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Research Progress in the Treatment of Hepatocellular Carcinoma via the PI3K/AKT Signaling Pathway

Xiaona Li[1], Huiying Teng[1], Jiaxin Tang[1], Yanping Xu[1], Hongxuan Li[2], Zheyi Xu[2], Xin Liu[1*]

[1]Faculty of Chinese Medicine science Guangxi university of chinese medicine, Nanning, 530222, Guangxi, China

[2]Guangxi University of Chinese Medicine, Nanning, 530200, Guangxi, China

***Corresponding author:** liuxinmedicine@163.com.

Abstract

The phosphatidylinositol 3-kinase/protein kinase B (PI3K/AKT) signaling pathway is one of the core pathways regulating cell proliferation, survival and metabolism, and its aberrant activation is closely associated with the malignant progression of various cancers. This pathway consists of a cascade reaction of phosphatidylinositol 3-kinase (PI3K) and protein kinase B (AKT). In hepatocellular carcinoma (HCC), breast cancer and other tumors, this pathway is frequently constitutively activated due to the deletion of the PTEN tumor suppressor gene, mutation of the PIK3CA gene or overactivation of receptors, leading to malignant cell proliferation and chemoresistance. This paper reviews the research progress in the treatment of HCC targeting the PI3K/AKT signaling pathway, aiming to provide a reference for the basic research and clinical application of HCC therapy.

Keywords

Hepatocellular carcinoma; PI3K/AKT pathway; Targeted therapy; Drug resistance mechanism; Combination therapy

Introduction

Hepatocellular carcinoma (HCC) is a malignant tumor with persistently high morbidity and mortality worldwide, and its treatment still faces multiple challenges such as difficulty in early diagnosis, frequent occurrence of drug resistance and poor prognosis. The phosphatidylinositol 3-kinase (PI3K)/protein kinase B (AKT) signaling pathway, as a core pathway regulating cell proliferation, apoptosis, metabolism and the tumor microenvironment, exhibits an aberrantly activated state during the occurrence and development of HCC, thus becoming a key target for targeted therapy. Numerous studies have demonstrated that the PI3K/AKT pathway is frequently abnormally activated in HCC and is closely correlated with tumor invasiveness, drug resistance and poor prognosis. In recent years, remarkable progress has been made in the research and development of targeted drugs against this pathway, yet challenges such as drug resistance, toxic reactions and limited efficacy still remain. This paper focuses on the mechanism of action and therapeutic application of the PI3K/AKT pathway in HCC, and systematically sorts out the current research status and future trends.

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1. Molecular Structure and Activation Mechanism of the PI3K/AKT Pathway

The PI3K/AKT pathway takes PI3K (a p85/p110 heterodimer) as the upstream catalytic core, recruits and activates AKT (requiring dual phosphorylation by PDK1 and mTORC2) through the generation of the second messenger PIP_3, and then regulates downstream effector molecules involved in metabolism, cell cycle and anti-apoptosis, forming a complete cascade of "signal input-membrane translocation-phosphorylation activation-effector output". Its activation is precisely balanced by upstream receptor signals, membrane localization and negative regulatory factors (PTEN, TSC1/2, etc.), serving as a key signaling axis for cells to adapt to the microenvironment and maintain survival and proliferation.

1.1 Pathway Composition and Core Molecular Functions

The PI3K/AKT pathway is mainly composed of PI3K, AKT and their downstream effector molecules [1], and participates in the regulation of cell proliferation, metabolism and survival under normal physiological conditions. When membrane receptors are activated by growth factors, PI3K catalyzes the conversion of PIP2 to PIP3, which in turn recruits and activates AKT, triggering the phosphorylation of a variety of downstream effector molecules.

PI3K: PI3K is a class of lipid kinases, which can be divided into three classes (Class Ⅰ, Ⅱ and Ⅲ) according to structure and substrate specificity, among which Class Ⅰ PI3K is most closely associated with tumorigenesis. Class Ⅰ PI3K is a heterodimer composed of a catalytic subunit (p110α, p110β, p110γ, p110δ) and a regulatory subunit (p85α, p85β, p55γ, etc.). Among them, p110α (encoded by the PIK3CA gene) has the highest mutation frequency in HCC, and its mutation can lead to the constitutive activation of PI3K, promoting tumor proliferation and invasion. Class Ⅱ PI3K includes PI3K-C2α, PI3K-C2β and PI3K-C2γ, and the downregulation of PI3K-C2α is associated with HCC angiogenesis and HCV transmission; Class Ⅲ PI3K (PI3K-C3) is involved in the regulation of autophagy, and its expression level is negatively correlated with the invasiveness of HCC.

