

# Phosphatidylinositol-3-kinase/Protein Kinase B Signaling Pathway: A Pivotal Factor for Stimulating Multidrug Resistance in Hepatocellular Carcinoma Cells

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**Abstract:** Hepatocellular carcinoma (HCC) is a common malignant tumor. Chemotherapy is one of its principal modes of treatment, but multidrug resistance (MDR) poses an obstacle in HCC treatment. MDR is mainly mediated through drug transmembrane transporter activity, apoptosis inhibitory pathway abnormality, and changes in the intracellular enzyme. On top of that, phosphatidylinositol-3-kinase (PI3K)/protein kinase B (Akt) pathway dysregulation is also one of the most common mechanisms of MDR. This signaling pathway plays critical roles in the differentiation, proliferation, and apoptosis of cancer cells, and is a new target of MDR treatment. This review article discusses the mechanisms of MDR in HCC with a strong emphasis on the significance and role of PI3K/Akt pathway in MDR, and the reversal of MDR by inhibiting PI3K/Akt pathway.

**Keywords:** Hepatocellular carcinoma, Multidrug resistance, PI3K/AKT pathway

## 1. Introduction

Hepatocellular carcinoma (HCC) is a malignant tumor of the digestive system with rapid progression, poor prognosis, and high recurrence, and its mortality ranks third among other tumors<sup>[1]</sup>. At present, drug therapy (chemotherapy) is one of the major therapeutic methods of HCC. However, the presence of multidrug resistance (MDR) in HCC usually causes poor chemotherapeutic effect or even treatment failure<sup>[2]</sup>.

The phosphatidylinositol-3-kinase (PI3K)/protein kinase B (Akt) pathway belongs to a very important and complex intracellular signal transduction network. This pathway promotes tumor cell invasion, proliferation, and migration as well as prevents apoptosis. This pathway plays indispensable roles in the occurrence and progression of HCC<sup>[3-5]</sup>. The role of PI3K/Akt pathway activation in the development of MDR in HCC has garnered much attention in recent years. Excessive activation of this pathway can promote the development of chemotherapy resistance in HCC. Clinical trials of pathway inhibitors may offer useful therapeutic approach for HCC patients. Some inhibitors such as SF1126, BKM120, and MK2206 targeting this pathway have been applied in the clinical treatment of cancers<sup>[6,7]</sup>.

This review article discusses the mechanisms of MDR in HCC with a strong emphasis on the significance and role of PI3K/Akt pathway in MDR, and the reversal of MDR by inhibiting PI3K/Akt pathway. In addition, the development of MDR in HCC and the association between PI3K/Akt signaling pathway and drug resistance of HCC are also reviewed.

## 2. Overview of the PI3K/Akt signaling pathway

PI3K is a group of phospholipid kinases capable of phosphorylating the 3'-OH group of the phosphatidylinositol ring. Its main components include the p85 regulatory subunit and the p110 catalytic subunit<sup>[8]</sup>. PI3K is usually classified into three subtypes, namely, subtypes I, II, and III. The type IA PI3K contains three isomers: p110 $\alpha$ , p110 $\beta$ , and p110 $\delta$ . These isomers are all activated by receptor tyrosine kinases (RTKs). The type IB includes the p110 $\gamma$  group, which is activated by G protein-coupled receptors<sup>[9]</sup>. Protein kinase B, a serine/threonine specific kinase, is a key downstream effector of PI3K and is also called Akt.

