


# Role of the HIF-1 $\alpha$ /BNIP3 Signaling Pathway in Recurrent Hepatocellular Carcinoma and the Mechanism of Traditional Chinese Medicine

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**Abstract:** Recurrence of hepatocellular carcinoma (HCC) negatively affects the quality of life of patients and leads to death. Studies have shown that recurrent hepatocellular carcinoma (RHCC) is closely related to tissue hypoxia and autophagy. It has been shown that hypoxia-inducible factor-1 $\alpha$  (HIF-1 $\alpha$ ) and its downstream factor BCL-2 19 kDa-interacting protein 3 (BNIP3) promote cellular autophagy under hypoxic conditions, resulting in metastasis and RHCC. In this article, the molecular structures of HIF-1 $\alpha$  and BNIP3 are described, and the significance of the HIF-1 $\alpha$ /BNIP3 signaling pathway in RHCC is explained. Moreover, the role and mechanism of traditional Chinese medicine (TCM) in treating RHCC by modulating the HIF-1 $\alpha$ /BNIP3 signaling pathway is discussed. Studies have shown that the HIF-1 $\alpha$ /BNIP3 signaling pathway is a potential target of TCM in the treatment of RHCC. The mechanism of the HIF-1 $\alpha$ /BNIP3 signaling pathway in RHCC and the progress achieved in TCM research on targeting and regulating this pathway are also reviewed in this article. The objective was to provide a theoretical basis for the prevention and treatment of RHCC, as well as further drug development.

**Keywords:** recurrent hepatocellular carcinoma, HIF-1 $\alpha$ /BNIP3 signaling pathway, traditional Chinese medicine, hypoxia, autophagy

## Introduction

Hepatocellular carcinoma (HCC) is the seventh most common type of cancer and the second leading cause of cancer-related mortality worldwide.<sup>1</sup> In China, >300,000 individuals expire due to HCC annually, accounting for approximately half of the liver cancer-related deaths globally.<sup>2</sup> Hepatitis B infection remains the main cause of HCC globally, while hepatitis C infection and nonalcoholic steatohepatitis associated with a high-fat diet are the major causes of HCC in Western countries.<sup>3</sup> Alcohol dependence, smoking, old age, obesity, insulin resistance, and type 2 diabetes are also risk factors for HCC.<sup>4</sup> The invasive potential, metastasis, lower surgical resection rate, higher recurrence rate, and insensitivity to chemotherapy are fundamental causes of the higher mortality rate linked to HCC compared with other types of cancer.<sup>5</sup> The higher recurrence rate of HCC after surgery is the main reason affecting the long-term survival rate of patients. Patients with HCC are at a 60–70% risk of recurrence after radical liver cancer surgery, and the 5-year survival rate after surgery is <30%.<sup>6</sup> Multiple factors influence the development of postsurgical recurrent hepatocellular carcinoma (RHCC). Oxidative stress is an important factor in RHCC.<sup>7</sup> Intratumorally hypoxia is a crucial feature in all solid tumors, particularly HCC.<sup>8</sup> The tumor hypoxic microenvironment is able to induce cascading metabolic changes in hypoxia-adapted HCC cells through hypoxia-inducible factor (HIF).<sup>9</sup> Alpha-functional subunit of HIF (HIF-1 $\alpha$ ) is a major transcription factor in the hypoxic response and able to activate the transcription of the gene encoding BCL-2 19 kDa-interacting protein 3 (BNIP3), thereby inducing mitochondrial selective autophagy.<sup>10</sup> BNIP3 is a stress sensor protein strongly induced by hypoxia. BNIP3 containing hypoxia-responsive element (HRE) is a direct target of HIF-1 $\alpha$  and implicated in hypoxia-induced cell death.<sup>11,12</sup> In this review, the currently available data on the interaction between HIF-1 $\alpha$  and BNIP3 in the development and progression of RHCC are discussed. In addition, the role of the HIF-1 $\alpha$ /

BNIP3 signaling pathway in RHCC and the progress achieved in traditional Chinese medicine (TCM) research on targeting and regulating this pathway are also reviewed.

## Biological Properties of HIF-1 $\alpha$ /BNIP3

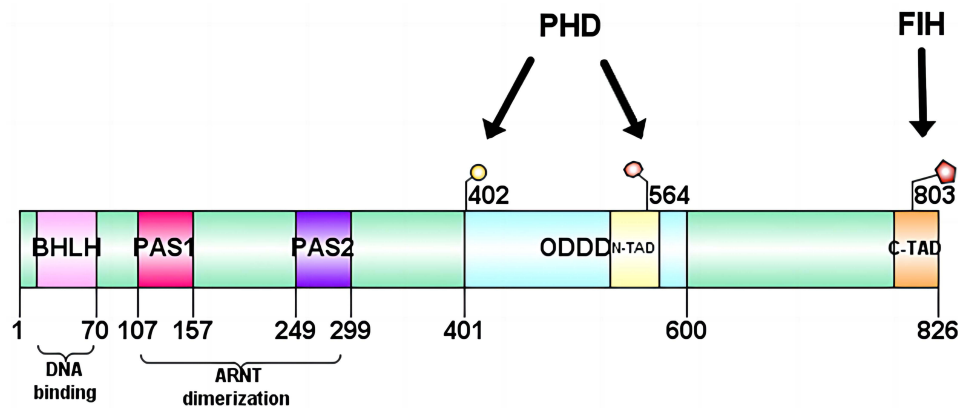
### HIF-1 $\alpha$ Structure and Characteristics

HIF-1 is an oligonucleotide sequence of a protein specifically bound to the erythropoietin gene enhancer. It was found in nuclear extracts of hypoxic HCC cell line Hep3B by Semenza et al in 1992.<sup>13</sup> HIF-1 is a member of the basic helix-loop-helix–PER-ARNT-SIM (bHLH–PAS) protein family and composed of  $\alpha$  and  $\beta$  subunits.<sup>14</sup> The bHLH–PAS is a class of dimeric transcription factors with diverse functions, that are highly conserved in vertebrates and invertebrates.<sup>15</sup> These proteins exhibit a relatively conserved structural domain structure, while sharing a common arrangement of structural domains: a bHLH DNA-binding domain; tandemly positioned PAS domains (PAS-A and PAS-B); and a variable trans-activating domain (TAD) or trans-repressive domain (TRD). bHLH domains are usually located at the N terminus of the protein and are responsible for DNA binding and dimerization by two alpha helices.<sup>16</sup> This is followed by a PAS structural domain comprising two structurally conserved regions.<sup>17</sup>

The  $\alpha$ -subunit is the functional subunit of HIF-1; it is sensitive to the concentration of oxygen and determines the activity of HIF-1. HIF-1 $\alpha$  is a 120-kDa polypeptide subunit containing an 826 amino acid oxygen-regulated polypeptide. The gene is located at 14q21-24 and has a DNA sequence of 3958 bp in length.<sup>18,19</sup> HIF-1 $\alpha$  contains four structural domains: bHLH; PAS; oxygen-dependent degradation domain (ODDD); and TAD. TAD consists of a transcriptional activation domain with the n-terminal transcriptional activation domain (N-TAD) and a c-terminal transcriptional activation domain (C-TAD), where the N-TAD partially overlaps with the ODDD. The bHLH is a structural domain of HIF-1 that heterodimerizes and binds to DNA, containing arginine and lysine residues. It is a structural domain of HIF-1 that activates gene transcription, particularly for genes involved in angiogenesis and epithelial–mesenchymal transition (EMT), by binding to HRE sequences in the promoters of different genes. PAS is a target gene-specific structural domain that mediates interprotein interactions upon stimulation or binds cofactors in its hydrophobic core to regulate interprotein interactions. The PAS structural domain consists of two very conserved hydrophobic repeats, termed PAS-A and PAS-B, spaced by a less conserved sequence. Under hypoxic conditions, the PAS-B structural domain of HIF-1 $\alpha$  heterodimerizes with PAS-B of ARNT. Notably, PAS-B is involved in transcriptional activation via a basic helix-loop-helix. ODDD is mainly associated with the ubiquitin-proteasome degradation pathway, and the region between the two TAD sequences is a repressive structural domain that mainly inhibits the transcriptional activity of TAD. The remaining regulatory regions include the nitrogen- and carbon-terminated nuclear localization signaling domains, proline-serine-threonine-rich protein stabilization domain, and the transcriptional repressor region.<sup>20</sup> The structure of HIF-1 $\alpha$  is shown in Figure 1.