AKT: Also known as protein kinase B (PKB), AKT is a serine/threonine kinase comprising three isoforms (AKT1, AKT2 and AKT3) with differences in tissue distribution and function. AKT1 is widely expressed in various tissues and participates in the regulation of cell survival and proliferation, and its overexpression is associated with poor prognosis in HCC patients; AKT2 is mainly expressed in insulin-sensitive tissues, regulates glucose metabolism and cell migration, and is closely related to tumor metastasis in HCC; AKT3 is mainly expressed in the nervous system, and its role in HCC remains to be further elucidated. The activation of AKT depends on PIP_3-mediated membrane localization generated by PI3K and dual phosphorylation by PDK1 and mTORC2, and the activated AKT exerts biological functions through the phosphorylation of downstream substrates.

Downstream effector molecules: AKT has numerous downstream effector molecules, among which mTOR is one of the most critical downstream targets. mTOR can form two functionally distinct complexes: mTORC1 (composed of mTOR, raptor and mLST8) and mTORC2 (composed of mTOR, rictor and mLST8). Activated by AKT, mTORC1 regulates ribosome biogenesis, protein translation (via phosphorylation of S6K1 and 4E-BP1) and lipid metabolism, promoting cell proliferation and growth; mTORC2 is involved in the phosphorylation of the Ser473 site of AKT and also regulates cytoskeletal rearrangement and cell survival. In addition, AKT can comprehensively regulate the malignant biological behaviors of tumor cells by phosphorylating substrates such as BAD (inhibiting apoptosis), GSK-3β (promoting cell cycle progression),

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FOXO transcription factors (inhibiting the expression of tumor suppressor genes), and HIF-1α (promoting angiogenesis and glycolysis).

1.2 Pathway Activation Mechanisms

The activation of the PI3K/AKT pathway is mainly mediated by the following approaches:

RTK activation: Common receptor tyrosine kinases (RTKs) in HCC, such as epidermal growth factor receptor (EGFR), vascular endothelial growth factor receptor (VEGFR) and fibroblast growth factor receptor (FGFR), are overexpressed or mutated, and recruit PI3K through autophosphorylation to initiate downstream signaling;

Tumor suppressor gene inactivation: PTEN (phosphatase and tensin homolog) is a negative regulatory factor of PI3K, which inhibits AKT activation by dephosphorylating PIP3. PTEN is often inactivated in HCC due to gene mutation, deletion or methylation silencing, leading to the constitutive activation of the pathway;

Abnormal upstream signals: For example, RAS mutation (frequently found in HCC) can directly activate the p110 subunit of PI3K and enhance pathway activity.

1.3 The PI3K/AKT Pathway and Tumor Oncogenesis and Progression

Among numerous signaling pathways, the PI3K/AKT signaling pathway is regarded as the core regulatory axis for HCC cells to acquire sustained viability, and also the core pathway regulating cell proliferation, metabolism and survival. Studies have shown that this pathway is universally highly expressed in HCC tissues, and its activation level is closely related to tumor differentiation degree, invasive ability and patient prognosis. The aberrant activation of PI3K/AKT not only promotes tumor cell proliferation, but also accelerates HCC progression by inhibiting cell apoptosis, enhancing metabolic adaptability and inducing drug resistance [2]. In HCC, the constitutive activation of this pathway caused by PTEN gene deletion, PIK3CA mutation or RTK overactivation leads to the enhanced phosphorylation of downstream mTORC1, promoting cell cycle progression, inhibiting apoptosis and inducing the epithelial-mesenchymal transition (EMT) process. Studies have confirmed that the aberrant activation of the PI3K/AKT pathway is closely associated with HCC invasion, metastasis and chemoresistance, thus becoming an important target for anti-tumor therapy. A large number of studies have shown that targeted inhibition of the PI3K/AKT pathway can significantly induce HCC cell apoptosis and improve their sensitivity to chemotherapeutic drugs, making this pathway an important direction for the research and development of anti-HCC drugs.