After RTK activation, receptor tyrosine residues and the Src homology 2 (SH2) domains of the PI3K regulatory subunit interact to initiate allosteric activation of the catalytic subunits to activate PI3K. Phosphatidylinositol-4,5-bisphosphate (PIP2) inside the plasma membrane is then converted into the second messenger phosphatidylinositol-3,4,5-trisphosphate (PIP3)<sup>[10]</sup>. PIP3 interacts with the pleckstrin homology (PH) domain of Akt to induce a conformational change of Akt and catalyze the phosphorylation at Ser473 and Thr308 of Akt<sup>[11]</sup>. Phosphorylated Akt (p-Akt) can induce changes in many downstream substrates, including Bcl-2-associated agonist of cell death (BAD), nuclear factor kappa B (NF- $\kappa$ B), forkhead box transcription factors (FOXO proteins), glycogen synthase kinase-3 (GSK-3), and mechanistic target of rapamycin (mTOR)<sup>[12,13]</sup>. p-Akt can phosphorylate Bcl-2 family of apoptosis regulator proteins, caspase-3, and caspase-9 to prevent apoptosis<sup>[14,15]</sup>. Through activation of NF- $\kappa$ B, p-Akt promotes migration and invasion of tumor cells<sup>[16]</sup>. GSK-3 mediates glycogen synthesis, inhibits glucose transport, and participates in the regulation of glycogen metabolism. p-Akt can reduce GSK-3 activity, promote glucose uptake, and enhance lipid metabolism<sup>[17,18]</sup>. p-Akt can also mediate phosphorylation of tuberous sclerosis 2 (TSC2) to relieve the inhibitory effect of TSC2 on mTORC1, leading to mTORC1 activation, stimulated cell growth, anabolic processes (including the synthesis of proteins, lipids, and nucleotides), and inhibition of the catabolic process of autophagy<sup>[19,20]</sup>. Therefore, the PI3K/Akt pathway is very important for many key cellular functions, including cancer cell growth, proliferation, differentiation, survival, glucose metabolism, cytoskeleton rearrangement, migration, and invasion.

## 3. Mechanisms of MDR in HCC

### 3.1. Transmembrane transporter protein-mediated drug efflux

Transmembrane transporter proteins can pump drugs out of HCC cells to decrease their concentrations in the cells. Transmembrane transporter proteins include ATP-binding cassette (ABC) transporters and cytoplasmic transporters. ABC transporter proteins can be classified as P-glycoproteins (P-gp/MDR1/ABCB1), MDR-associated proteins (MRP/ABCC), and breast cancer resistance proteins (BCRP/ABCG2)<sup>[21-23]</sup>.

P-gp is encoded by the *MDR1/ABCB1* gene. As a transmembrane transporter with energy-dependent drug efflux function, P-gp can pump positively charged drugs out of cells across a reverse concentration gradient. P-gp is divided into two homologous segments. Each fragment is composed of one ATP domain and a hydrophobic transmembrane region containing six  $\alpha$ -helices<sup>[24]</sup>. Meng *et al.* showed that, compared to parental HepG2 cells, HepG2/ADM cells had enhanced resistance to many chemotherapeutic drugs, such as adriamycin, mitomycin, and vincristine<sup>[25]</sup>. Treatment with the MEK inhibitor U0126 can reduce the resistance of cells to these drugs. Further analyses showed that P-gp expression in drug-resistant cells was 5.37 times that of parental HepG2 cells, and its expression in drug-resistant cells was 2.68 times that of HepG2 cells after U0126 treatment, U0126 downregulated P-gp expression in drug-resistant HCC cells to further reverse MDR. On the other hand, Yahya *et al.* found that miR-122 overexpression might further regulate the sensitivity of HCC to drugs by downregulating the MDR-related gene ABCB1<sup>[26]</sup>. The above findings indicate that P-gp is closely associated with MDR in HCC cells. Upregulation of P-gp promotes MDR development in HCC cells, and its downregulation holds promise in MDR reversal.

Being a half-transporter, BCRP/ABCG2 contains only one hydrophobic transmembrane domain and one ATP-binding domain<sup>[27]</sup>. The *in vivo* and *in vitro* findings of Guo *et al.* showed that augmenter of liver regeneration downregulates the protein and mRNA expression of ABCG2 and ABCB1 in HCC cells to effectively inhibit efflux of doxorubicin, thereby enhancing the sensitivity of HCC cells to doxorubicin and reversing drug resistance in HCC cells<sup>[28]</sup>. In an experiment using RNA interference to knock down BCRP expression in HCC cells, Li *et al.* found that the drug sensitivity of HCC cells was greatly increased after treatment with pSUPER-BCRPs<sup>[29]</sup>, indicating that BCRP knockdown can overcome the BCRP-mediated MDR in HCC cells.

MRP/ABCC is a transmembrane transporter that can pump negatively charged drugs out of cells. It is speculated that the efflux of glutathione-coupled substrates through MRP, as mediated by drug-glutathione

complex, further reduces the intracellular drug concentration, leading to MRP-induced MDR<sup>[30]</sup>. Huang *et al.* found that ABCC1 might serve as the direct target of the long noncoding RNA (lncRNA) NR2F1 antisense RNA 1 (NR2F1-AS1)<sup>[31]</sup>. NR2F1-AS1 targets ABCC1 through microRNA-363 (miR-363) to regulate oxaliplatin resistance in HCC. Kataoka *et al.* reported that loss of Runt-related transcription factor 3 induced the expression of MRP5, MRP3, MRP2, and MRP1 and upregulated MRP to promote resistance of HCC cells to 5-fluorouracil and cisplatin and stimulate MDR<sup>[32]</sup>.

