Critical signaling pathways governing hepatocellular carcinoma behavior; small molecule-based approaches

Zahra Farzaneh1*, Massoud Vosough2, Tarun Agarwal3 and Maryam Farzaneh4*

Abstract
Hepatocellular carcinoma (HCC) is the second leading cause of death due to cancer. Although there are different treatment options, these strategies are not efficient in terms of restricting the tumor cell's proliferation and metastasis. The liver tumor microenvironment contains the non-parenchymal cells with supportive or inhibitory effects on the cancerous phenotype of HCC. Several signaling pathways are dis-regulated in HCC and cause uncontrolled cell propagation, metastasis, and recurrence of liver carcinoma cells. Recent studies have established new approaches for the prevention and treatment of HCC using small molecules. Small molecules are compounds with a low molecular weight that usually inhibit the specific targets in signal transduction pathways. These components can induce cell cycle arrest, apoptosis, block metastasis, and tumor growth. Devising strategies for simultaneously targeting HCC and the non-parenchymal population of the tumor could lead to more relevant research outcomes. These strategies may open new avenues for the treatment of HCC with minimal cytotoxic effects on healthy cells. This study provides the latest findings on critical signaling pathways governing HCC behavior and using small molecules in the control of HCC both in vitro and in vivo models.

Keywords: Hepatocellular carcinoma, Cancer, Signaling pathways, Small molecules, Carcinoma

Background
Hepatocellular carcinoma (HCC) or hepatoma is the most type of cancer in the tissues of the liver and the second leading cause of cancer-related death around the world [1, 2]. Hepatitis B/C virus and alcohol consumption are two important and independent risk factors that increase the risk of HCC [3–5]. Liver transplantation or surgical liver resection are two main options for the treatment of HCC [6, 7]. In addition to other surgical treatment options, some non-surgical methods such as chemotherapy or radiotherapy are effective treatments for HCC [8, 9]. However, these methods are not able to restrict the growth, progression, and metastasis of HCC [10]. On the other hand, these treatments cause side effects on the surrounding healthy cells [11]. Several signaling pathways are dis-regulated in HCC and lead to uncontrolled cell division and metastasis [12, 13]. Targeting specific signaling pathways that are involved in HCC phenotypes such as non-stopped cell proliferation, migration, and metastasis may control the progress of the disease [14, 15]. Recent studies have established a new approach for the prevention and treatment of HCC using small molecules [16]. Small molecules are compounds with a low molecular weight that usually inhibit the specific targets in signal transduction pathways [14, 17]. Targeting cancer-specific signaling pathways using small
molecules can be novel therapeutic strategies against HCC (Table 1). Inhibition of these signaling pathways or common downstream effectors by different anti-cancer agents leads to increase apoptosis and autophagy along with a decrease in the survival, metastasis, EMT, proliferation, and colony formation of HCC cell lines and animal models [18, 19]. This study provides the latest findings on using small molecules in the control of HCC both in vitro and in vivo models.

Characterization of HCC
Hepatocytes as the most functionally liver cells have been reported to participate in HCC [20, 21].

Disruption of intracellular regulators or extracellular signals in the tumor microenvironment (TME) leads to inappropriate activation of certain signaling pathways [22–24]. Thus, aberrant molecular signaling increases levels of abnormal epigenetic modification and gene expression in the cancerous hepatocytes [25]. The outcome of these events is the loss of mature or differentiated hepatocytes (a phenomenon termed cellular dedifferentiation) [26, 27]. Under these conditions, the expression of E-cadherin (an epithelial marker) is downregulated and the cytoskeleton is reorganized [28]. The expression of Snail, Twist, and ZEB as the major transcription factors associated with mesenchymal cellular phenotype are up-regulated and induce an epithelial-to-mesenchymal transition (EMT) state in HCC [29]. Matrix metalloproteinases (MMPs) are also expressed at a high level in HCC and promote cellular migration and angiogenesis [30]. In HCC, the telomerase activity increases by up to 90%, checkpoints of the cell cycle are inactivated, and apoptosis is suppressed [31, 32]. All of these events cause uncontrolled cell proliferation, prolonged cell viability, and metastasis in HCC [33]. In HCC, several growth factors are released from non-parenchymal cells around the hepatocytes [14]. This event triggers cancerous phenotypes include EMT, metastasis, checkpoints aberration, uncontrolled proliferation, immortalization, and neovascularization in hepatocytes [34]. Other stimulators of hepatocyte malignancy come from micro-environmental cues such as hypoxia [35].

Critical signaling pathways in HCC
Several signaling pathways, including TGF-β, Wnt/B-catenin, Hh, Notch, EGF, HGF, VEGF, JAK/STAT, Hippo, and HIF are dis-regulated in HCC and lead to uncontrolled cell division and metastasis (Fig. 1).