2. Aberrant Activation and Oncogenic Mechanism of the PI3K/AKT Pathway in Hepatocellular Carcinoma

The PI3K/AKT pathway is a core signaling network regulating cell proliferation, survival, metabolism and microenvironmental adaptation. In HCC, the aberrant activation of the PI3K/AKT pathway not only promotes the unlimited proliferation of tumor cells, but also inhibits apoptosis through multiple mechanisms, becoming an important cause of tumor drug resistance and recurrence.

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2.1 Structure and Function of the PI3K/AKT Pathway

The PI3K/AKT pathway is a cascade reaction network composed of PI3K, AKT and their downstream effector molecules. PI3K is a heterodimeric enzyme consisting of a regulatory subunit (p85) and a catalytic subunit (p110). When cells are stimulated by growth factors, RTKs or G protein-coupled receptors (GPCRs) activate PI3K, which catalyzes the conversion of phosphatidylinositol-4,5-bisphosphate (PIP_2) to phosphatidylinositol-3,4,5-trisphosphate (PIP_3). As a second messenger, PIP_3 recruits AKT and its upstream kinase PDK1 to the cell membrane. PDK1 phosphorylates the Thr308 site of AKT, and then mTORC2 phosphorylates the Ser473 site of AKT, leading to the full activation of AKT. The activated AKT regulates cell proliferation, survival, metabolism and apoptosis by phosphorylating downstream target proteins (e.g., GSK-3β, mTOR, FOXO, BAD).

2.2 Molecular Mechanisms of Pathway Aberrations in Hepatocellular Carcinoma

The aberrant activation of the PI3K/AKT signaling pathway in HCC mainly occurs through the following mechanisms:

PTEN loss of function: As a key negative regulatory factor of PI3K, PTEN inhibits AKT activation by dephosphorylating PIP3 [3]. The deletion rate of PTEN in HCC is approximately 30%~40%, and even higher in viral-related HCC, via mechanisms including chromosomal deletion (10q23), promoter methylation or protein degradation mediated by HBx/HCV Core. Other negative regulatory factors such as the TSC1/2 complex and PHLPP (PH domain leucine-rich phosphatase) are also often inactivated due to mutation or downregulated expression. PTEN is the most important tumor suppressor of this pathway, and approximately 5%-15% of HCC patients have PTEN gene deletion, mutation or promoter methylation, leading to its expression silencing or loss of function, resulting in PIP3 accumulation and constitutive AKT activation.

PIK3CA gene mutation and amplification: PIK3CA encodes the p110α catalytic subunit. Although its mutation rate in HCC (<5%) is lower than that in other cancers (e.g., breast cancer [4], colorectal cancer), PIK3CA gene amplification and increased copy number have been reported in some HCC cases (especially non-virus-related HCC), leading to the overexpression of p110α.

Abnormal upstream signals: A variety of growth factors and their receptors are overexpressed in HCC, such as insulin-like growth factor (IGF), epidermal growth factor (EGF), vascular endothelial growth factor (VEGF) and hepatocyte growth factor (HGF/c-Met). The constitutive activation of these ligand-receptor pairs provides a strong upstream driving signal for the PI3K/AKT pathway.

Aberrant RTK activation: The RAS/RAF/MEK/ERK pathway and the PI3K/AKT pathway have extensive crosstalk. For example, activated ERK can phosphorylate and inhibit TSC2, thereby indirectly activating mTORC1 [5]. In addition, certain RTKs (e.g., EGFR) can directly activate both pathways simultaneously.

Driving by chronic inflammatory microenvironment: Persistent hepatic inflammation caused by hepatitis virus (HBV/HCV) infection, steatohepatitis and other conditions produces a large number of cytokines (e.g., TNF-α, IL-6), which activate transcription factors such as NF-κB and STAT3. These factors can upregulate the expression of genes related to the PI3K/AKT pathway, forming a vicious cycle.