### BNIP3 Structure and Characteristics

BNIP3 is a stress sensor protein strongly induced by hypoxia and associated with autophagy and apoptosis.<sup>21</sup> It mediates apoptosis in a cysteine-dependent or cysteine-independent manner and promotes autophagy causing cell death through various mechanisms in different cells. BNIP3 belongs to the BCL-2 family and contains a BCL-2 homology structural domain 3 (BH3). The BNIP3 protein consists of 194 amino acids, has a predicted molecular weight of 21.5 kDa, is located at locus 10q26.3, and comprises six exons of approximately 15 kilobases in size.<sup>22</sup> It consists of four major structural domains: proline-glutamic acid-serine-threonine-rich structural domain; BH3 structural domain; conserved structural domain; and transmembrane (TM) structural domain. The BH3 structural domain in BNIP3 is a functional structural domain of BNIP3, which forms a heterodimer with the anti-apoptotic molecules BCL2 and BCL-XL, further enabling the freeing and activation of BCL2 associated X (BAX) and BCL2 antagonist/killer (BAK). BAX and BAK are key regulators of the apoptotic mitochondrial pathway, prompting the release of cytochrome C from mitochondria and activating the caspase 9-dependent apoptosis pathway. The BH3 structural domain plays a dominant role in the caspase-dependent mitochondrial apoptotic pathway mediated by BNIP3. However, it is not required for the induction of cell death. Unlike deletion of the BH3 domain, deletion of the TM domain prevents BNIP3-induced cell death. The TM



**Figure 1** Structure of HIF-1 $\alpha$ . HIF-1 $\alpha$  contains four structural domains: bHLH, PAS, ODDD and TAD. The TAD in turn consists of a transcriptional activation domain with the N-TAD and a C-TAD, where the N-TAD partially overlaps with the ODDD.

**Abbreviations:** HIF-1 $\alpha$ , hypoxia-inducible factor-1 $\alpha$ ; bHLH - PAS, basic helix - loop - helix - PER - ARNT - SIM; ODDD, oxygen-dependent degradation; TAD, trans-activating domain; N-TAD, n-terminal transcriptional activation domain; C-TAD, c-terminal transcriptional activation domain.



**Figure 2** Structure of BNIP3. BNIP3 consists of four major structural domains: the PEST, the BH3, the CD, and the TM structural domain.

**Abbreviations:** BNIP3, BCL-2 19 kDa-interacting protein 3; BH3, Bcl-2 homology 3; CD, conserved structural domain; TM, transmembrane structural domain.

domain is required for dimerization, mitochondrial targeting, and pro-apoptotic activity, and plays a role in the promotion of cell death by BNIP3. When the cell receives foreign apoptotic signals or introduces exogenous BNIP3, BNIP3 forms dimers through the TM domain and is dispersed on the outer mitochondrial membrane surface. After binding tightly to the outer mitochondrial membrane, it opens the mitochondrial permeability transition pore (MPTP). This leads to mitochondrial depolarization and a decrease in the proton electrochemical gradient, followed by chromosome condensation and DNA breakage. This process can be blocked by MPTP inhibitors; however, caspase inhibitors are unable to inhibit the apoptotic process.<sup>23</sup> The structure of BNIP3 is illustrated in Figure 2.

## Transduction of the HIF-1 $\alpha$ /BNIP3 Signaling Pathway

Hypoxia-mediated apoptosis is closely associated with the induction of pro-death BCL-2 family proteins through stimulation of HIF-1.<sup>24</sup> BNIP3, which belongs to the BCL-2 family, is a downstream factor of HIF-1 $\alpha$ . In vivo and in vitro experiments have shown that it is associated with the initiation of autophagy. BNIP3 is a target molecule of HIF-1 $\alpha$  and can be induced by hypoxia or ischemia.<sup>25</sup> Under normoxic conditions, HIF-1 $\alpha$  is rapidly hydrolyzed by the hypoxia-inducible factor proline hydroxylase (HPH). Following a decrease in oxygen concentration, HPH is inactivated and HIF-1 $\alpha$  expression is increased. The bHLH structural domain in HIF-1 $\alpha$  is able to bind HRE sequences in different gene promoters. This structure is an important target for the transcriptional regulation of HIF-1.<sup>26</sup> The BNIP3 promoter region contains two HREs. HIF-1 $\alpha$  binds tightly to HRE2 under hypoxic conditions, thereby stimulating BNIP3 expression.<sup>27</sup> Zhang et al<sup>28</sup> determined the area of myocardial tissue damage in rats after coronary artery ligation by hematoxylin-eosin staining, the cell viability level of myocardial tissue by the 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium (MTS) method, and the expression of HIF-1 $\alpha$  and BNIP3 proteins in rat myocardial tissue by Western blotting (WB). It was confirmed that the HIF-1 $\alpha$ /BNIP3 pathway could activate mitochondrial autophagy and cause myocardial injury. He et al<sup>29</sup> determined the expression levels of HIF-1 $\alpha$  and BNIP3 in osteosarcoma cells by co-immunoprecipitation combined with WB. A tibial in situ operating system model

was also established in nude mice, and the survival rate was recorded. Moreover, the number of lung lymph nodes in mice was counted by microscopic observation. It was shown that the HIF-1 $\alpha$ /BNIP3 pathway inhibits the growth and metastasis of osteosarcoma through the downregulation of  $\beta$ -linked protein. HIF-1 $\alpha$  may maintain cell survival by activating the downstream protein BNIP3 and, subsequently, inducing mitochondrial autophagy. It has been shown that HIF-1 $\alpha$  activates a functional hypoxia response element (HRE) within the BNIP3 promoter. In hypoxia, the proteasomal degradation of HIF-1 $\alpha$  is reduced, leading to the accumulation and translocation of HIF-1 $\alpha$  to the nucleus. In the nucleus, HIF-1 $\alpha$  binds to the HRE within the BNIP3 promoter to activate the transcription of pro-death target genes.