Transforming growth factor-β (TGF-β) signaling
Cancer-associated fibroblast (CAF), derived from either stromal cells or hepatocytes is the main source of TGF-β secretion in the liver tumor [36]. TGF-β binds to the heterodimer of receptors, TβRII and TβRI, phosphorylates and activates Smad2/3 that further translocate to the nucleus in association with Smad4 [37]. TGF-β upregulates the expression of Snail, downregulates E-cadherin in the polarized hepatocytes, and promotes EMT and metastasis [38]. The role of the TGF-β signaling pathway is also the preservation of CSC subpopulation and the promotion of HCC proliferation [39]. This pathway has been shown to induce VEGF expression in HCC and recruit endothelial cells at the tumor site [40]. TGF-β with the EGF, Wnt, and SHH pathways can promote the mesenchymal features of HCC cell lines [41]. TGF-β converts tumor-associated macrophages (TAM) to M2-like macrophages and improves proliferation, metastasis, and neoangiogenesis of HCC [38], suppresses MHC-I and II expression on HCC and modulates the immune cell defense in HCC [39]. Accumulating evidence shows that HCC cell lines represent different levels of TGF-β activity (Sk-Hep1 cells with low expression and HepG2 cells with high expression of TGF-β) [42]. Suppression of TGF-β receptors by LY2109761 or SB431542 increases E-cadherin expression, decreases migration, and invasion of HCC [43]. Recently, LY2157299 (Galunisertib) was shown to decrease both the canonical and non-canonical TGF-β pathway in HCC [42]. Galunisertib with Sorafenib has entered into the phase II clinical trial [44]. FGFR or MAPK/ERK inhibitors (such as PD98059) can also be used for inhibition of TGF-β and metastasis in HCC [45]. A combination of TGF-β inhibitor and atezolizumab (a programmed cell death ligand 1 (PD-L1) inhibitor) can overcome the immune escape of HCC [46].

Wnt/B-catenin signaling
In the liver tumor, HCC cells, and macrophages are emerging sources of Wnt ligand [47]. Besides, some of the environmental risk factors cause mutations in different components of the Wnt pathway, leading to overactivation of Wnt signaling in HCC [48, 49]. Binding of Wnt ligand to the Frizzled (Fzd) and low-density lipoprotein receptor-related protein (LRP) receptors causes phosphorylation of the Disheveled [50]. Activated receptors and Disheveled inhibit the destruction of complex proteins (glycogen synthase kinase 3β (GSK3β), axis inhibition protein (Axin), and adenomatous polyposis coli (APC)), thereby causing the release of β-catenin [51, 52]. Activated β-catenin further translocates to the nucleus, binds with other co-activators (like lymphoid enhancer factor (LEF)/ T-cell factor (TCF) proteins or histone acetyltransferase CREB-binding protein (CBP)/p300), and activates the transcription of several target genes [53]. These genes are involved in CSC maintenance (CD44, EpCAM), proliferation (cyclin D1, c-Myc), and EMT [54]. Leucine-rich repeat-containing G (LGR5) is a receptor related
| Pathway | Small molecule | Phase | Target | Cell line | Animal model | Result | Ref. |
|---------|---------------|-------|--------|-----------|--------------|--------|------|
| TGF-B   | Galunisertib (LY2157299) | Phase II/III in HCC | Receptor | SK-HEP1, HepG2, Hep3B, Huh7 | – | Decrease proliferation, increase apoptosis In combination with Sorafenib, the anti-cancer effects was increased in concentration dependent manner | [42] |
|         | PD98059       |       | ERK    | HepG2    | 7 x 10⁵ HepG2 intraperitoneal into nude mice | Inhibit proliferation, migration, invasion, and tumor growth | – | [45] |
| Wnt     | IC-2          |       | TCF/β-catenin | Huh7, HepG2, HLF | Huh7 spheres to flank of NOD/SCID mice | Decrease the CSC subpopulation | – | [63] |
|         | CGP049090/ PKF115-854 | +/- | TCF/β-catenin | Huh7, HepG2, | 1 x 10⁷ HepG2 subcutaneously to nude mice | Induce apoptosis, cell cycle arrest, inhibit tumor growth | – | [191] |
| Hh      | Cyclopamine   | Phase III | SMO receptor | Huh7, PLC, SM-7721, | 5 x 10⁵ Mistheton Lectin-1 into the left liver of mice | Induce apoptosis, inhibit tumor growth | – | [74, 192] |
| GANT61  | –             |       | Gli    | Huh7, Hep3B, HepG2 | 1 x 10⁷ Huh7 cells to flank of SCID mice | Induce the autophagy and apoptosis, Inhibit the HCC tumor growth Similar to Sorafenib, increase the apoptosis | – | [71] |
| GDC-0449| –             |       | SMO receptor | Huh7, MHCC97 | 5 x 10⁶ MHCC97 subcutaneously to syngeneic rat | Decrease the angiogenesis Combined with Sorafenib can modulate the VEGF expression | – | [69] |
| Notch   | PF-4014       | Phase II | γ-secretase | MHCC97, Huh7 | 1 x 10⁶ MHCC97-H or 4 x 10⁵ CSC subcutaneously to nude or SCID mice then tumor cubes were then implanted into nude mice liver lobes | Inhibited the proliferation of HCC and CSC self-renewal, decrease the tumor volume, and suppress the liver tumor metastasis PF-03084014 in combination with cisplatin or doxorubicin increase the anti-cancer effects | – | [80] |
| GSI     | –             | Phase II | γ-secretase | Bel7404, HepG2 | – | Decrease the HCC proliferation and colony formation | – | [81] |
**Table 1 (continued)**