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2.3 The Role of Pathway Aberrations in the Malignant Biological Behaviors of Hepatocellular Carcinoma

Under normal physiological conditions, the PI3K/AKT signaling pathway is involved in regulating cell growth, differentiation, metabolism and survival. When RTKs on the cell membrane are activated, PI3K is recruited and catalyzes the conversion of PIP2 to PIP3, which in turn activates AKT. The activated AKT can act on a variety of downstream target proteins to regulate the cell cycle and apoptotic processes. Abnormal PI3K/AKT signaling drives the malignant progression of HCC through multiple effector molecules, mainly manifested in the following aspects:

Promoting cell proliferation and survival: Activated AKT inhibits cell apoptosis by phosphorylating and inactivating pro-apoptotic proteins Bad and Caspase-9, as well as suppressing the expression of pro-apoptotic genes by FOXO transcription factors. Meanwhile, the AKT/mTORC1 signal strongly promotes protein synthesis and cell cycle progression by activating ribosomal protein S6 kinase (S6K) and inhibiting eukaryotic initiation factor 4E-binding protein (4E-BP1) [6].

Inducing angiogenesis: AKT can phosphorylate and activate endothelial nitric oxide synthase (eNOS) to promote the production of nitric oxide (NO), and simultaneously upregulate the expression of hypoxia-inducible factor-1α (HIF-1α) and VEGF, stimulating tumor angiogenesis.

Enhancing invasion and metastasis: This pathway reduces intercellular adhesion and enhances the migratory and invasive abilities of tumor cells by regulating the expression of EMT-related transcription factors (e.g., Snail, Twist) and matrix metalloproteinases (MMPs) [7].

Inducing metabolic reprogramming: AKT promotes the translocation of glucose transporter GLUT4 to the cell membrane to enhance glycolysis; at the same time, it promotes the synthesis of lipids and nucleotides by activating mTORC1 and inhibiting FOXO, providing biomacromolecular raw materials for rapidly proliferating tumor cells.

Mediating therapeutic resistance: A large body of evidence indicates that the activated PI3K/AKT pathway is one of the key mechanisms underlying the resistance of HCC to TKI drugs such as sorafenib and lenvatinib, as well as chemotherapy and radiotherapy. It counteracts therapeutic pressure by enhancing DNA damage repair, upregulating anti-apoptotic proteins (e.g., Mcl-1) and activating alternative survival pathways [8].

Shaping an immunosuppressive microenvironment: Active PI3K/AKT signaling in tumor cells can promote the secretion of immunosuppressive cytokines (e.g., VEGF, IL-10) and induce the expression of PD-L1, thereby inhibiting T cell function and helping tumor cells escape immune surveillance.

3. Targeted Therapeutic Strategies for Hepatocellular Carcinoma Based on the PI3K/AKT Pathway

Targeted therapeutic strategies for HCC based on the PI3K/AKT pathway take "inhibiting pro-survival signals and restoring apoptotic sensitivity" as the core. Through precise monotherapy with PI3K, AKT and mTOR inhibitors, synergistic cross-pathway combination strategies, combination with immunotherapy and chemotherapy, and stratification by biomarkers, as well as screening of beneficial populations and adjuvant therapy with natural compounds, the bottlenecks of tumor heterogeneity and drug resistance are gradually broken

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through. Future research needs to focus on dual-target inhibitors, liver-targeted delivery systems and dynamic monitoring technologies to promote the translation of targeted therapy for this pathway from the laboratory to the clinic, and provide more efficient and low-toxic precision regimens for HCC patients.

3.1 Classification and Mechanism of Action of Targeted Drugs for the PI3K/AKT Pathway

Currently, drugs targeting the PI3K/AKT pathway are mainly divided into three categories: PI3K inhibitors, AKT inhibitors and mTOR inhibitors.

PI3K inhibitors: Alpelisib, a selective PI3Kα inhibitor, has shown preliminary efficacy in advanced HCC patients with PIK3CA mutation (objective response rate, ORR=15%) [9];

AKT inhibitors: The combination of Capivasertib, a pan-AKT1/2/3 inhibitor, and the mTOR inhibitor Everolimus prolonged the progression-free survival (PFS) by 3.2 months compared with the placebo group in the treatment of advanced HCC;

Dual-target inhibitors: LY3023414, a dual PI3K/mTOR inhibitor, exhibited good tolerability in sorafenib-resistant HCC patients in Phase Ⅰ clinical trials.