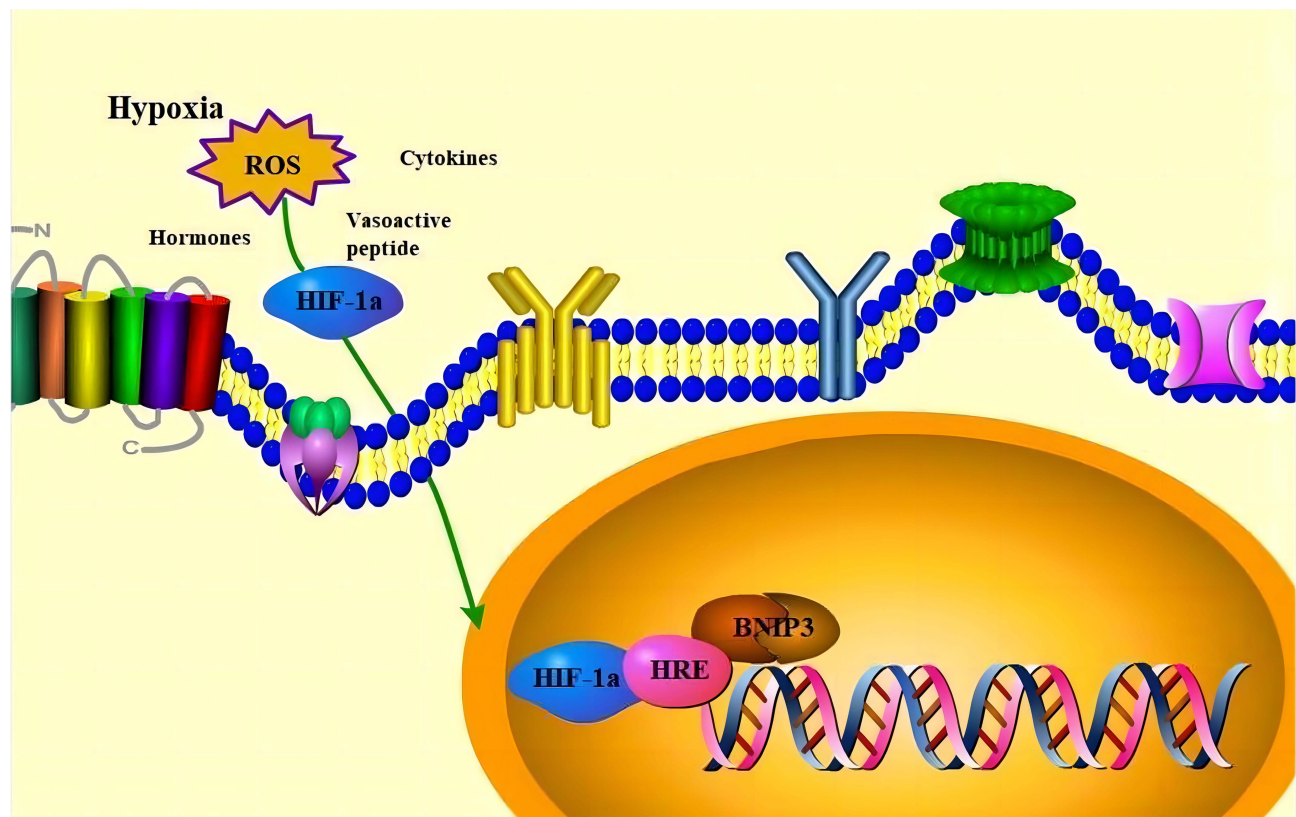
Zhu et al<sup>30</sup> collected serum samples from neonates with hypoxic-ischemic encephalopathy and healthy full-term neonates within 24 h of birth. They measured the levels of BNIP3 in serum using the enzyme-linked reaction adsorption assay. The results showed that the concentration of BNIP3 in serum was significantly higher in neonates with hypoxic-ischemic encephalopathy versus healthy neonates ( $P < 0.001$ ). Under hypoxic conditions (1%), the levels of BNIP3 mRNA and protein were significantly increased in a time-dependent manner. Following pretreatment with a HIF1 $\alpha$  inhibitor and hypoxia, BNIP3 expression was significantly lower than that observed in cells cultured under hypoxic conditions only. The number of apoptotic AGE1.HN cells was significantly increased after BNIP3 treatment ( $P < 0.05$ ). After pretreatment with HIF1 $\alpha$  or HIF2 $\alpha$  inhibitors and hypoxic culture, BNIP3 expression was significantly lower than that noted in cells cultured under hypoxia only. The results of this clinical trial support the ability of HIF-1 $\alpha$  to modulate BNIP3 and induce neuronal injury under hypoxic conditions. BNIP3 has limited homology to the BCL-2 family in the BH3 and C-terminal TM structural domains. However, unlike other BH3-containing proteins, deletion of the BH-3 structural domain in BNIP3 does not eliminate its pro-apoptotic activity. The TM structural domain is essential for the translocation of BNIP3 to mitochondria, where it neutralizes its anti-apoptotic function with the pro-survival proteins BCL-2 and BCL-X heterodimerization L. In addition, it has been shown that HIF-1 also responds to various non-hypoxic stimuli, including vasoactive peptides, cytokines, and hormones. These stimuli can initiate the production of reactive oxygen species, which subsequently activate the HIF-1 cascade response. Thus, non-hypoxic stimuli can also activate HIF-1 $\alpha$  through redox signaling by activating specific kinases or inactivating phosphatases. BNIP3 acts as a receptor for mitochondrial autophagy and induces autophagy through direct binding to LC3 (microtubule-associated protein one light chain 3)<sup>31</sup> Figure 3 depicts the mechanism of HIF-1 $\alpha$ /BNIP3 signaling.

## Role of the HIF-1 $\alpha$ /BNIP3 Signaling Pathway in RHCC

HCC is characterized by a high recurrence rate.<sup>32</sup> RHCC is primarily caused by cellular autophagy and hypoxia.<sup>33</sup> Tumor cells gain energy through autophagy to enhance their survival and promote outward cell migration.<sup>34</sup> Autophagy can induce changes in cell adhesion signaling and promote tumor cell invasion and migration. Moreover, tumor cells contain specific inhibitory Focal adhesion kinase (FAK) that activates Src family kinase (SRC), thereby inhibiting autophagy.<sup>35</sup> Autophagy plays an important role in RHCC. Furthermore, it acts as an important mediator of HCC cell invasion, inducing changes in cell adhesion signaling and promoting HCC cell migration and glycolytic pathways. In addition, it has been shown that autophagy promotes the expression and invasion of EMT markers in HCC cells. This is an important intermediate stage of cancer metastasis and a major cause of intrahepatic dissemination and metastasis of HCC cells.<sup>36</sup> According to the theory of angiogenesis proposed by Judah Folkman, RHCC is also associated with the growth of blood vessels in the tumor. The formation of new blood vessels from endothelial precursors is the prerequisite for the growth and progression of solid malignancies. Access to the host vascular system and the generation of tumor blood supply are rate-limiting steps in tumor growth and progression. Vascular endothelial growth factor (VEGF) refers to a subfamily of growth factors that promote tumor angiogenesis under hypoxic conditions.<sup>37</sup> The HIF-1 $\alpha$ /BNIP3 signaling pathway is closely related to the above-mentioned tumor cell autophagy, hypoxia, and tumor angiogenic processes.

HIF-1 $\alpha$  is an upstream factor of BNIP3. Under hypoxic conditions, HIF-1 $\alpha$ -driven BNIP3 expression helps to reduce overall cellular oxygen consumption by triggering mitochondrial autophagy, thereby facilitating metabolic adaptation to low oxygen concentrations.<sup>38</sup> High BNIP3 and HIF-1 $\alpha$  levels are commonly associated with poor prognosis in patients with cancer (eg, melanoma lung, liver). The HIF-1 $\alpha$ /BNIP3 pathway promotes cellular autophagy under hypoxic conditions. HCC cells gain energy to migrate outward through autophagy and enhance the degree of cell adhesion, thus contributing to metastasis and RHCC.<sup>39</sup> Autophagy is also an important factor in the induction of EMT, which is activated by the HIF-1 $\alpha$ /BNIP3 pathway