| Pathway | Small molecule | Target | Cell line | Animal model | Result | Ref. |
|---------|----------------|--------|-----------|--------------|--------|-----|
| EGF     | Brivanib       | Tyrosine kinase receptor | Hep3B, HepG2, Huh7 | DEN to rat | HCC apoptosis, cell cycle arrest, inhibit the liver tumor growth | [193] |
|         | U0126          | Erk    | HCCLM3, HepG2 | –            | Decrease proliferation | – |
|         | BEZ-235/ SHBM1009 | PI3K  | –          | –            | –      | – |
| HGF     | PHA665752      | c-met  | MHCC97, Huh7, Hep3B | 3 x 10^5 MHCC97 subcutaneously to nude mice | Inhibit proliferation, tumor growth, and CSC, increase apoptosis | [93] |
| AMG 337 | –             | c-met  | MHCC97, HCCLM3, Hep3B, SNU, JHH5 | human primary HCC tumor tissues | Subcutaneously injecting nude mice | Decrease proliferation, tumor growth | [95] |
| Indo5   | –             | c-met  | HepG2, A549, SMMC-7721, MHCC97H | 2 x 10^8 HepG2, 4 x 10^6 MHCC 97H, 4 x 10^6 MHCC 97 L, 2 x 10^6 A549 cells, or 5 x 10^6 SMMC-7721 subcutaneously to flank of SCID mouse MHCC97H subcutaneously to flank of SCID mouse then insert tumor into liver | Inhibit proliferation, migration, and metastasis Similar or better result in animal model recovery compared with Sorafenib In contrast to Sorafenib without body weight lost | [94] |
| VEGF    | Bufalin        | VEGFR/VEGFR | SMMC-7721, PLC | 5 x 10^6 SMMC-7721 subcutaneously to flank of nude mice | Inhibit angiogenesis, HCC migration, and proliferation The anti-cancer effects of Bufalin improved in combination with Sorafenib | [194] |
| Pathway | Small molecule | Target | Cell line | Animal model | Result | Ref |
|---------|----------------|--------|-----------|--------------|--------|-----|
| Stat3   | Jaki – Jak     | Huh7, Hep3B, HepG2 | –         | Increase apoptosis | Sensitize the HCC to anti-cancer effects of Sorafenib | [112] |
| C188-9  | –              | Stat3  | PLC, HepG2, Huh7 | HepPten- mice | Decrease the survival of HCC, reduce the HCC proliferation, decrease the secretion of inflammatory factors | [113] |
| S3i-201 | –              | Stat3  | Huh7, Hep3B, HepG2 | – | Induce HCC apoptosis and enhance the sorafenib effects | [112] |
| UA      | –              | Stat3  | Huh7, HepG2, SM-7721, Hep3B | 1 × 10⁷ HepG2 subcutaneously into flank of nude mice | Increase the HCC apoptosis, inhibit the tumor growth | [114] |
| 2-Ethoxystypandrone | Stat3 | HepG2 | – | Induce apoptosis and cell cycle arrest, inhibit the CSC self-renewal | – | [115] |
| YAP/TAZ | verteporfin    | YAP/TEAD | Huh7, MLP29 | IP injection of DENA to Rats | Decrease the colony formation, survival, and tumor colony | – | [125] |