### 3.2. Enzyme-mediated drug resistance

Telomerase is a nuclear reverse transcriptase with three subunits: human telomerase RNA, human telomerase-related proteins, and human telomerase reverse transcriptase (hTERT)<sup>[33]</sup>. hTERT can add a six-base repetitive DNA sequence to chromosomal ends to prevent their shortening during the process of cell division. Telomerase is associated with tumor cell immortality, which might be the result of the ability of hTERT to stabilize telomeres and prevent apoptosis<sup>[34,35]</sup>. Some studies have shown that telomerase is related to drug resistance. Dong *et al.* found that inhibition of telomerase activity reduced adriamycin resistance in cancer cells<sup>[36]</sup>. Smith *et al.* found that tumor cell lines that are resistant to gemcitabine, vindesine, and cisplatin showed changes in telomere length and telomerase activity compared with their parental cell lines<sup>[37]</sup>. Kuranaga *et al.* found that telomerase might induce MDR by improving the stability of chromosome and the expression of ABCB1 and ABCC genes<sup>[38]</sup>.

In an experiment, Ling *et al.* treated HCC cells with cisplatin to establish a drug-resistant cell line SK-Hep1/CDDP, which is resistant to cisplatin, but also cross-resistant to doxorubicin and 5-fluorouracil<sup>[39]</sup>. The mitochondrial translocation of hTERT had a protective effect on the mitochondria and could shorten the telomere length in drug-resistant SK-Hep1/CDDP to reduce mitochondrial DNA damage. Apoptosis of SK-Hep1/CDDP was prevented through hTERT-mediated mitochondrial protection<sup>[39]</sup>. These findings imply that mitochondrial translocation of hTERT might be one of the potential mechanisms of MDR in HCC.

Abnormal expression of glutathione S-transferase (GST) might also induce MDR in HCC cells. GST is a Phase II metabolic enzyme that can be roughly classified into five types:  $\alpha$ ,  $\beta$ ,  $\theta$ ,  $\mu$ , and  $\pi$ . MDR in HCC is closely associated with GST- $\pi$ , which can catalyze interactions between lipophilic drugs and glutathione to enhance their water solubility and pump them out of the cells. GST- $\pi$  also has peroxidase activity, reducing the cytotoxic effect of drugs by converting toxic peroxides into less toxic alcohols<sup>[40]</sup>. Lu *et al.* found that tissue factor pathway inhibitor-2 (TFPI-2) reduced the resistance of BEL-7402/FU cells to 5-fluorouracil<sup>[41]</sup>. The underlying mechanism behind the drug resistance is associated not only with

downregulation of drug resistance genes such as *MDR1*, *LRP*, and *MRP1* but also with a significant reduction in the expression level and the activity of GST- $\pi$ .

### 3.3. Drug resistance mediated by dysregulated apoptosis pathway

MDR development in HCC cells is often associated with abnormal expression of apoptosis-related genes. HCC cells can be killed by the drugs that induce apoptosis. However, resistance to apoptosis will render the drugs ineffective and lead to MDR. B-cell lymphoma 2 (Bcl-2) and survivin are key factors in the anti-apoptotic mechanism. The upregulation of Bcl-2 and survivin gene expression will cause apoptosis resistance and further worsen MDR<sup>[42,43]</sup>.