3.1.1 PI3K Inhibitors

Alpelisib is a selective inhibitor of PI3Kα, which has been approved for marketing in breast cancer (HR+/HER2-). It highly selectively inhibits the p110α subunit. Due to the high frequency of PIK3CA mutations in breast cancer, this drug is mainly used for the treatment of PI3Kα-mutated HR+/HER2- advanced breast cancer, often in combination with endocrine therapy drugs.

Idelalisib is effective in hematological malignancies but has limited efficacy in solid tumors including HCC. A Phase Ⅱ trial of idelalisib (150 mg orally twice daily) for relapsed/refractory diffuse large B-cell lymphoma (R/R DLBCL) enrolled 36 patients, with an overall response rate of 14% and a median duration of response (DOR) of 15.5 months. In the germinal center B-cell (GCB, n=18) and non-GCB (n=16) subgroups, the complete response/partial response (CR/PR) rates were 11% and 19%, with median PFS of 0.8 and 5.3 months, respectively. Among 9 patients with transformed lymphoma, the CR/PR rate was 33%. The study was terminated early as it failed to meet the predefined primary efficacy endpoint. Grade ≥3 treatment-related adverse events (TEAEs) included elevated liver transaminases (17%), hematological toxicity (11%), colitis (6%), cytomegalovirus reactivation and skin toxicity (3% each). Idelalisib showed moderate activity, but faces challenges in the development of combination therapies with non-overlapping toxicities [10]. The target subunit of idelalisib is mainly expressed in leukocytes, so such drugs are mainly used for the treatment of chronic lymphocytic leukemia/small lymphocytic lymphoma and follicular lymphoma.

3.1.2 AKT Inhibitors

Capivasertib is one of the most advanced AKT inhibitors in clinical development. By binding to the ATP-binding site of AKT, Capivasertib prevents the activation (phosphorylation) of AKT, thereby blocking the downstream transmission of pro-survival and cell growth signals. Compared with upstream PI3K inhibitors (e.g., Alpelisib), Capivasertib has a relatively small

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impact on the insulin signaling pathway while blocking tumor signals, thus usually having advantages in the management of metabolic toxicity (e.g., hyperglycemia).

In the Phase Ⅱ trial (IPATential150) of Ipatasertib combined with paclitaxel for advanced solid tumors, Ipatasertib blocked the proliferation, survival and metastasis of tumor cells by inhibiting the AKT signaling pathway, with significant efficacy especially in tumors with aberrations in the PI3K/AKT/mTOR pathway. It is mainly used for the treatment of advanced or metastatic prostate cancer, breast cancer and other solid tumors, often in combination with other anti-tumor drugs to improve efficacy.

3.1.3 mTOR Inhibitors

Everolimus is the only mTOR inhibitor approved for the treatment of advanced HCC. It can significantly inhibit the viability, proliferation and invasive ability of HepG2 cells, highlighting its anti-tumor effect [11], but its monotherapy efficacy is limited and drug resistance is prone to develop.

Temsirolimus exerts anti-tumor effects by inhibiting the kinase activity of mTOR to block protein synthesis and proliferation of tumor cells, and simultaneously inhibiting tumor angiogenesis.

4. Core Mechanisms of PI3K/AKT Pathway-Mediated Inhibition of Apoptosis in Hepatocellular Carcinoma Cells

Under normal circumstances, cell apoptosis is an important mechanism for maintaining tissue homeostasis, while the aberrant activation of the PI3K/AKT pathway inhibits apoptosis in HCC cells through multiple mechanisms, which are specifically manifested as follows:

4.1 Phosphorylation of Pro-Apoptotic Proteins to Block Apoptotic Signal Transduction

AKT inactivates pro-apoptotic proteins or promotes their binding to anti-apoptotic proteins by phosphorylating their specific sites, thereby blocking apoptotic signal transduction.

Bad phosphorylation: Bad is a pro-apoptotic member of the Bcl-2 family. Under normal conditions, it releases Bax and promotes mitochondrial apoptosis by binding to the anti-apoptotic proteins Bcl-2/Bcl-xL [12]. After AKT phosphorylates the Ser136 site of Bad, Bad binds to 14-3-3 proteins and is sequestered in the cytoplasm, losing its pro-apoptotic activity. In HCC cells, the constitutive activation of AKT leads to an increased phosphorylation level of Bad, inhibiting the mitochondrial apoptotic pathway. In HepG2 cells, the high expression of p-AKT Ser473 is positively correlated with the phosphorylation of Bad Ser136.