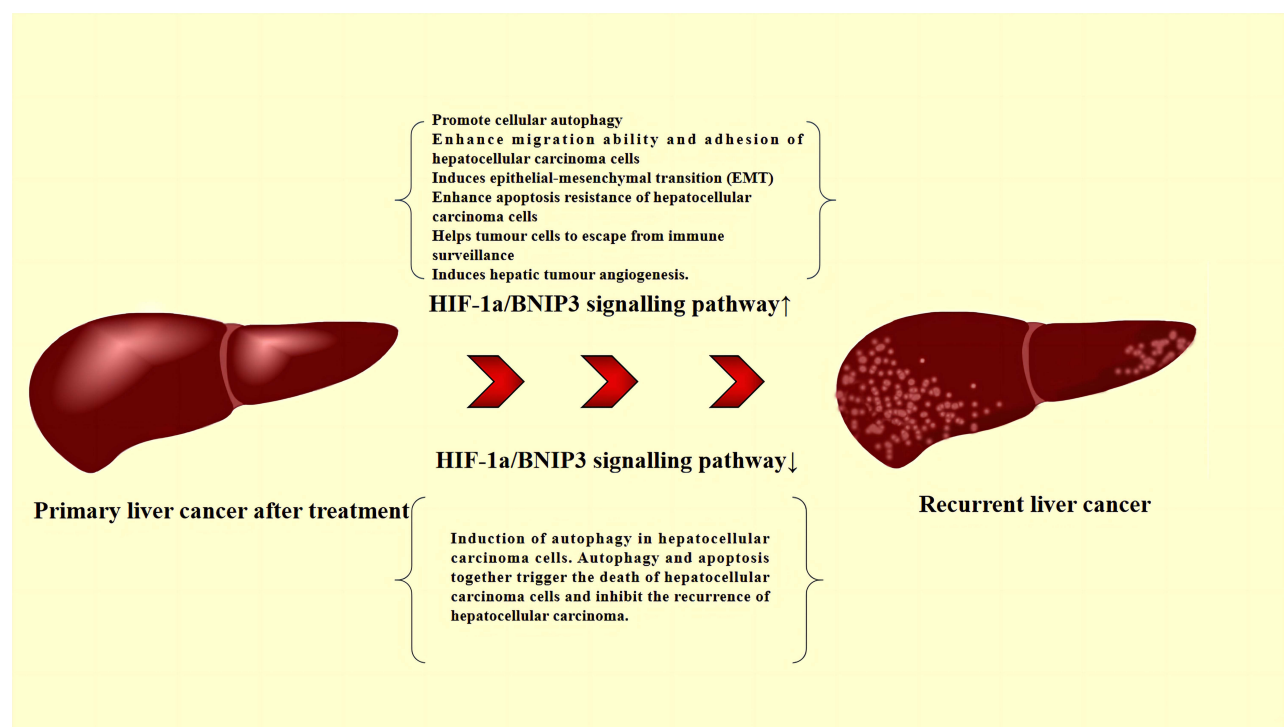
**Figure 3** HIF-1 $\alpha$ /BNIP3 signaling mechanism. Stimulation by external factors activates HIF-1 $\alpha$ , which binds to the HRE within the BNIP3 promoter region and stimulates BNIP3 expression.

**Abbreviations:** BNIP3, BCL-2 19 kDa-interacting protein 3; HIF-1 $\alpha$ , hypoxia-inducible factor-1 $\alpha$ ; HRE, hypoxia response element.

through autophagy in HCC cells. This process produces extracellular matrix, degrades enzymes, and increases the resistance of HCC cells to apoptosis.<sup>40</sup> HIF-1 $\alpha$  can also promote endothelial cell growth and migration by regulating the hypoxic upregulation of VEGF, thereby inducing liver tumor angiogenesis and causing RHCC.<sup>41</sup>

By transfecting HepG2 cells with BNIP3 small interfering RNA, followed by treatment with incomplete radio-frequency ablation (IRFA), Xu et al<sup>42</sup> performed cell proliferation, migration, and invasion assays, as well as electron microscopy and protein blotting on residual tumor cells. Following in vitro simulated IRFA, the residual HepG2 cells showed increased autophagic vesicles, proliferation, migration, and invasion, as well as increased expression of HIF-1 $\alpha$  and BNIP3 proteins. These findings demonstrated that IRFA can induce autophagy through the HIF-1 $\alpha$ /BNIP3 signaling pathway and promote RHCC. Méndez-Blanco et al<sup>43</sup> developed an in vitro model of acquired resistance to sorafenib in HCC to illustrate the role of HIFs and BNIP3 in HCC resistance to treatment and recurrence. HIF-1 $\alpha$  was silenced using siRNA, and its effect on cell viability was tested 24 h after the induction of hypoxia. HIF-1 $\alpha$  knockdown significantly reduced cell viability in both HepG2S1 and HepG2S3 sorafenib-resistant cells. The expression of BNIP3 in these drug-resistant cells was also measured under hypoxic conditions. The experiments showed that the protein and mRNA levels of BNIP3 in HepG2 cells tended to decrease following treatment with sorafenib.

The role of autophagy in cancer is bidirectional. Although autophagy is a mechanism involved in cancer cell survival in response to environmental and cellular stresses, it may be associated with cancer cell death in some cases. In a study conducted by Wang et al,<sup>44</sup> the induction of autophagy in HCC cells (Huh7, Hep3B, and HepG2) was prevented using sonic hedgehog (Shh) or smoothened agonist (SAG) and violet morpholino. In contrast, autophagy in HCC cells (Huh7, Hep3B and HepG2) was induced using the GLI-selective inhibitor GANT61. Hedgehog inhibition-induced autophagy caused apoptosis in HCC cells (Huh7, Hep3B, and HepG2), as determined by measuring the mouse liver holotype index, monoalkyl cadaverine staining, and transmission electron microscopy. Analyses through WB and quantitative real-time



**Figure 4** Role of the HIF-1α/BNIP3 signaling pathway in the RHCC.

**Abbreviations:** BNIP3, BCL-2 19 kDa-interacting protein 3; HIF-1α, hypoxia-inducible factor-1α; RHCC, recurrent hepatocellular carcinoma.

polymerase chain reaction revealed that autophagy was associated with BNIP3. These experiments demonstrated that GANT61 induced autophagy through the upregulation of BNIP3 and that this mechanism contributed to apoptosis, thereby inhibiting the metastasis of HCC. Figure 4 describes in detail the mechanism by which the HIF-1α/BNIP3 signaling pathway causes RHCC through autophagy.

## TCM to Combat Recurrent Liver Cancer Through Targeted Regulation of the HIF-1α/BNIP3 Pathway

In TCM, RHCC belongs to the categories of “jaundice”, “meteorism”, and “aggregation”.<sup>45</sup> The basic pathogenesis of HCC is liver stagnation, deficiency in the liver and kidneys, deficiency in qi and blood, and dampness-heat. The toxins produced by the above factors stagnate in the liver and develop into HCC; in TCM theory, this process is termed cancer toxicity (Ai-Du).<sup>46</sup> Hepatic yin deficiency, syndrome of qi stagnation and blood stasis, hepatospleen transport has no right, and damp heat produces phlegm are factors associated with RHCC and metastatic HCC.<sup>47</sup> Patients often exhibit symptoms of yin deficiency, such as low fever, night sweats, dry mouth and throat, and weight loss due to prolonged illness. Due to the rapid progression of the disease, some patients may experience abdominal distension or pain, refusal to press, bulging masses, and a lean body.<sup>48</sup> Owing to its multi-targeted intervention, low toxicity, and cost-effectiveness, Chinese medicine offers significant advantages in preventing and treating the recurrence of liver cancer. The HIF-1α/BNIP3 signaling pathway is related to autophagy, which is essential for RHCC. Therefore, it may be a target for intervention to prevent and treat disease recurrence through TCM.