The Bcl-2 protein family is usually divided into two types. One of them is belonging to anti-apoptotic factor including Bcl-XL and Bcl-2, whereas the other type is belonging to apoptosis-promoting factor containing Bcl-2 homologous antagonist/killer (BAK), BAD, and Bcl-2-associated X (BAX). Survival of cancer cells can be determined by the Bcl-2/BAX ratio<sup>[44]</sup>. Bcl-2 inhibits the activation of downstream caspase-3 by blocking the release of cytochrome c, and effectively inhibits the occurrence of apoptosis<sup>[45]</sup>. Li *et al.* indicated that Bcl-2 upregulation causes the IC<sub>50</sub> value of doxorubicin in Bel7402/5-FU cells to be 20 – 50 times higher than that in Bel7402 cells<sup>[46]</sup>. Due to the doxorubicin resistance, the anticancer efficacy of the drug is reduced in the HCC drug-resistant Bel7402/5-FU cell model. Fang *et al.* confirmed that downregulation the expression of UBC9 was able to reduce the expression of anti-apoptotic factors Bcl-2 and Bcl-XL and increased the expression of pro-apoptotic factor caspase-3 to increase the chemosensitivity of HCC cells to doxorubicin and to reverse their drug resistance<sup>[47]</sup>. Yang *et al.* confirmed that the downregulation of Bcl-2 by miR-503 enhances chemosensitivity of HCC cells to cisplatin, thereby reducing the proliferation rate of tumor cells and reversing their drug resistance<sup>[48]</sup>. The above findings all indicate that increased expression of anti-apoptotic protein Bcl-2 positively correlates with the development of drug resistance in HCC and conversely, a reduction of Bcl-2 expression positively correlates with reversal of MDR in HCC cells. Therefore, the expression level of the anti-apoptotic protein Bcl-2 is closely associated with the development of MDR in HCC.

Survivin is an apoptosis-inhibiting protein. Its amino-terminus contains a BIR sequence, which can interact with the apoptosis effector molecules of the caspase family to prevent tumor cell apoptosis<sup>[49]</sup>. Survivin inhibits the activity of downstream terminators caspase-7 and caspase-3 in the caspases cascade pathway by preventing the latter from fully hydrolyzing microtubule associated proteins (MAPs), arresting the cells in G/M phase, and exerting anti-apoptotic functions; all of which lead to MDR<sup>[50]</sup>. Ho *et al.* showed that folate deficiency (FD) upregulated the expression of survivin, which synergistically promoted

MDR in HCC cells by reducing FD-induced reactive oxygen species generation<sup>[51]</sup>. Liu *et al.* found that the expression of survivin was significantly increased in cancer stem cells, showing resistance to carboplatin, temozolomide and paclitaxel<sup>[52]</sup>.

### 3.4. Transcription factor-mediated drug resistance

NF- $\kappa$ B is a dimeric transcription factor that consists of RelB, RelA (p65), NF- $\kappa$ B2 (p52), NF- $\kappa$ B1 (p50), and c-Rel subunits<sup>[53]</sup>. NF- $\kappa$ B has two different activation pathways. The canonical NF- $\kappa$ B activation mainly utilizes the catalytic activity of the I $\kappa$ B kinase (IKK) complex to phosphorylate I $\kappa$ B $\alpha$ . Phosphorylated I $\kappa$ B $\alpha$  is further ubiquitinated for degradation by proteases to cause nuclear translocation of the p50-p65 heterodimer. The other pathway is activation through the NF- $\kappa$ B-induced kinase IKK $\alpha$ . Activated IKK $\alpha$  phosphorylates the NF- $\kappa$ B2 (p100) precursor, which subsequently degrades its inhibitory region to be further processed into NF- $\kappa$ B2 (p52)<sup>[54]</sup>. Under normal conditions, the NF- $\kappa$ B heterodimer interacts with specific promoters to induce genes associated with cell death, survival, and inflammatory responses. However, persistent NF- $\kappa$ B