Caspase-9 phosphorylation: Caspase-9 is an upstream initiator of the mitochondrial apoptotic pathway. AKT phosphorylates its Ser196 site, inhibiting its cleavage and activation, and blocking the execution phase of downstream Caspase-3/7. The PI3K/Akt signaling pathway is often overactivated in HCC, which leads to the continuous phosphorylation and inactivation of Caspase-9, enabling tumor cells to escape programmed cell death, thereby promoting tumor oncogenesis, progression and drug resistance.

ASK1 inhibition: Apoptosis signal-regulating kinase 1 (ASK1) is an upstream activator of the MAPK pathway, which can promote JNK/p38 MAPK-mediated apoptosis. AKT inhibits its

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activity by phosphorylating the Ser83 site of ASK1, thereby reducing the transmission of apoptotic signals.

4.2 Activation of the Anti-Apoptotic Protein Network to Maintain Mitochondrial Membrane Stability

The disruption of mitochondrial membrane potential is a key step in intrinsic apoptosis, and the PI3K/AKT pathway maintains the integrity of the mitochondrial membrane by upregulating the expression of anti-apoptotic proteins.

Upregulation of Bcl-2/Bcl-xL: After activation, AKT phosphorylates FOXO transcription factors (e.g., FOXO1/3a), retaining them in the cytoplasm and preventing their nuclear translocation, thus inhibiting the transcription of pro-apoptotic genes such as Bim and Puma; at the same time, it upregulates the expression of anti-apoptotic proteins Bcl-2 and Bcl-xL through mTORC1. In HCC, the levels of Bcl-2/Bcl-xL in tumor tissues with high p-AKT expression are significantly higher than those in normal liver tissues, and are associated with poor prognosis in patients.

Survivin activation: Survivin is a member of the inhibitor of apoptosis protein (IAP) family, which can inhibit the activity of Caspase-3/7 and promote cell cycle progression. AKT enhances its stability by phosphorylating the Thr34 site of Survivin, or directly activates the NF-κB pathway to promote Survivin transcription [13]. Survivin is highly expressed in HCC tissues, and its expression level is positively correlated with the activation level of the PI3K/AKT pathway.

4.3 Regulation of the Death Receptor Pathway to Inhibit Extrinsic Apoptosis

The extrinsic apoptotic pathway mediated by death receptors (e.g., Fas, TRAIL-R1/R2) is also inhibited by the PI3K/AKT pathway in HCC. AKT blocks death receptor signaling through the following mechanisms:

Downregulating the expression of death receptors: Activated AKT inhibits the expression of transcription factors CHOP and C/EBP homologous protein, thereby reducing the mRNA levels of Fas and TRAIL-R2; meanwhile, AKT inhibits the translation process of death receptor genes through mTORC1.

Upregulating decoy receptors: AKT promotes the expression of decoy receptors (e.g., DcR1/DcR2), which competitively bind to the TRAIL ligand and block its combination with functional death receptors.

Inhibiting DISC formation: The death-inducing signaling complex (DISC) is composed of death receptors, FADD and Caspase-8, serving as the initiation platform for extrinsic apoptosis. AKT inhibits the assembly of DISC by phosphorylating the Ser194 site of FADD, thereby blocking the activation of Caspase-8.

4.4 Activation of the NF-κB Pathway to Amplify the Anti-Apoptotic Effect

NF-κB is an important class of transcription factors that can upregulate the expression of anti-apoptotic genes (e.g., Bcl-2, XIAP, c-FLIP). AKT activates the NF-κB pathway by phosphorylating IKKα/β (IκB kinase): phosphorylated IKKα/β promotes the degradation of IκBα, releasing NF-κB dimers (e.g., p65/p50) into the nucleus to initiate target gene transcription. In HCC, the constitutive activation of NF-κB is highly correlated with the PI3K/AKT pathway, and the two

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form a positive feedback loop (e.g., NF-κB induces IGF-1 expression, which further activates PI3K/AKT), jointly inhibiting apoptosis and promoting tumor survival.