## TCM Interferes with the HIF-1α/BNIP3 Pathway Through Its Active Ingredient to Inhibit RHCC

In recent years, several studies demonstrated that the active ingredients contained in some Chinese herbal medicines can effectively inhibit RHCC by interfering with the HIF-1α/BNIP3 pathway. The primary herbal medicines with modulating effects include Shuifeiji, Jiegeng, Wutou, Danshen, and Honghua. Table 1 lists common herbal medicines that interfere

**Table I** Common Herbal Medicines That Inhibit Recurrent Hepatocellular Carcinoma by Modulating the HIF-1 $\alpha$ /BNIP3 Pathway

Drug	Latin Name	Active Ingredient	Mechanism of Action	Reference Number
Shuifeiji	<i>Silybum marianum</i>	Silymarin	Upregulation of BNIP3 expression in Hep G2 cells induces autophagic death of tumor cells	[49]
Jiegeng	<i>Platycodon grandiflorus</i>	Platycodon saponin D	Upregulation of BNIP3 expression in Hep G2 cells results in mitochondrial swelling, and decreases membrane potential and Increased of tumor cells	[50]
Wutou	<i>Aconitum carmichaeli</i> Debx	Aconitine	Downregulation of BNIP3 expression reduces inflammatory response caused by autophagy, and inhibits the proliferation, invasion, and migration of HCC cells	[51]
Danshen	<i>Salvia miltiorrhiza</i>	Tanshinone 2A	Induction of BNIP3/N-mediated mitochondrial autophagy and reprogramming of mitochondrial metabolism in tumor cells promotes apoptosis	[52]
Honghua	<i>Carthamus tinctorius</i> L.	Hydroxysafflor Yellow A	Downregulation of HIF-1 $\alpha$ expression, inhibition of EMT, and promotion of apoptosis in liver tumor cells	[53]
Guanyejinsitao	<i>Hypericum perforatum</i> L.	Hypericin	Inhibition of HIF-1 $\alpha$ and BNIP3 expression attenuate hypoxic response	[54]
Jiaogulan	<i>Gynostemma pentaphyllum</i>	Gynostemin	Inhibition of HIF-1 $\alpha$ expression blocks anaerobic glycolysis in HepG2 cells	[55]
Renshen	<i>Ginseng Radix et Rhizoma</i>	Ginsenoside	Upregulation of BNIP3 expression in Hep G2 cells induces autophagic death in tumor cells	[56]
Huangqi	<i>Astragali Radix</i>	Astragalus polysaccharide	Activation of HIF-1 $\alpha$ /BNIP3 axis promotes autophagy and induces apoptosis in HCC cells	[57]
Sanqi	<i>Notoginseng Radix</i>	Panax notoginseng saponin	Activation of the HIF-1 $\alpha$ /BNIP3 axis promotes autophagy and induces apoptosis in HCC cells	[58]
Suoluozhi	<i>Semen Aesculi</i>	Sodium Aescinate	Downregulation of BNIP3 through inhibition of HIF-1 $\alpha$ expression inhibits anaerobic glycolysis in HepG2 cells, and downregulates VEGF expression (an angiogenesis-promoting factor)	[59]
Chaihu	<i>Bupleuri Radix</i>	Saikosaponin	Inhibition of HIF1 $\alpha$ -regulated glycolytic pathway through upregulation of SIRT6 protein expression results in inhibition of HCC cell proliferation	[60]
Dahuang	<i>Rhei Radix et Rhizoma</i>	Rhododendrin	Downregulation of HIF-1 $\alpha$ expression reduces inflammatory response caused by autophagy, and inhibits the proliferation, invasion and migration of HCC cells	[61]
Baihuadan	<i>Plumbago zeylanica</i>	Phellodendron	Downregulation of the HIF-1 $\alpha$ /BNIP3 axis inhibits anaerobic glycolysis in HepG2 cells and downregulates VEGF expression (a neovascularization-promoting factor)	[62]
Qinghao	<i>Artemisia annua</i> L.	Dihydroartemisinin	Inhibits HIF-1 $\alpha$ expression and downregulates VEGF	[63]
Banmao	<i>Lytta vesicatoria</i>	Zanthoxanthin	Reduces HIF-1 $\alpha$ and BNIP3 expression, thereby inhibiting immune escape of tumor cells	[64]
Tiandong	<i>Asparagus cochinchinensis</i>	Aspartame polysaccharide	Downregulation of HIF-1 $\alpha$ expression inhibits tumor angiogenesis, thereby promoting VEGF downregulation	[65]
Leigongteng	<i>Tripterygium wilfordii</i>	Radicin	Downregulation of the HIF-1 $\alpha$ /BNIP axis inhibits anaerobic glycolysis in HepG2 cells	[66]

(Continued)

**Table 1** (Continued).

Drug	Latin Name	Active Ingredient	Mechanism of Action	Reference Number
Huangqin	<i>Scutellaria baicalensis</i> Georgi	Baicalein	Activation of the HIF-1 $\alpha$ /BNIP3 axis promotes autophagy and induces apoptosis in HCC cells	[67]
Lihao	<i>Artemisia selengensis</i> Turcz. ex Bess.	Artemisinin flavonoids	Downregulation of the HIF-1 $\alpha$ /BNIP axis inhibits anaerobic glycolysis in HepG2 cells	[68]
Loulu	<i>Rhapontici Radix</i>	RUE	Downregulation of HIF-1 $\alpha$ expression promotes VEGF downregulation and inhibits tumor angiogenesis	[69]
Luole	<i>Ocimum basilicum</i>	Basil polysaccharide	Downregulation of the HIF-1 $\alpha$ /BNIP axis inhibits anaerobic glycolysis in HepG2 cells	[70]
Chanpi	<i>Bufo gargarizans</i>	Huachanin	Downregulation of HIF-1 $\alpha$ expression promotes VEGF downregulation and inhibits tumor angiogenesis	[71]
Baitouweng	<i>Pulsatilla chinensis</i> (Bunge) Regel	Baicalein	Reduction of HIF-1 $\alpha$ protein expression reduces tumor cell energy metabolism and inhibits HCC recurrence	[72]
Jianghuang	<i>Curcuma Longae Rhizoma</i>	Curcumin	Downregulation of the COX-2/PGE2 pathway reduces HIF-1 $\alpha$ protein expression and promotes VEGF downregulation	[73]

with the HIF-1 $\alpha$ /BNIP3 pathway and can be used to treat a recurrence of liver cancer recurrence, as well as their active ingredients and intervention mechanisms. Chinese herbal medicines can biphasically regulate the HIF-1 $\alpha$ /BNIP3 signaling pathway to inhibit the recurrence of liver cancer. Activation of the HIF-1 $\alpha$ /BNIP3 signaling pathway can enhance autophagy of HCC cells, promote apoptosis of tumor cells, and inhibit the recurrence of liver cancer. Xuan et al found that silymarin could induce tumor cell autophagy and apoptosis by activating BNIP3 protein expression in Hep G2 cells. Inhibition of the HIF-1 $\alpha$ /BNIP3 signaling pathway could block anaerobic glycolysis in HCC cells, as well as the occurrence of EMT mechanism. Li et al showed that Rehmannia methyl estradiol could inhibit the process of anaerobic glycolysis by downregulating the HIF-1 $\alpha$  /BNIP3 signaling pathway to suppress hepatocarcinogenesis. This evidence suggests that the active ingredients of Chinese herbal medicine modulating the HIF-1 $\alpha$  /BNIP3 signaling pathway may be effective in inhibiting RHCC.

## Chinese Herbal Compound Interferes with the HIF-1 $\alpha$ /BNIP3 Pathway to Inhibit RHCC

TCM compounds can be combined with other drugs to exert synergistic effects. In TCM theory, this process is known as mutual promotion or mutual assistance. It has been reported that HCC relapses can be inhibited by targeting and modulating the HIF-1 $\alpha$  /BNIP3 pathway. We summarize the common groups associated with the HIF-1 $\alpha$ /BNIP3 pathway for the treatment of RHCC, as shown in Table 2. The Fuzheng Yiliu Decoction, Jiedu Xiaozheng Decoction, Xuefu Zhuyu Decoction, Huang Qin Decoction, and Yi Qi Hua Yu Jie Du Prescription are discussed below.

