cell lines, while treated with the same dose of doxorubicin, the apoptosis rate of the cells were significantly lower of overexpressing AFP than low expression of AFP, and HCC cells which overexpressing AFP presented a drug-resistant phenotype^[75,76]. The immunosuppressive receptors PD-1 and PD-L1 are already proven inhibitory checkpoints^[77, 78], and inhibiting immune checkpoint signaling is the primary cause of HCC immune tolerance^[79,80]. PD-1 expression is observed on human T cells, B cells, and natural killer(NK) cells, for example, which can interact with its ligands PD-L1 and PD-L2 to inhibit the activation of antigen-specific T cells. AFP can influence the immune suppressive checkpoint, reduce the antigenicity and obtain immune escape of HCC cells^[81,82], it maybe a mechanism that prompts patients to develop resistance to immune checkpoint drugs. Therefore, exploring the mechanisms by which AFP promotes drugs resistance is an important way to improve drug efficacy in treatment of patients loading HCC.

Figure 3. Mechanism of AFP promote the resistance of HCC cells to Sorafenib.

Epidermal growth factor (EGF) can activate PI3K/AKT signaling by binding to the epidermal growth factor receptor (EGFR) on the cellular membrane, Sorafenib is able to inhibit this signaling pathway, but AFP has trait to activating PI3K/AKT signaling pathway to antagonize the role of Sorafenib.

5.2. Role Mechanism of AFP in Regulating Drugs Resistance of HCC

High expression of drugs resistance genes in tumor cells leads to drugs resistance and reduces the therapeutic effect of drugs. AFP can regulate drugs resistance genes, leading to high expression of drugs resistance genes and promoting HCC cells drugs resistance (shown in **Figure 4**). Immune escape and resistance were key factor for HCC cells resisted the therapeutic of immune check point drugs, such as anti-PD-L1 or anti-PD-1^[83-85]. Previously, studies displayed that AFP has a function to impairs activation of NK cells by inhibiting the function of dendritic cells (DC cells), and tumor-derived AFP impairs the differentiation and T cell stimulatory activity of human dendritic cells^[86-92], AFP induces apoptosis in antigen-presenting cells, reduces antigenicity of tumor cells, and further impair immune response in HCC patients. AFP can upregulate the expression of promoting apoptotic genes (such as Bax, Bid, Bad, *etc.*), and induce

apoptosis of immune cells (*e.g.*, lymphocytes, dendritic cells)^[93,94]. AFP can also upregulate the expression of proapoptotic factor genes in immune-infiltrating cells in the tumor microenvironment. In addition to promoting apoptosis of immune cells, AFP also inhibited the proliferation of NK cells and T lymphocytes. Lymphocytes have immune attack function to HCC cells, Fas is expressed by lymphocytes, AFP suppresses Fas expression while promoting FasL expression in HCC cells, Fas can highly specifically bind to FasL to reduce the killing role of lymphocytes to tumor cells^[95]. AFP has the role of immunosuppressors that can inhibit macrophage-induced cell cleavage and promotes HCC cell escape from lymphocyte-mediated immune surveillance^[96,97]. DC cells have a function to extract antigen information, identify antigens and present antigen information. AFP suppresses the maturation of DC cells and promotes its apoptosis, enabling HCC cells to evade immune surveillance^[98,99]. AFP enhanced apoptosis factors and caspase-3 expression in DC cells and induce apoptosis of DC cells. These results showed that the expression of AFP in HCC cells led to immune escape and resistance, and subtractive immune attack of immune cells. It is suggested that AFP inhibits immune response, which is an important factor for the drugs resistance of HCC cells.

Figure 4. The role mechanism of AFP in promoting drugs resistance of HCC cells.

Sorafenib can inhibit angiogenesis, AFP participates in angiogenesis and reduce drug efficacy; AFP is able to up-regulate expression of MDR 1, MRP, BCRP, and ABC transporters, to cause drug efflux.

Autophagy is a highly conserved process of cellular decomposition and metabolism for cellular degradation after fusion of autophagosomes and lysosomes. Autophagy can provide regenerative metabolites to promote cancer cells growth and cancer cells survival through mitochondria energy metabolism, and promote cancer cells migration and metastasis^[100-102]. Autophagy has been shown to promote survival and drugs resistance of cancer cells^[103-105]. Lower mTOR activity is a key factor of autophagy inhibition and is particularly activation of PI3K/AKT signaling pathway^[106,107]. Autophagy can be activated by resistance chemotherapy, and inhibition of autophagy can regain sensitivity of cancer cells to drugs and enhance the effects of chemotherapy. Evidences have indicated that multiple resistance occurs after autophagy, and that enhanced levels of autophagy were monitored in patients with poor prognosis. Autophagy behavior may lead to increased cancer cells resistance to paclitaxel^[108], tamoxifen^[109]. It is shown that chemotherapeutic agents of HCC can increase autophagy in HCC cells, this phenomenon occurs may be enhance drugs resistance and promote cancer cells survival^[110]. Therefore, at the stage of tumor development, autophagy enhances the drugs resistance of cancer cells, while the inhibition of

autophagy can promote cancer cell death^[111-113]. AFP can inhibit autophagy to promote malignant behavior in HCC cells, this effects was mediated by AFP to activate the PI3K/Akt/mTOR signaling pathways^[114]. Low expression of autophagy-related genes are associated with resistance to sorafenib, and with activation of the AKT/mTOR and AMPK signaling pathways^[115,116].