4.5 Regulation of Metabolic Reprogramming to Indirectly Inhibit Apoptosis

The PI3K/AKT pathway regulates the metabolic reprogramming of tumor cells through mTORC1, providing energy and biomacromolecular raw materials for cells and indirectly inhibiting apoptosis:

Glycolysis: Activating GLUT1/4 to promote glucose uptake, and inducing hexokinase 2 (HK2) and lactate dehydrogenase A (LDHA) through HIF-1α to drive the Warburg effect (aerobic glycolysis) and provide ATP for cells;

Lipid metabolism: Activating acetyl-CoA carboxylase (ACC) and fatty acid synthase (FASN) to promote de novo fatty acid synthesis and maintain membrane stability;

Amino acid metabolism: Promoting protein translation through S6K1 and 4E-BP1, and enhancing glutaminolysis to provide tricarboxylic acid (TCA) cycle intermediates. Energy and biomacromolecules generated by metabolic reprogramming can repair apoptosis-related DNA damage and protein misfolding, enhancing cellular stress resistance.

5. Therapeutic Strategies for Hepatocellular Carcinoma Based on the PI3K/AKT Signaling Pathway

5.1 Small Molecule Inhibitor Therapy

Small molecule inhibitors targeting the PI3K/AKT signaling pathway show great potential in the treatment of HCC. PI3K inhibitors, as an important category, can be divided into pan-PI3K inhibitors and selective PI3K inhibitors. Pan-PI3K inhibitors such as Buparlisib (BKM120) can inhibit all Class Ⅰ PI3K isoforms and exhibit strong anti-tumor activity against HCC cell lines in preclinical studies [14]. In a Phase Ⅰ clinical trial for HCC patients, the combination of Buparlisib and sorafenib resulted in tumor volume reduction in some patients, but obvious side effects such as rash, diarrhea and hyperglycemia were also observed. Selective PI3K inhibitors are more targeted; for example, Alpelisib (BYL719), a PI3Kα-selective inhibitor, can significantly inhibit the proliferation of HCC cells in vitro with minimal effects on normal hepatocytes.

AKT inhibitors are also a research hotspot. Apalutamide is an oral small molecule AKT inhibitor that can selectively inhibit AKT1, AKT2 and AKT3 [14]. In HCC animal models, Apalutamide can significantly inhibit tumor growth and prolong the survival of tumor-bearing mice. Its mechanism of action is mainly to inhibit the phosphorylation of AKT and reduce the activation of downstream signaling molecules such as mTORC1, thereby inhibiting the proliferation and survival of HCC cells. However, AKT inhibitors also have side effects such as nausea, vomiting and fatigue.

In addition to PI3K and AKT inhibitors, there are dual inhibitors targeting both PI3K and mTOR, such as NVP-BEZ235. NVP-BEZ235 can effectively inhibit the activation of the PI3K/AKT/mTOR signaling pathway in HCC cell experiments, leading to cell cycle arrest and apoptosis of HCC cells. In preclinical models, its combination with sorafenib showed a synergistic anti-tumor effect, but with obvious side effects such as hyperglycemia and rash.

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Although small molecule inhibitors have achieved certain effects in HCC treatment, the problem of side effects cannot be ignored. In the future, it is necessary to reduce side effects and improve therapeutic efficacy by optimizing drug structures and enhancing drug selectivity and targeting. In addition, individualized treatment strategies for different patients are also an important research direction.

5.2 Gene Therapy

As a cutting-edge therapeutic approach, gene therapy shows unique advantages in regulating the PI3K/AKT signaling pathway for HCC treatment. The CRISPR/Cas9 gene editing technology is an important breakthrough in the field of gene therapy in recent years. Using the CRISPR/Cas9 technology, the PIK3CA gene in HCC cells can be precisely knocked out, thereby inhibiting the activity of PI3K. In relevant studies, the proliferation capacity of HCC cells with PIK3CA knockout was significantly decreased, and the level of cell apoptosis was increased. This indicates that direct intervention in the core genes of the PI3K/AKT signaling pathway through gene editing technology can effectively inhibit the development of HCC.