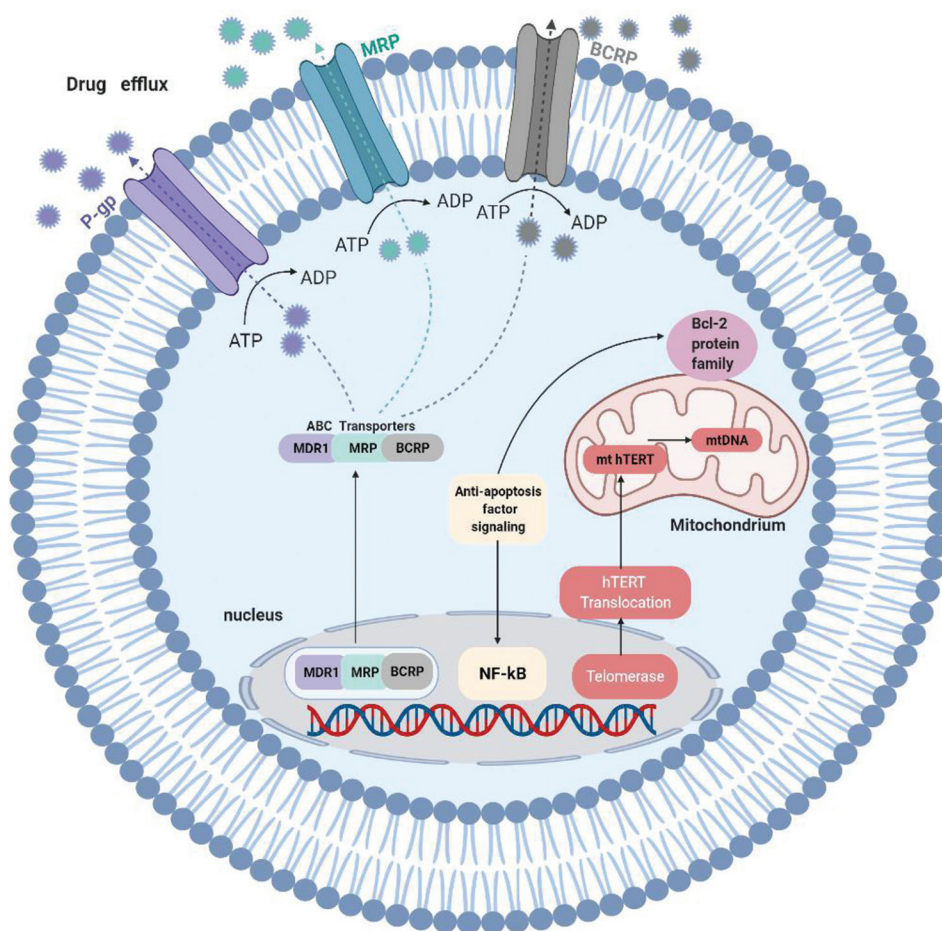
activation results in the occurrence and progression of tumors and their resistance to anti-tumor drugs.

Bonavida *et al.* found that induction of RKIP expression inhibits NF- $\kappa$ B activation to increase the drug sensitivity of drug-resistant tumor cells<sup>[55]</sup>. Therefore, NF- $\kappa$ B expression positively correlates with MDR in HCC cells. Other studies further suggested that NF- $\kappa$ B influences HCC drug resistance by regulating P-gp (MDR1) expression. Yang *et al.* found that Oroxylin A can inhibit P-gp expression by inhibiting the NF- $\kappa$ B signaling pathway, thus reversing the MDR in HCC cells<sup>[56]</sup>. Liu *et al.* found that the mRNA expression levels of MDR1 and NF- $\kappa$ B in cultured HepG2 parental cells were very low but their expression increased significantly in drug-resistant HepG2/ADM cells<sup>[57]</sup>. The MDR-related mechanisms in HCC cells are shown in **Figure 1**.

## 4. Relationship between the PI3K/Akt pathway and MDR in HCC cells

### 4.1. MDR results from drug efflux promoted by the PI3K/Akt pathway

The PI3K/Akt pathway not only can regulate transmembrane transporter protein expression but also can influence the



**Figure 1.** Schematic diagram of multidrug resistance-related mechanisms in hepatocellular carcinoma cells

localization of transmembrane transport proteins to the cell membrane to regulate MDR in tumor cells. The activation of PI3K/Akt pathway can result in an upregulation of the expression of many drug resistance-associated proteins, such as MRP, P-gp, and BCRP, to induce drug resistance<sup>[58]</sup>.

Cheng *et al.* found that MDR in HCC cells mediated by fucosyltransferase 4 (FUT4), FUT6, or FUT8 was associated with activation of the PI3K/Akt pathway and expression of MRP1<sup>[59]</sup>. Inhibition of the PI3K/Akt pathway by Wortmannin or Akt small interfering RNA (siRNA) reduces MDR in Bel-7402/FU cells, which is partly due to the downregulation of MRP1<sup>[59]</sup>. Yang *et al.* reported that HCC cell lines HepG2/ADM and BEL-7402/FU were resistant to many anti-tumor drugs, such as adriamycin, cisplatin, and 5-fluorouracil<sup>[60]</sup>. They also demonstrated that these drugs were able to block the PI3K/Akt signaling pathway, and Kanglaite induced apoptosis and cell cycle arrest, inhibited P-gp expression, and reduced drug resistance to reverse MDR. Xie *et al.* found that astrocyte elevated gene-1 (AEG-1) stimulates the PI3K/Akt/HIF-1 $\alpha$  signaling pathway to upregulate MDR1 expression in hypoxic HCC cells, paving the way for MDR development in HCC cells<sup>[61]</sup>.