| HIF     | PT2385        | HIF-2a | HepG2, Sk-hep1 | of 1 × 10⁸ SK·Hep1 intrahepatic injections to nude mice | Increase the efficiency of sorafenib treatment, decrease invasion and survival | [136] |
| Cell cycle | Dinaciclib | Cdk1,2,5,9 | Hep3B, HLE | 1 × 10⁶ Huh7 cells or 2 × 10⁶ PLC BALB/c subcutaneously to nude mice | Decrease the colony formation, survival, induce cell cycle arrest, decrease the tumor size | [143] |
| Ribociclib | – | CyclinD/cdk4,6 | Huh7, HepG2, Hep3B, PLC | – | Decrease cell proliferation Synergist effects with sorafenib and anti-cancer effects on sorafenib resistance-HCC lines | [144] |
| Pathway | Small molecule | Target | Cell line | Animal model | Result | Ref |
|---------|---------------|--------|-----------|--------------|--------|-----|
| Apoptosis | Tumstatin | – | Akt/mTOR | Huh7, Hep3B | $5 \times 10^6$ Hep3B cells subcutaneously to armpit of nude mice | Induce apoptosis, cell cycle arrest, autophagy, decrease the tumor growth, increase the apoptotic proteins | [171] |
| | Brivanib | – | FGF, VEGf, P53 | Huh7, HepG2, Hep3B, Rat with DENA | Induce cell cycle arrest and apoptosis | – | [82] |
| | Nutlin | – | MDM | Huh7, SM-7721, | Inhibit proliferation and survival | – | [161] |
| | Rubone | – | miR-34a, Bcl2, cyclinD | HepG2, HuH7, Hep3B | $5 \times 10^6$ HepG2 to dorsal flanks of nude mice | Activate the miR34 and inhibit the TGF-B pathway and tumor growth Stronger than Sorafenib | [162] |
| Autophagy | Verteporfin | – | lysosom | HepG2, HuH7 | $2 \times 10^6$ HepG2 to dorsal flanks of nude mice | Induce autophagy Increase the anti-cancer effects with Sorafenib | [173] |
| | NVP-BGT226 | – | mTOR | Hep3B, HepG2, SNU475, Mahlavu | – | Induce autophagy More sensitive to Sorafenib | [174] |
| | Mitoxantrone | – | mTOR | HepG2, HuH7 | – | Induce autophagy | [170] |
| ROS | Propyl gallate | – | ROS formation | HepJ5, Hep3B, Mahlava | 200 HepJ5 or Hep3B injected to yolk of zebrafish embryos | Decrease proliferation, increase apoptosis and autophagy | [186] |
| | Auranofin | – | TXNRD | Hep3B | – | Increase apoptosis | [184] |
to the Wnt/β-catenin pathway and metastasis of HCC [55, 56]. High expression of LGR5 has been found in the PLC and HepG2 lines [57]. Wnt/β-catenin also regulates angiogenesis in the liver tumor [58]. TGF-β, HGF, and environmental cues (such as hypoxia condition) can activate β-catenin [59, 60]. Targeting this pathway at the receptors-ligand level or downstream effectors modulate its activation in HCC [61, 62]. It has been reported that CGP049090 and PKF115-854 can block TCF/LEF/β-catenin interactions [58]. A recent study reported that IC-2 can decrease CSC subpopulation by sphere formation assay [63]. Some of the inhibitors of β-catenin-CBP interaction can induce the differentiation of CSCs [58].