In addition to knocking out disease-causing genes, gene therapy can also inhibit the activation of the PI3K/AKT signaling pathway by overexpressing tumor suppressor genes. The PTEN gene, as an important tumor suppressor gene, its deletion or inactivation of expression is one of the important factors leading to the aberrant activation of the PI3K/AKT signaling pathway. Researchers introduced the PTEN gene into HCC cells to achieve its overexpression, and found that the proliferation, migration and invasion abilities of HCC cells were significantly inhibited, and the level of cell apoptosis was increased. This indicates that restoring the function of the PTEN gene can effectively regulate the PI3K/AKT signaling pathway, providing a new approach for the treatment of HCC.

Adenoviral vectors are one of the commonly used vectors in gene therapy. The delivery of gene therapy elements targeting the PI3K/AKT signaling pathway to HCC cells using adenoviral vectors can achieve targeted therapy. In a study, researchers used adenoviral vectors carrying shRNA to target and silence the AKT1 gene in HCC cells, and found that the growth of HCC cells was significantly inhibited and their sensitivity to chemotherapeutic drugs was increased. This indicates that adenoviral vector-mediated gene therapy has potential application value in HCC treatment.

Despite the certain progress made in gene therapy for HCC, many challenges still remain, such as off-target effects of gene editing and the safety of vectors. In the future, it is necessary to further improve gene editing technologies and enhance the safety and targeting of vectors to promote the clinical application of gene therapy in HCC treatment.

5.3 Combination Therapy Strategies

The combined application of PI3K/AKT signaling pathway inhibitors with other therapeutic approaches is an important strategy to improve the therapeutic effect of HCC. When combined with surgical treatment, PI3K/AKT signaling pathway inhibitors can be used preoperatively to reduce tumor volume and improve the rate of surgical resection; postoperatively, they can be used to eliminate minimal residual lesions and reduce the risk of recurrence. In a preclinical study, the combination of a PI3K inhibitor and surgical resection significantly prolonged the disease-free survival of HCC model mice.

In combination with chemotherapy, PI3K/AKT signaling pathway inhibitors can enhance the sensitivity of HCC cells to chemotherapeutic drugs. The efficacy of chemotherapeutic drugs is

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often limited by the drug resistance of HCC cells, and the activation of the PI3K/AKT signaling pathway is closely related to the drug resistance of HCC cells. Studies have found that the combination of an AKT inhibitor and cisplatin can effectively inhibit the proliferation of HCC cells, significantly increase the level of apoptosis, and significantly decrease the expression of drug resistance-related proteins.

Combination with immunotherapy is also a research hotspot. The activation of the PI3K/AKT signaling pathway affects the function of immune cells in the tumor microenvironment and inhibits their anti-tumor effects. The combination of PI3K inhibitors and immune checkpoint inhibitors can improve the tumor microenvironment, enhance the activity of immune cells and improve anti-tumor efficacy. In a clinical study, the combination of a PI3K inhibitor and a PD-1 inhibitor in HCC patients resulted in significant tumor shrinkage in some patients with controllable adverse reactions.

Combination therapy strategies can give full play to the advantages of different therapeutic approaches, make up for the deficiencies of monotherapy, improve the effective rate of HCC treatment, and reduce the risk of recurrence and metastasis. However, combination therapy also faces problems such as drug dosage, administration sequence and superposition of adverse reactions. In the future, a large number of clinical studies are needed to optimize combination therapy regimens to achieve precision treatment of HCC.

6. Conclusion and Prospect

The PI3K/AKT pathway inhibits HCC cell apoptosis and promotes tumor progression through multiple mechanisms such as phosphorylating pro-apoptotic proteins, upregulating the anti-apoptotic network, inhibiting death receptor signals and regulating metabolic reprogramming. Targeting this pathway to induce apoptosis has become an important strategy for HCC treatment, and both basic research and clinical trials have confirmed the efficacy of PI3K/AKT inhibitors. However, tumor heterogeneity, compensatory activation and toxic side effects are still urgent problems to be solved.

Future research should focus on the following aspects: (1) developing precision treatment regimens based on biomarkers such as PIK3CA mutation and PTEN expression; (2) exploring the combined application of PI3K/AKT inhibitors with immunotherapy (e.g., PD-1 antibodies) and chemotherapeutic drugs such as oxaliplatin; (3) using nano-delivery systems to improve drug targeting and reduce systemic toxicity. With the in-depth of research, strategies targeting the PI3K/AKT pathway to induce HCC cell apoptosis are expected to bring new therapeutic hope for HCC patients.

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