#### 4.2. Induction of MDR in HCC cells by PI3K/Akt through activation of telomerase

Akt can mediate hTERT phosphorylation to upregulate telomerase activity. Chebel *et al.* found that PI3K can activate Akt to phosphorylate hTERT<sup>[62]</sup>. The latter forms a complex with hTR and the chaperone proteins Hsp90 and p23. This complex translocates into the nucleus and reverse-transcribe during telomere maintenance. Dogan *et al.* found that excessive hTERT activation confers a replicative feature and immortality to cancer stem cells (CSCs)<sup>[63]</sup>. Therefore, the stemness and replicative properties of CSCs depend on the telomerase activity. The PI3K/Akt/mTOR signaling pathway and hTERT upregulation has been associated with cancer stemness features and drug resistance. The PI3K/Akt/mTOR pathway can promote hTERT activation to further promote self-renewal and drug resistance of CSCs. Tahtouh *et al.* found that inhibition of the PI3K/Akt/mTOR signaling pathway can reduce hTERT expression and activity, suggesting that the PI3K/Akt signaling pathway in HCC cells is a key factor inducing hTERT to promote MDR in HCC cells<sup>[64]</sup>. These findings show that telomerase and PI3K/Akt are functionally connected and that telomerase is a new target of PI3K/Akt activity.

#### 4.3. Development of MDR in HCC cells mediated by anti-apoptotic function of PI3K/Akt pathway

Regulation of the Bcl-2 family and caspase family by the PI3K/Akt pathway further lower the apoptosis of cancer cells. Akt inhibits several downstream signaling molecules,

including caspase-9, and induces phosphorylation of the Bcl-2-related pro-apoptotic protein BAD on serine 136 (Ser136) site to cause dissociation of Bcl-2 on the mitochondrial membrane and inhibit apoptosis<sup>[65]</sup>. It can also induce BAX phosphorylation on Ser184 to cause inactivation and eventually inhibit apoptosis. Tang *et al.* found that Paris saponin VII (PS VII) downregulates the expression of drug resistance gene by inhibiting the PI3K/Akt/MAPK pathway to increase adriamycin accumulation in HepG2/ADR cells, upregulates the expression levels of pro-apoptotic factors caspase-3 and BAX, and downregulates the anti-apoptotic factor Bcl-2 to eventually cause apoptosis and enhance the sensitivity of HepG2/ADM cells to adriamycin<sup>[66]</sup>. These results suggest that the PI3K/Akt pathway can downregulate BAX and caspase-3 to promote MDR development and block apoptosis.

Abnormal activation of the PI3K/Akt pathway can also prevent the transcription of pro-apoptotic genes through phosphorylation of FOXO to further prevent tumor cell apoptosis<sup>[67]</sup>. The FOXO family is mainly composed of FOXO1, FOXO3, FOXO4, and FOXO6. FOXO translocates to the nucleus to activate transcription and exert pro-apoptotic functions. Jiang *et al.* found that trifluoperazine increases the expression level of FOXO1 in the nucleus of HCC cells to increase BAX/Bcl-2 expression and promote HCC cell apoptosis<sup>[68]</sup>. Akt can phosphorylate FOXO, which then translocates from the nucleus to the cytoplasm in HCC cells, where it interacts with 14-3-3 protein to block its nuclear translocation and promote its accumulation in the cytoplasm, thereby inactivating its transcription, promoting tumor proliferation, and inhibiting apoptosis<sup>[69,70]</sup>. These results show that the PI3K/Akt pathway causes MDR development in HCC cells through its anti-apoptotic effects.

#### 4.4. Development of MDR through the interaction between PI3K/Akt and NF- $\kappa$ B

The PI3K/NF- $\kappa$ B interaction is induced through a multistep cascade reaction triggered by the release of pro-inflammatory cytokines, followed by the interaction between the regulatory p85 subunit of PI3K and cytokine receptors in the cytoplasm, which, in turn, increases PI3K 110 $\alpha$  catalytic activity. Activated PI3K 110 $\alpha$  phosphorylates downstream p65RelA of NF- $\kappa$ B, inducing its nuclear translocation<sup>[71]</sup>. Activation of Akt can lead to the activation of IKK $\alpha$ , which induces I $\kappa$ B $\alpha$  ubiquitination and phosphorylation. Ubiquitinated I $\kappa$ B $\alpha$  is further degraded by 26S proteasome complex. Since I $\kappa$ B $\alpha$  is a specific inhibitory protein, I $\kappa$ B $\alpha$  degradation can lead to NF- $\kappa$ B activation and the entry of released p50-p65 into the nucleus from cytoplasm. Akt can also directly phosphorylate the transactivation domain TAD1 of the p65/RelA subunit to activate NF- $\kappa$ B independently of IKK<sup>[58]</sup>.