Hedgehog (Hh) signaling
In the liver, hepatocytes and kupffer cells are able to secrete SHH ligands after injury [64, 65]. Hepatitis B virus also activates the SHH pathway [66]. SHH interacts with Patched (Ptch) receptor and triggers Smoothened (Smo) receptor, initiates the signaling cascade, and subsequent nuclear translocation of the transcription factor, and the glioma protein (Gli) [67, 68]. SHH causes the expression of cell cycle-related genes (cyclin D, c-Myc), invasion-related genes (especially MMPs), and CSC-specific genes (like CD133) in HCC [65]. Gli enhances the expression of VEGF in HCC and tumor angiogenesis [69]. SHH can bind to the TGF-β, Wnt, or Notch pathways to promote EMT and metastasis in HCC [65]. Smo and Gli can be increased in several HCC cell lines such as Hep3B, Huh7, Sk-Hep1, and HepG2 [70]. Cyclopamine is a small molecule that inhibits SMO and GANT61 [70–72].

Notch signaling
Activation of the Notch pathway is regulated via the interaction of two receptors on adjacent cells, wherein one of them acts as a ligand (majorly from macrophages) and the other as a receptor, known as the Notch receptor (on hepatocytes) [73, 74]. The intracellular domain (NICD) of the Notch receptor is then cleaved by γ-secretase, which further translocates to the nucleus and binds to the DNA binding transcription factors [75]. The main target genes of the Notch pathway such as Hes1, P53, cyclin-D, and c-Myc control the expression of cancer cell proliferation, invasion, and apoptosis markers [76, 77]. However, it is notable that the Notch pathway has controversial effects on HCC [75]. This pathway crosstalks with the Wnt and SHH pathways for CSC maintenance, the PI3K and mTOR pathways for HCC proliferation, and the VEGF pathway for angiogenesis [78]. The level of the Notch pathway activity in various HCC cell lines depends on their invasion character [79]. For instance, activation of Notch signaling in an invasive MHCC97 cell line is more than the HepG2 cell line [79]. Small molecules like GSI or PF-03084014 (4014) are known to suppress γ-secretase activity [80, 81]. Bivananib is a tyrosine kinase and a Notch3 inhibitor that
promotes the intracellular accumulation of P53 protein and enhances HCC apoptosis [82].

**Epidermal growth factor (EGF) signaling**

The EGF pathway can be abnormally activated in HCC via autocrine or paracrine secretion, which promotes cell proliferation and migration [83]. EGF binds to the EGF receptors and activates PI3K/Akt, MAPK/ERK, P38/MAPK, or NF-kB proteins via a series of downstream signal transduction events [84, 85]. Overexpression and overactivation of EGFR are often observed in HCC [86]. EGF pathway is involved in the recruitment of the inflammatory cells for the secretion of interleukins (IL-1, 6, 8) and tumor progression [87]. U0126 is a small molecule inhibitor of ERK; while BEZ-235 and SHBM1009 are the antagonists of PI3K [87]. EGCG can suppress the EGFR, PI3K/Akt, and MAPK/ERK pathways [88].

**Hepatocyte growth factor (HGF) signaling**

HGF was found to regulate HCC proliferation, survival, and metastasis [89, 90]. HGF binds to the c-met receptor and activates PI3K, ERK, and Jnk/Stat3 pathways [91]. c-Met inhibitors such as capmatinib and tepotinib have been assessed in liver tumor clinical trials [89, 92]. c-Met is overexpressed in the MHCC97 and HCCLM3 cell lines [89]. It has been confirmed that 3-(1H-benzimidazole-2-methylene)-5-(2-methylphenylaminosulfo)-2-indolone (Indo5), PHA665752, and AMG 337 as selective c-MET inhibitors decrease HCC proliferation, migration, and tumor growth [93–96].

**Vascular endothelial growth factor (VEGF) signaling**

In order to ensure efficient nutrient and oxygen supply in the solid tumors, the liver tumor cells secrete growth factors that promote angiogenesis [97]. Angiogenic signals can be triggered via several pathways like HGF, PDGF, FGF, and VEGF [98]. VEGF, as the main angiogenic factor, not only induces angiogenesis, but also interacts with RTK in an autocrine manner, and activates PI3K/Akt pathway in HCC [99, 100]. Sorafenib is known to inhibit the VEGF, PDGF, and FGF pathways, thereby suppressing neoangiogenesis in HCC [101, 102]. LY2109761 (TGF-β inhibitor) can suppress VEGF secretion and neovascularization in HCC [103].

**Targeting common downstream proteins in HCC**

Several growth factors or environmental signaling pathways can activate the common targets in HCC [104, 105]. Signal transducer and activator of transcription 3 (Stat3), Hippo, and HIF are the main downstream proteins that are activated in HCC [106]. Inhibition of these proteins can suppress or weaken the activated signal pathway, thereby modulating the tumorigenicity of HCC [107, 108].

**Janus kinases (Jak)/Stat3 signaling**

The Jak/Stat3 pathway can be stimulated by inflammatory cytokines (such as interleukins, tumor necrosis factor (TNF), HGF, TGF-β, and EGF) [109, 110]. Stat3 as a transcription factor can promote HCC proliferation, metastasis, tumor survival, and angiogenesis [111]. The Jak inhibitors such as Jaki and S3i-201, or Stat3 inhibitor-related small molecules such as C188-9, ursolic acid (UA), and 2-Ethoxystypandrone can induce apoptosis, cell cycle arrest, and block CSC self-renewal in HCC [112–115].

**Hippo signaling**

Several growth factors such as Wnt, Notch, EGF, and SHH can activate the YAP (Yes-associated protein) pathway [116, 117]. Activated YAP translocates to the nucleus and interacts with a transcriptional coactivator, PDZ-binding motif (TAZ), and transcriptional enhanced associate domain (TEAD) to promote proliferation, metastasis, and inhibition of apoptosis and autophagy in HCC [118]. YAP or TAZ are highly expressed in HCC cell lines such as HLF and HepG2 and also primary liver tumor samples [119, 120]. Hippo protein activates several kinases and negatively regulates the expression of oncoprotein YAP [121]. Inhibition of YAP/TAZ/TEAD transcriptional activity is often used for anti-cancer treatment [122–124]. Verteporfin is a small molecule that inhibits YAP/TEAD complex interaction [125].