The effect of HCC proliferation-related genes pattern can be divided into two subtypes, one proliferative type and one non-proliferative^[117,118]. The proliferation type showed high expression of signaling pathways molecules, such as TGF- β , MET, AKT, and non-proliferative biological manifestations are usually low invasive forms, low serum AFP levels, and a better prognosis^[119]. HCC was divided into three subtypes based on metabolic process and prognostic correlation indicators, C1 showed low serum AFP levels and good prognosis, C2 showed high immune checkpoint gene expression, and C3 showed high serum AFP level and poor prognosis^[120]. Metabolic features suggests that C1 patients are more sensitive to metabolic therapy performance, such as metabolically-related drugs metformin. Patients with C2 exhibit a high mutation rate and are more sensitive to related immunotherapeutic agents, such as PD-1 immune checkpoint inhibitors and CTLA-4 inhibitors. The different phenotypes of HCC cells show different therapeutic effects on the drugs. Drugs mismatch, has a driving the effect of HCC cells drugs resistance, and drives a subtype transition in liver cancer. Reduce the

production of drugs resistance, improve the patient survival rate, targeted differentiation of liver cancer patients, personalized drug use, is also an important condition to reduce HCC cells drugs resistance.

Different phenotypes of HCC cells also exhibit different DNA methylation. In contrast to the normal tissues, methylation of p16, RASSF1A, APC, GSTP1, CDH1, p15, RUNX3, SOCS1, SFRP1, PRDM2, p14, RAR β and p73 genes in liver cancer tissues is upregulated^[121]. Abnormal methylation of gene is recognized as contributing to adverse biological outcomes. It is also considered as one of the reasons for the development of HCC^[122], aberrant methylation of genes shows reduced sensitivity to drugs, induction of phenotypic changes in cells, promote HCC cells to development of drugs resistance. Studies have indicated that high level of AFP positive associated with low methylation of drugs resistance-related genes^[123-125], and high methylation of drugs sensitivity associated genes, such as p53 and p73 were able to inhibit the expression of AFP^[126]. These evidences indicated that AFP influence the aberrant methylation of genes, contribute to the drugs resistance of HCC cells.

6. Conclusions and Outlook

HCC cells drugs resistance involve with variety factors, and multiple signaling pathway cause drugs resistance in HCC cells, such as sorafenib^[127]. Cancer stem cells(CSC) can enter a stealth quiescence during systemic treatment, pending treatment cessation, reactivation of CSC causes cancer recurrence. Explain the possible origin cause of drugs resistance from CSC, contribute to clarify the mechanisms of drugs resistance. In immunosuppression aspect, elaborates the reduced antigenicity of HCC cells and suppresses the immune response lead to cancer cells immune escape, participate in HCC cells drugs resistance. This review describes the multifaceted mechanisms of drugs resistance of HCC, to whole analyze the generation and influence of drugs resistance in HCC cells. The malignant phenotype of HCC cells is a crucial factor for the drugs resistance. AFP has a regulatory effect on the malignant phenotype of hepatoma cells, stimulates the proliferation and invasion of cancer cells, promotes HCC cells drugs resistance, and affects the therapeutic effect of HCC, the engineering nonviral

polymers to function as gene delivery vectors with increased efficiency and specificity, and co-delivery of therapeutic genes and drugs show great therapeutic effect against liver tumors, including drug-resistant tumors^[128]. AFP reduces immune rejection and the killing effect of immune cells on HCC cells, contribute to the drugs resistance of HCC cells. As the study progressed, the role of AFP and its role mechanisms became more clear, found the novel function of AFP in promoting malignant phenotype of HCC cells, provide new therapeutic targets for the treatment of HCC. It has been studied that AFP acting vaccines can improve the poor prognosis of HCC, and AFP can carry targeted drugs to provide new therapeutic ideas for drugs treatment. The continuous exploring of the drugs resistance mechanism of HCC cells and the role mechanism of AFP in affecting drugs resistance, is bound to provide new development ideas for the drugs treatment of HCC.

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Authors' Contributions

XS, MZ, HL and MZ gathered the related literature, XS prepared the figures and drafted the manuscript. MZ and ML participated in the design of the review and drafted the manuscript. All authors read and approved the final manuscript.

Ethics Approval and Consent to Participate

Not applicable.

Consent for Publication

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Competing Interests

The authors declare that this article content has no conflict of interest. All authors of this paper have read and consented to publication of the final version.

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