After phosphorylation of downstream I $\kappa$ B caused by PI3K/Akt pathway activation, NF- $\kappa$ B translocates into the

nucleus and interacts with the *ABCB1* gene promoter to activate gene transcription, causing P-gp overexpression and eventually the occurrence and progression of MDR<sup>[72]</sup>. Wang *et al.* suggested that miR-503 can block Akt phosphorylation and reduce PI3K/Akt pathway activity in HCC cells to further inhibit the transcription activity of NF- $\kappa$ B<sup>[73]</sup>. The negative regulatory function of this process on NF- $\kappa$ B activity reverses MDR in HepG2/ADM cells, suggesting that regulation of NF- $\kappa$ B by the PI3K/Akt pathway might be a way to reverse drug resistance in HCC cells. The mechanisms by which the PI3K/Akt pathway promotes MDR in HCC cells are shown in **Figure 2**.

## 5. Reversal of MDR in HCC cells through the inhibition of PI3K/Akt pathway

### 5.1. PI3K inhibitors

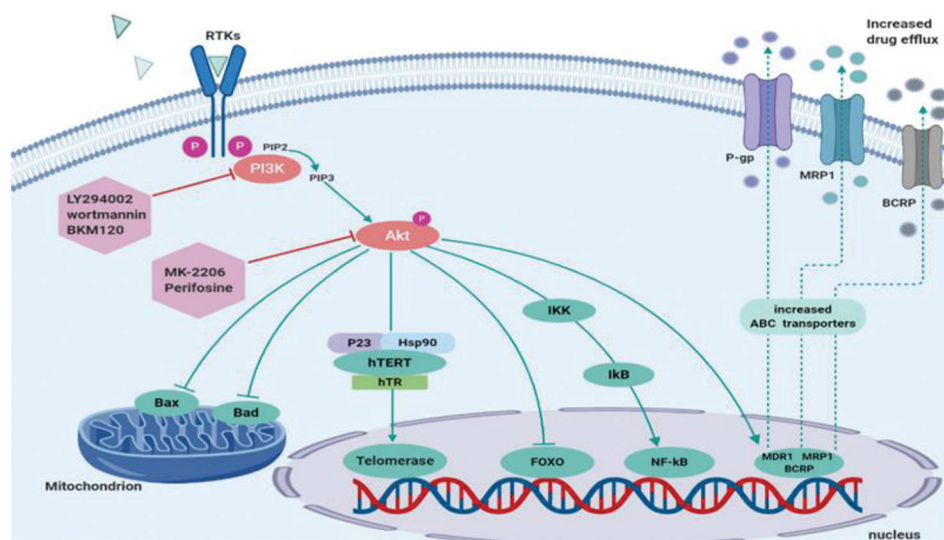
Wortmannin and LY294002 are pan-PI3K related inhibitors. The function of LY294002 is reversible, but the function of wortmannin is irreversible. Zhang *et al.* showed that LY294002 inhibited the activation of extracellular signal-regulated protein kinases (ERKs) and induced the expression of apoptotic proteins, including BAX, to reduce cell viability, migration and invasion ability of drug-resistant HCC cells, as well as decrease the drug resistance of HCC patients to sorafenib<sup>[74]</sup>. He *et al.* showed that the combined use of baicalein and LY294002 could regulate the PI3K/Akt signaling pathway, inhibit HCC cell proliferation, and promote apoptosis<sup>[75]</sup>. Sun *et al.* found that basic fibroblast growth factor could inhibit the apoptosis rate of HCC cells and reduce the cell number in G2/M phase of the cell cycle, whereas wortmannin blocked this function to induce HCC cell apoptosis<sup>[76]</sup>. In addition, the mRNA level of survivin also decreased.