### Fuzheng Yiliu Decoction

Fuzheng Yiliu Decoction is a classic formula used for the treatment of tumors. It consists of 10 herbs, namely Sanleng (10 g), Ezhu (15 g), Longkui (15 g), Baiying (10 g), Banzhilian (30 g), Baihuasheshacao (30 g), Huangqi (9 g), Danggui (30 g), Fuling (30 g), and Baizhu (10 g). *Astragalus* polysaccharide in Huangqi activates the HIF-1 $\alpha$ /BNIP3 axis, promotes autophagy, and induces apoptosis in HCC cells. The combination of Ezhu and Huangqi can exert a synergistic effect. Moreover, the combination of Curcumol and *Astragalus* saponin<sup>75</sup> reduces the expression of HIF-1 $\alpha$  in HCC cells and inhibits aerobic glycolysis and tumor angiogenesis in RHCC.



**Table 2** A Formulas to Inhibit RHCC by Modulating the HIF-1 $\alpha$ /BNIP3 Pathway

Formulas	Component Drugs	Active Ingredient	Mechanism of Action	Reference Number
Fuzheng Yiliu Decoction	Sanleng (10 g), Ezhu (15 g), Longkui (15 g), Baiying (10 g), Banzhilian (30 g), Baihuasheshecao (30 g), Huangqi (9 g), Danggui (30 g), Fuling (30 g), Baizhu (10 g)	Quercetin, naringenin, $\beta$ -sitosterol	It inhibits tumor cell growth, induces apoptosis, activates Bnip3 expression in hepatocellular carcinoma cells, induces autophagy in tumor cells, and inhibits RHCC.	[74]
Jiedu Xiaozheng Decoction	Baihuasheshecao (30 g), Xiakucao (15 g), Shancigu (15 g), Kushen (15 g)	Xiakucao total flavonoids, bitter ginseng, quercetin, $\beta$ -sitosterol, asparagine	Down-regulation of HIF-1 $\alpha$ protein and glucose uptake inhibits the proliferation of HCC cells, promotes apoptosis and migration of HCC cells, and inhibits the RHCC.	[75]
Xuefu Zhuyu Decoction	Taoren (12 g), Honghua (9 g), Danggui (9 g), Shudi (9 g), Niuxi (9 g), Chuanxiong (4.5 g), Jiegeng (4.5 g), Baishao (6 g), Zhishi (6 g), Gancao (6 g), Chaihu (3 g).	Hydroxysafflor Yellow A, Radix Bupleurum Saponin, Hyoscyamus Saponin, Catechin, Palmitic Acid	Down-regulation of serum HIF-1 $\alpha$ expression inhibits the growth and migration of HCC cells, thereby reducing the probability of RHCC.	[76]
Huang Qin Decoction	Huangqin (9 g), Gancao (6 g), Shaoyao (6 g) and 12 Dazao.	Paeoniflorin, baicalin, glycyrrhizic acid, baicalein	Downregulating HIF-1 $\alpha$ expression promotes VEGF downregulation and inhibits tumor angiogenesis and RHCC.	[77]
Yiqi Huayu Jiedu Prescription	Baishen (10 g), Huangqi (30 g), Ezhu (15 g), Chonglou (15 g), Hanzhilian (30 g), Gancao (5 g)	Ginsenoside, Astragalus polysaccharide, Curcumin, Curcuminoid, Chrysoide	Down-regulation of HIF-1 $\alpha$ and VEGF expression in tumor tissues improves the state of tumor tissue hypoxic microenvironment and inhibits tumor angiogenesis and HCC recurrence.	[78]
Ai Tong Xiao Prescription	Baihuasheshecao (20 g), Jieducao (20 g), Sanleng (10 g), Banzhilian (15 g), Muli (30 g), Xiangfu (10 g), Dangshen (10 g), Huangqi (20 g), Jiaogulan (12 g), Ezhu (10 g), Yanhusuo (20 g), Zhigancao (10 g)	Astragalus polysaccharide, curcumol, ginsenoside, fenugreek ethereine	Down-regulating HIF-1 $\alpha$ expression and promoting VEGF down-regulation inhibited tumor angiogenesis and RHCC.	[79]
Shu Yu Pill	Shanyao (24g), Danggui (6g), Guizhi (6g), Shenqu (6g), Shengdi (12 g), Renshen(6 g), Gancao (12 g), Chuanxiong (6 g), Baishao (6 g), Baizhu (6 g), Maidong (6 g), Kuxingren (6 g), Chaihu (6 g), Jiegeng (3 g), Fulin (6 g), Ejiao (6 g), Ganjiang (3g), Fangfeng (6g), Bailian (6 g), Dazao (24 g)	Diosgenin, Ginsenoside, Ginsenoside, Chaihuoside, Cinnamaldehyde	Inhibit the effect of HIF-1 expression, improve the energy metabolism of tumor microenvironment and structural damage from mitochondria, to play a role in treating RHCC.	[80]
Di Dang Decoction	Shuizhi (6 g), Mangchong (6 g), Taoren (9 g), Dahuang (9 g)	Rhodopsin, Hirudin	Reduce mitochondrial damage, lower ROS content, inhibit apoptosis, and upregulate HIF-1 $\alpha$ and BNIP3 protein expression.	[81]
Si Ni Decoction	Fuzi (35g), Ganjiang (15g), Mahuang (10g), Wuzhuyu (15g), Gancao (15g)	Aconitine, hyaconitine, glycyrrhizin, glycyrrhetic acid	Down-regulating HIF-1 $\alpha$ expression and promoting VEGF down-regulation inhibited tumor angiogenesis and RHCC.	[82]

(Continued)

**Table 2** (Continued).

Formulas	Component Drugs	Active Ingredient	Mechanism of Action	Reference Number
Yi Qi Jian Pi Decoction	Huangqi (30g), Taizishen (30g), Danggui (30g), Baizhu (15g), Fulin (15g), Cangzhu (10g), Chenpi (10g), Huangqin (10g), Gancao (3g)	Atractylonone, Astragalus polysaccharide, Ginsenoside, Glycyrrhetic acid, Poria polysaccharide	It can alleviate hepatocyte injury, promote HIF-1 $\alpha$ protein expression, and protect against hypoxic injury while reducing mitochondrial ROS production and inhibiting hepatocyte apoptosis.	[83]
Yi Ai Prescription	Huangqi (30g), Baizhu (30g), Tengligen (30g), Ezhu (30g), Dongguazi (30g)	Astragaloside IV, Atractyliside III, Isocurcumenol, Oleanolic Acid, Trigonelline	Down-regulate HIF-1 $\alpha$ expression in hepatocellular carcinoma cells, promote VEGF down-regulation, prevent tumor angiogenesis and inhibit RHCC.	[84]

**Abbreviations:** BNIP3, BCL-2 19 kDa-interacting protein 3; COX-2, cyclooxygenase-2; HCC, hepatocellular carcinoma; HIF-1 $\alpha$ , hypoxia-inducible factor-1 $\alpha$ ; EMT, epithelial–mesenchymal transition; PGE2, prostaglandin E2; SIRT6, sirtuin 6; VEGF, vascular endothelial growth; RHCC, recurrent hepatocellular carcinoma.