**Hypoxia signaling**

It has been confirmed that HCC cells rapidly use environmental oxygen [126]. In the center of the liver tumor, hypoxic conditions activate major transcription factors and inducing factors such as HIF-1A, HIF-2A [127]. HIF induces the expression of TGF-β and Snail and enhances EMT in tumor cells [126]. HIF via MMP expression helps in ECM remodeling and tumor cell invasion [128]. It also increases c-Myc expression, HCC proliferation, and escape of HCC from the immune destruction [129]. HIF also inhibits P53 (a tumor suppressor gene), enhances the activity of anti-apoptotic proteins (like Bcl-2, caspases), and prevents HCC apoptosis [126]. HIF-1A promotes CSCs maintenance in liver tumors [130]. HSP90, a general oncogene protein, stabilizes HIF-1A and positively modulates the survival, growth, and metastasis of the tumor cells [131]. Under hypoxia conditions, the cells transition from aerobic to anaerobic metabolism [132]. HIF-1A promotes glycolysis metabolism and increases lactate production in HCC [133]. The components of this pathway also interact with other pathways to promote
tumorigenicity [134]. HIF-1A impacts on downstream signal transduction and increases VEGF expression and angiogenesis in HCC [98]. HIF-1A also stimulates TGF-β interaction with its receptors, enhances HCC survival, and proliferation [128]. Hypoxia activates the expression of Notch downstream genes and recruits HIF-1A for HCC metastasis [130]. Recent studies have suggested that hypoxia can regulate the Hh pathway [130]. The Wnt pathway also increases the expression of HIF-1A in HCC [130]. YAP interacts and stabilizes HIF-1A in HCC [135]. HIF-1A regulates the metabolism of HCC, increases the expression of glycolysis enzymes and glucose uptake receptors for adaptation to the hypoxic condition [130]. Besides, HIF-1A changes the activity of the macrophages and hepatic stellate cells (HSC) to promote HCC survival, growth, and angiogenesis [130]. PT2385 as a small molecule can suppress HIF-activated proteins such as Stat3, VEGF, PDGF, and ERK [136].

**Cell division signaling**

Uncontrolled cell cycle program and telomerase activity in the hepatocytes increase carcinogenesis [137]. The cell cycle is regulated by cyclin-dependent kinases (CDK)/Cyclin complex at different stages [138]. Downstream of signaling pathways such as EGF, TGF-β, TNF, and IL6 can stimulate CDK/CyclinD complex and phosphorylated retinoblastoma (pRb) to promote HCC proliferation [139]. P53, an anti-proliferation protein, activates P16, P21, and P27 tumor suppressor proteins at the G1 phase, thereby hindering the pRb and CDK/Cyclin proteins [140]. Notably, P21 via inhibition of pro-caspase 3 has contradictory effects in cancers [141]. Normal hepatocytes have a cell cycle arrest in the G0 phase; however, in the case of HCC, P21, and P27 are usually degraded [142]. Mutations of β-catenin or P53 lead to sustained expression of c-Myc, misregulation of PI3K and ERK pathways, and uncontrolled cell cycle progression in HCC [138].

In this regard, Dinaciclib and Ribociclib are CDK/pRb inhibitors that upregulate P53 to control HCC proliferation [143, 144].

**Apoptosis signaling**

Targeted activation of the apoptosis pathway in cancer cells is another crucial way in cancer therapy [145, 146]. In normal cells, apoptosis may initiate via the extrinsic (owing to the attachment of external ligands to the receptors) or intrinsic (owing to mitochondrial factors) pathways [147]. Cellular FLICE/caspase-8-inhibitory protein (cFLIP) and Bcl-2 are negative regulators of the apoptosis pathway, while PPARγ acts as an apoptosis inducer [148, 149].