BKM120 is a newly developed oral pan-class I PI3K inhibitor that is a 2,6-dimorpholino pyrimidine derivative. It selectively inhibits PI3K $\alpha$ , - $\beta$ , - $\gamma$ , and - $\delta$  as well as mutant PI3K but does not act on mTOR. BKM120 inhibits the phosphorylation of the PI3K target Akt and exhibits anti-proliferation activities in several tumor cell lines<sup>[77]</sup>. BKM120 blocks PI3K/Akt/NF- $\kappa$ B expression to activate caspase-3/7 and caspase-9 and alter the expression of several apoptosis-related genes, thus effectively eliminating the CSC subpopulation, reducing sphere formation by these drug-resistant cells and overcoming the MDR phenotype in chemoresistant tumor cells<sup>[78]</sup>.

### 5.2. Akt inhibitors

MK-2206 is an Akt allosteric inhibitor. When Akt is in an inactive state, the PH domain takes on the “PH-in” conformation. MK-2206 interacts with the allosteric site generated through the interaction between the PH domain and the kinase domain to lock Akt in the “PH-in” conformation and thereby inhibit Akt phosphorylation<sup>[20]</sup>. MK-2206 has exhibited excellent preclinical anti-tumor activity and is currently in the second phase of clinical evaluation<sup>[79]</sup>. Although the specific mechanism of the anti-tumor activity of MK-2206 is still unclear, MK-2206 can induce cell cycle arrest and apoptosis<sup>[80]</sup>. Simioni *et al.* found that MK-2206 downregulated the synergistic effect of Akt-1 and the phosphorylation levels of its downstream targets GSK 3 $\alpha/\beta$  and FOXO3a in a concentration-dependent manner; therefore, it is considered a potentially effective treatment for HCC<sup>[81]</sup>.

Perifosine is an alkylphosphocholine analog. It can target the PH domain of Akt and block its plasma membrane translocation to keep Akt in the cytoplasm, thereby blocking Akt phosphorylation and activation. It is a PH domain inhibitor<sup>[82]</sup>. Fei *et al.* confirmed that



**Figure 2.** Mechanisms by which the phosphatidylinositol-3-kinase/protein kinase pathway promotes multiple drug actions in hepatocellular carcinoma cells

perifosine inhibits HCC cell growth in a dose-dependent manner and causes cell cycle arrest at G2<sup>[83]</sup>. Combined use of perifosine and other drugs can increase drug sensitivity and reduce adverse effects of chemotherapeutic drugs such as cisplatin in HCC. Downregulation of Bcl-2 and upregulation of Bax through inhibition of Akt phosphorylation might be the underlying mechanism of this synergistic effect. Therefore, it is speculated that combined use of chemotherapeutic drugs and Akt inhibitors could improve the chemotherapy resistance of tumor cells to reduce adverse reactions caused by the use of single drugs.

## 6. Conclusions and perspectives

MDR, one of the causes of HCC chemotherapy failure, results from multiple factors, multiple mechanisms, and multiple protein interactions. Despite many years of research, the relevant mechanisms underlying MDR development in HCC are still not completely understood, and further in-depth studies are urgently needed. The PI3K/Akt pathway is closely associated with MDR development in HCC cells and thus, becomes an important target in HCC treatment. Some studies recently reported that alpha-fetoprotein (AFP), which has high expression in HCC cells, can interact with the tumor suppressor protein phosphatase and tensin homolog (PTEN) to block the inhibitory effect of PTEN on the PI3K/Akt signaling pathway<sup>[84-87]</sup>. On the account of the high expression of AFP in HCC cells, AFP is suspected to be the driver of MDR development in HCC cells. Therefore, in-depth studies targeting the association between AFP and PI3K/Akt can further elucidate how AFP regulates MDR development in HCC cells through the activation of the PI3K/Akt pathway. In addition, vaccines can be developed targeting AFP, and the PI3K/Akt-specific pathway inhibitors can be developed into novel anti-tumor drugs that offer alternative modes of treatment for effectively reversing MDR in HCC. Because the efficacy of single-pathway inhibitors is limited, multidrug combinations can be developed to block downstream signaling molecules of the PI3K/Akt signaling pathway to achieve better curative effects. Furthermore, the therapeutic model of the targeted blocking of signaling molecules can reduce toxic side effects in normal cells and help diminish the effect of PI3K/Akt signaling in HCC treatment.

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## Conflicts of interest

None to declare.

## Author contributions

M.L. and M.Z. conceived the idea of this review. C.Z. gathered data and information for this review. C.Z. wrote the manuscript. M.L. and M.Z. reviewed and edited the manuscript.

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