Zhang et al<sup>74</sup> prepared a model of HCC in nude mice through the transplantation of Hu-7 cells. They observed the formation of autophagosomes in the model and drug-treated mice by electron microscopy. The expression of BNIP3 protein was measured by WB. The results showed that, compared with the model group, the Fuzheng Yiliu Decoction induced apoptosis and inhibited the growth rate of tumors in the treatment group. WB analysis showed that the expression of autophagy-related proteins beclin-1 (BECN1), BNIP3, and LC3 was upregulated in the Fuzheng Yiliu Decoction group compared with the Fuzheng Yiliu Decoction treatment group. These findings suggested that Fuzheng Yiliu Decoction can inhibit the growth of Hu-7 cells and induce apoptosis of tumor cells in nude mice. Moreover, it can activate the expression of BECN1, BNIP3, and LC3 proteins in liver cancer cells to induce autophagy. Yin et al<sup>85</sup> evaluated 80 patients treated after radical surgery for HCC with conventional radiotherapy (n=40) and Fuzheng Yiliu Decoction (n=40). The postoperative survival rate, tumor recurrence rate, and vascular tumor marker levels were compared between the two groups. The results showed that patients in the Fuzheng Yiliu Decoction group had a higher survival rate and lower recurrence rate at 3 years after surgery compared with the conventional radiotherapy group. Moreover, the levels of alpha-fetoprotein (AFP), carcinoembryonic antigen (CEA), and des-gamma-carboxy prothrombin (DCP) were lower in the former group versus the latter group. This evidence indicates that Fuzheng Yiliu Decoction effectively improves the survival rate and reduces the rate of disease recurrence in patients with HCC. Chen et al<sup>86</sup> determined the effects of different concentrations of the ethyl acetate extract of the Fuzheng Yiliu Decoction on the survival rate and HIF-1 $\alpha$  protein expression in HepG2 HCC cells under hypoxic conditions. The results showed that the survival rate of HepG2 HCC cells under hypoxic condition was gradually decreased with the increase in the concentration of ethyl acetate extract of Fuzheng Yiliu Decoction. Furthermore, the expression of HIF-1 $\alpha$  protein was also inhibited in a dose-dependent manner ( $P<0.05$  or  $P<0.01$ ). Cao et al<sup>87</sup> prepared a nude mouse model of HCC through transplantation of Hu-7 cells and administration of different concentrations of Fuzheng Yiliu Decoction. The results showed that the mean mass of HCC transplanted tumors in the Fuzheng Yiliu Decoction group was lower than that measured in the control group. In addition, the number of bilayer autophagic vesicles was increased and the expression of BNIP3 was increased in the treatment group, as observed through electron microscopy; these effects were dose-dependent ( $P<0.05$ ). Based on the available evidence, Fuzheng Yiliu Decoction can significantly enhance the autophagic activity of tumor cells and inhibit the proliferation, invasion, and metastasis of HCC cells.

### Jiedu Xiaozheng Decoction

Jiedu Xiaozheng Decoction is composed of four herbs, namely, Baihuasheshecao (30 g), Xiakucao (15 g), Shancigu (15 g), and Kushen (15 g); these herbs possess detoxifying and anti-inflammatory properties. The alcoholic extracts of *Bupleurum* enhance cellular and humoral immunity and inhibit the growth of liver cancer cells.<sup>88</sup> The total flavonoids of Xiakucao reduce the expression of HIF-1 $\alpha$  in tumor cells and inhibit RHCC by blocking the aerobic glycolytic process in HCC cells. Cao et al<sup>89</sup> successfully prepared a liver metastasis tumor model by subcutaneously transplanting HepG2 cells into BALB/c nude mice. This was followed by treatment using ethyl acetate extract of Jiedu Xiaozheng Decoction. The

tumor volume and weight were measured, and the apoptotic index and the expression levels of BAX and cytochrome C were determined by immunohistochemistry (IHC). The results showed that the treatment significantly reduced the proliferation of HepG2 cells and significantly increased the apoptosis index and the expression of intracellular BAX protein. This evidence indicates that the ethyl acetate extract of Jiedu Xiaozheng Decoction can treat HCC by inducing apoptosis and inhibit RHCC. Lu et al<sup>75</sup> explored the changes in glucose metabolism after intervention with Jiedu Xiaozheng Decoction in different HCC cell lines by establishing in vitro hypoxic HepG2, Hep3B, and Huh7 HCC cell models. The effects of different concentrations (0, 0.05, 0.1, and 0.2 mg/mL) of Jiedu Xiaozheng Decoction on the survival rate and apoptosis of HCC cells were examined by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) and Hoechst staining. The proliferation of HCC cells and HIF-1 $\alpha$  protein expression under hypoxic and normoxic conditions were detected using MTT assay and WB, respectively. The results showed that Jiedu Xiaozheng Decoction promoted the apoptosis of HCC cells, downregulated the expression of HIF-1 $\alpha$  and inhibited the glycolytic ability of cells, thus inhibiting the proliferation, invasion, and metastasis of HCC cells.

### Xuefu Zhuyu Decoction

Xuefu Zhuyu Decoction is composed of Taoren (12 g), Honghua (9 g), Danggui (9 g), Shudi (9 g), Niuxi (9 g), Chuanxiong (4.5 g), Jiegeng (4.5 g), Baishao (6 g), Zhishi (6 g), Gancao (6 g), and Chaihu (3 g). Hydroxysafflor yellow A, the main component in Honghua, can downregulate the expression of HIF-1 $\alpha$ , inhibit the EMT process, and promote the apoptosis of liver tumor cells<sup>90</sup> Chaihu saponin can interfere with the glycolytic pathway of liver cancer cells by downregulating HIF-1 $\alpha$  expression, thus inhibiting the proliferation of liver cancer cells.<sup>88</sup> *Platycodon* saponin was able to upregulate BNIP3 expression in HCC cells. This caused mitochondrial swelling and decreased membrane potential in tumor cells, thereby triggering autophagy and death of HCC cells.

Meng et al<sup>91</sup> investigated 70 patients with advanced HCC; patients in the experimental group received a combination of Xuefu Zhuyu Decoction with XELOX (capecitabine plus oxaliplatin) chemotherapy. The XELOX regimen comprised capecitabine (1000 mg/m<sup>2</sup> twice daily, administered orally on days 1–14) and oxaliplatin (135 mg/m<sup>2</sup> administered intravenously on day 1). One cycle included 4 weeks of therapy, and three cycles of treatment were performed. The results showed that clinical symptoms (eg, pain in the liver area, bloating, and poor appetite) were improved. In addition, the numbers of CD3+ and CD4+ cells were decreased, whereas those of CD8+ and CD4+/CD8+ cells were increased. The serum levels of HIF-1 $\alpha$  were decreased by the combination treatment, indicating that XELOX chemotherapy with Xuefu Zhuyu Decoction improves immunity and serum HIF-1 $\alpha$  expression, as well as prognosis in patients with advanced HCC.