The extrinsic pathway is activated when immune cells secrete TNF-related apoptosis-inducing ligand (TRAIL) that binds to death receptors (DR) on the cell surface [150]. This cascade causes the recruitment of a complex of FAAD-procaspase 8 (DISC complex), and subsequent activation of caspase 8 (an endonuclease and protease), leading to apoptosis [148]. The proteasome complex causes the degradation of tumor suppressor proteins and activation of NF-kB and c-FLIP, thereby promoting the survival and proliferation of HCC [148]. Additionally, NF-kB regulates MMP9 expression and HCC metastasis [151]. On the other hand, in the intrinsic apoptosis pathway, DNA damage in the cells activates P53 protein, triggers the activation of Bax, and mitochondria-mediated caspase activity [152]. P53 is crucial for cell cycle arrest, cell senescence, and cell autophagy [153, 154]. In HCC, mutations or deletion in the P53 gene or increase of its inhibitors such as a ubiquitin ligase DM2 (Double Minute 2) obligate the apoptosis pathway [155]. Snail inhibits the TRAIL pathway and P53 in cancer cells [156]. HCC cell lines express P53 at different levels. For instance, Hep3B, HepG2, and Huh7 have no, normal, and high levels of P53, respectively [157]. PPARγ also positively modulates the components of these pathways and inhibits HCC survival [158]. The strategies that upregulate TRAIL receptors or ligands (via recombinant protein or agonist receptor antibodies) were shown to cause selective apoptosis in HCC cell lines [159, 160]. Co-treatment of HCC cell lines with recombinant TRAIL and Bortezomib (as proteasome inhibitors) increased the apoptosis induction in the Huh7 cells, compared to the primary hepatocytes [159]. Nutlin, an inhibitor of DM, was reported to stabilize P53 and decrease Bcl-2 expression [161]. Rubone can downregulate the expression of Notch, cyclin D1, Bcl-2, while increase P53 level in HCC [162].

**Autophagy signaling**

Autophagy, a type II cell death, is lysosome-dependent and initiated by surrounding the intracellular organelle with a double membrane (autophagosome) and self-degradation of cells [163]. ATG7, LC3, and beclin are the major proteins involved in this process [164]. Depends on the stage of cancer, autophagy either negatively or positively regulates cancer progression [165, 166]. In HCC late stages, autophagy promotes survival, metastasis, and EMT via activation of the TGFβ pathway, P53 degradation, and chemotherapy resistance of HCC [167]. Inhibitors of main signaling pathways such as PI3K/Akt, MAPK/ERK, and JAK/Stat3 can induce autophagy and cell death in HCC [168, 169]. Small molecules like rapamycin, Mitoxantrone (PI3K/mTOR inhibitors), and Erlotinib/Cetuximab (EGFR inhibitors) are thought to activate cellular autophagy and apoptosis in various HCC cell lines [167, 170]. Tumstatin was previously shown to increase the expression of Bax, Fas, and Fasl to induce
apoptosis and autophagy in HCC [171]. However, some studies have found that the suppression of autophagy via 3-MA leads to inhibit HCC growth [167, 172]. Vertepeporfin, mitoxantrone, and NVP-BGT226 are small molecules that trigger autophagy in HCC [170, 173, 174].

Oxidative stress signaling
Both the intrinsic and extrinsic apoptotic pathways affect the mitochondrial respiratory chain and cause the generation of reactive oxygen species (ROS) in the cells [152, 175]. In cancer cells, ROS may play as a double-edged sword in the induction or suppression of tumor growth in a concentration-dependent manner [176]. A low level of ROS is normal in all the cell types, while its moderate level leads to promote cancer development [176]. ROS, via activation of the TGF-β pathway along with an increase in MMP expression, causes EMT, metastasis, and invasion of cancer cells [176]. ROS can stimulate VEGF or the hypoxia pathway to promote angiogenesis in HCC [177, 178] and mediates cell cycle activation and CSC maintenance in cancer [179]. ROS-mediated signaling events mediate chemoresistance to the cancer cells [180]. Though, excessive ROS can disrupt the proteins in mitochondria and promote the DNA mutations, causing the release of pro-apoptotic factors into the cytoplasm of the cancer cells [178]. Accordingly, agents that restore the intracellular REDOX balance or elevate the ROS content cannot be useful in cancer treatment [181, 182]. In this regard, vitamin C as a natural antioxidant can increase ROS production in HCC and stimulate apoptosis, cell cycle arrest, and suppress CSC self-renewal [183]. Auranofin, a thioredoxin reductase (TXNRD) inhibitor, increases ROS in HCC and suppresses both the extrinsic and intrinsic apoptotic pathways [184]. Morin, a flavonoid from Ficus carica, in combination with Auranofin caused apoptosis in HCC [185]. Propyl gallate (PG), a synthetic antioxidant, activates superoxide and ROS formation in HCC, thereby causing autophagy and apoptosis [186]. N-acetylcycteine (NAC) acts as a potent ROS inhibitor [187]. ART, a YAP inhibitor, promotes ROS formation in HCC [188].