### Huang Qin Decoction

Huang Qin decoction is composed of Huangqin (9 g), Gancao (6 g), Shaoyao (6 g), and 12 Dazao. Han baicalin in Huangqin activates the HIF-1 $\alpha$ /BNIP3 axis and promotes autophagy and apoptosis of liver cancer cells. Paeoniflorin (PF) contained in *Paeonia lactiflora* exerts a wide range of anti-inflammatory and immunomodulatory effects.<sup>92</sup> Studies have reported that paeoniflorin downregulates HIF-1 $\alpha$  expression in vivo, affects the expression of downstream factors BNIP3 and VEGF, and inhibits autophagy and angiogenesis in tumor cells.<sup>93</sup> Hu et al<sup>94</sup> investigated the effect of Huang Qin Decoction on the expression of HIF-1 $\alpha$  in human hepatoma HepG2 cells and the potential mechanism involved in this process. The results of WB analysis showed that, after 24 h of treatment with 5-fluorouracil and baicalin soup, the HIF-1 $\alpha$  protein expression in HepG2 cells was decreased in all intervention groups. Notably, the lowest levels of HIF-1 $\alpha$  were detected in the group treated with high-dose baicalin. This evidence indicates that Huang Qin Decoction dose-dependently decreased HIF-1 $\alpha$  protein expression in human HCC HepG2 cells, inhibited the autophagy-activated EMT pathway and the production of extracellular matrix degrading enzymes in HCC cells, and induced apoptosis. Li et al<sup>67</sup> assigned 112 patients with primary liver cancer into control and observation groups (n=56 per group). Patients in both groups were treated with conventional treatment, such as hepatic artery chemoembolization. In the observation group, patients were also treated with Huang Qin Decoction for 4 months. Treatment effectiveness was significantly higher in the observation group versus the control group (67.86% vs 48.21%, respectively;  $P<0.01$ ). Furthermore, the levels of HIF-1 $\alpha$  and AFP and tumor volume were significantly lower in the observation group compared with the control group

( $P < 0.01$ ). These results showed that treatment with Huang Qin Decoction in combination with hepatic artery chemoembolization could inhibit the growth and spread of HCC cells by reducing HIF-1 $\alpha$  and AFP expression in patients with HCC. Wang et al<sup>95</sup> examined 120 patients with HCC and classified them into observation and control groups. There were 60 patients in each of the two groups. Patients in the control group were treated with sequential hepatic artery chemoembolization, while those in the observation group were treated with Huang Qin Decoction for 8 weeks. The results showed that the levels of aspartate transaminase, aspartate transaminase, total bilirubin, AFP, and HIF-1 $\alpha$  were decreased in the observation group compared with the control group after treatment ( $P < 0.05$ ). This evidence demonstrates that Huang Qin Decoction combined with sequential hepatic artery chemoembolization can reduce the serum levels of HIF-1 $\alpha$  and effectively improve liver function in patients with HCC.

### Yiqi Huayu Jiedu Prescription

The formula of Yiqi Huayu Jiedu is composed of Baishen (10 g), Huangqi (30 g), Ezhu (15 g), Chonglou (15 g), Hanzhilian (30 g), and Gancan (5 g), and improves qi and blood stasis. This formula is rich in ginsenosides, *Astragalus* polysaccharides, curcuminoids, curcuminoids, shigella saponins, glycoproteins, and other anti-tumor active components. Curcumin can inhibit the HIF-1 $\alpha$ /BNIP3 axis and block the EMT of HCC, thus exerting anti-metastatic and anti-invasive effects. Modern pharmacological analysis shows that Yiqi Huayu Jiedu Prescription can improve immunity, promote phagocytosis, and enhance the function of the adrenal cortex. These effects can stabilize tumors, prevent recurrence and metastasis, and improve the quality of life of patients.

Zeng et al<sup>78</sup> evaluated the effect of Yiqi Huayu Jiedu Prescription on human liver tumors and HIF-1 $\alpha$  expression by establishing a liver cancer transplantation tumor model in nude rats. IHC was performed on tumor tissues after 21 days of intervention with Yiqi Huayu Jiedu Prescription. The results showed that the tumor size was significantly reduced and HIF-1 $\alpha$  expression was decreased in the treatment group versus the control group. Wang et al<sup>96</sup> established a transplantation tumor model of human HCC sorafenib-resistant cells in nude mice. The purpose was to evaluate the effects of the combination of Yiqi Huayu Jiedu Prescription with sorafenib on the size of the transplantation tumors and HIF-1 $\alpha$  expression in tumor tissues. Mice in the experimental group were treated with Yiqi Huayu Jiedu Prescription (7.5 g/kg) combined with sorafenib (15 mg/kg) through gavage. The tumor volume was measured after 21 days of drug administration, and the expression of HIF-1 $\alpha$  was detected by IHC. The results showed that the treatment decreased the tumor volume and expression of HIF-1 $\alpha$  in experimental mice. Hence, this formula can improve the prognosis of HCC by downregulating the expression of HIF-1 $\alpha$ , improving the hypoxic microenvironment of tumor tissues, and preventing the development of resistance to sorafenib.

## Conclusion and Future Perspective

This article summarizes the biological characteristics of HIF-1 $\alpha$  and BNIP3, and discusses the role of the HIF-1 $\alpha$ /BNIP3 signaling pathway in RHCC. The hypoxic or ischemic state of the body activates HIF-1 $\alpha$  and the downstream factor BNIP3, which mediates cellular autophagy. Autophagy promotes HCC cell migration, glycolysis, and EMT process, but can also directly kill tumor cells. The article also outlines currently available information on potential herbs/extracts/combinations for treating RHCC. These agents inhibit disease recurrence through biphasic modulation of the HIF-1 $\alpha$ /BNIP3 signaling pathway. The main components of these formulations include saponins, flavonoids, and polysaccharides. These components enhance autophagy and promote apoptosis in HCC cells by activating the HIF-1 $\alpha$ /BNIP3 signaling pathway, or inhibiting anaerobic glycolysis and the EMT mechanism in tumor cells by downregulating the expression of the HIF-1 $\alpha$ /BNIP3 signaling pathway.

TCM is widely used in China as an essential complement or alternative to pharmacological therapy for improving the clinical outcomes of patients with recurrent liver cancer. TCM formulations are effective in modulating the HIF-1 $\alpha$ /BNIP3 signaling pathway to prevent the recurrence of liver cancer. However, as most clinical trials are conducted in China with a limited sample size, additional detailed animal and clinical studies are warranted to confirm their efficacy and safety. It is also essential to elucidate the molecular mechanism underlying the effects of the above herbs/extracts/combinations in the treatment of recurrent liver cancer by targeting HIF-1 $\alpha$ /BNIP3 signaling. Of note, there are few

studies on the toxicity of the above herbs/extracts/combinations and their target organs. Therefore, our future research studies will investigate the side effects and toxicity of TCM in RHCC.

Overall, this article provides the latest information on natural agents used in the treatment of recurrent liver cancer through modulation of the HIF-1 $\alpha$ /BNIP3 signaling pathway. This review may provide new directions for the development of natural agents targeting RHCC.

## Abbreviations

HCC, Hepatocellular carcinoma; RHCC, recurrent hepatocellular carcinoma; HIF-1 $\alpha$ , Hypoxia-inducible factor-1 $\alpha$ ; BNIP3, BCL-2 19 kDa interacting protein 3; TCM, traditional Chinese medicine; NASH, nonalcoholic steatohepatitis; bHLH - PAS, basic helix - loop - helix - PER - ARNT - SIM; ODDD, oxygen-dependent degradation; TAD, trans-activating domain; TRD, trans-repressive domain; N-TAD, N-terminal transcriptional activation domain; MTS, 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulphophenyl)-2H-tetrazolium; HRE, hypoxia response element; C-TAD, C-terminal transcriptional activation domain; NLS-N, nuclear localization signaling domains-nitrogen; NLS-C, carbon-terminated nuclear localization signaling domains; PSTD, proline-serine-threonine-rich protein stabilization domain; BH3, Bcl-2 homology 3; CD, conserved structural domain; TM, transmembrane; MPTP, Mitochondrial Permeability Transition Pore; HPH, hypoxia-inducible factor proline hydroxylase; WB, Western Blot; LC3, microtubule-associated protein one light chain 3; ROS, reactive oxygen species; FAK, Focal adhesion kinase; specific inhibitory adhesion kinase; SRC, Src family kinase; EMT, Epithelial-mesenchymal transition; VEGF, Vascular endothelial growth; IRFA, incomplete radiofrequency ablation; Shh, Sonic hedgehog; SAG, Smoothed Agonist.

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## Disclosure

The authors report no conflicts of interest in this work.

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