Conclusion and perspective
Several important signaling pathways such as TGF-β, Wnt, SHH, Notch, and RTK are misregulated in HCC, compared to the normal hepatocytes. These pathways initiate differential networks that consequently result in HCC cell cycle promotion, EMT, metastasis, vasculogenesis, and anti-apoptotic mechanisms. Suppression of these pathways with small molecules, herbal drugs, and miRNA stimulates cell cycle arrest, apoptosis, and inhibits the invasion of HCC [189, 190]. Simultaneously targeting different signaling pathways or common downstream proteins would facilitate control over malignant HCC. Induction of differentiation in transformed mesenchymal HCC to the epithelial state would also help in regulating the tumorigenesis of HCC. Smart delivery of anti-cancer agents to the liver tumor could facilitate the targeted therapy in this solid tumor.

Abbreviations
APC: Adenomatous polyposis coli; CAI: Cancer-associated fibroblast; CBP: CREB-binding protein; CDK: Cyclin-dependent kinases; Cflp: Cellular NICE/caspase-8-inhibitory protein; DM2: Double Minute 2; DR: Death receptors; HSC: Hepatic stem cells; ROS: Reactive oxygen species; EGF: Epidermal growth factor; EMT: Epithelial-to-mesenchymal transition; Fzd: Fzdized; Gli: Glioma protein; GSK3β: Glycogen synthase kinase 3β; HGF: Hepatocyte growth factor; HCC: Hepatocellular carcinoma; Hh: Hedgehog; Indo5: 3-(1H-benzimidazole-2-methylene)-5-(2methylphenylaminosulfo)-2-indolone; Jak: Janus kinases; LEM: Lymphoid enhancer factor; LGR5: Leucine-rich repeat-containing G, LRP: Lipoprotein receptor-related protein; MMPs: Matrix metalloproteinases; PD-L1: Programmed cell death ligand 1; PRb: Phosphorylated retinoblastoma; Ptch: Patched; Smo: Smoothened; TAM: Tumor-associated macrophages; TCF: T-cell factor; TEAD: Transcriptional enhanced associate domain; TGF-β: Transforming growth factor-β; TME: Tumor microenvironment; TRAIL: TNF-related apoptosis-inducing ligand; TXNRD: Thioredoxin reductase; UA: Ursolic acid; VEGF: Vascular endothelial growth factor; YAP: Yes-associated protein.

Acknowledgements
Not applicable.

Authors’ contributions
ZF has been involved in drafting the manuscript. MV and TA have made substantial contributions to the revisions of the manuscript. MF has made a substantial contribution to the writing and revising of the manuscript and the design of the Figures. All authors read and approved the final manuscript.

Funding
Not applicable.

Availability of data and materials
The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations
Ethics approval and consent to participate
Not applicable.
Consent for publication
Not applicable.

Competing interests
The authors declare that there is no competing interests.

Author details
1 Department of Stem Cells and Developmental Biology, Cell Science Research Center, Royan Institute for Stem Cell Biology and Technology, ACECR, Tehran, Iran. 2 Department of Regenerative Medicine, Cell Science Research Center, Royan Institute for Stem Cell Biology and Technology, ACECR, Tehran, Iran. 3 Department of Biotechnology, Indian Institute of Technology Kharagpur, Kharagpur, West Bengal 721302, India. 4 Fertility, Infertility and Perinatology Research Center, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran.

Received: 5 February 2021   Accepted: 7 April 2021
Published online: 13 April 2021
References
1. Chen S, Cao Q, Wen W, Wang H. Targeted therapy for hepatocellular carcinoma: Challenges and opportunities. Cancer Lett. 2019;4601–9.
2. Balogh J, Victor D 3rd, Asham EH, Burnough GS, Boktour M, Saharia A, Li X, Ghoobial RM, Monsour HP. Jr. Hepatocellular carcinoma: a review. J Hepatol Oncol. 2016;3:41–53.
3. Iida-Ueno A, Enotomo M, Tamori A, Kawada N. Hepatitis B virus infection and alcohol consumption. World J Gastroenterol. 2017;23:2651–9.
4. Midorikawa Y, Takayama T, Nakayama H, Higaki T, Morishita M, Moriya K, Kanda T, Matsuoka S, Moriyama M. Prior hepatitis B virus infection as a co-factor of chronic hepatitis C patient survival after resection of hepatocellular carcinoma. BMC Gastroenterol. 2019;19:147.
5. Li W, Deng R, Liu S, Wang K, Sun J. Hepatitis B virus-related hepatocellular carcinoma in the era of antiviral therapy: The emerging role of non-viral risk factors. Liver Int. 2020;40:2316–25.
6. Kemuri R, Sahu MK, Tripathy A, Uthanshingik K, Behera M. Hepatocellular carcinoma treatment: hurdles, advances and prospects. Hepat Oncol. 2018;5:HEP08.
7. Raza A, Sood GK. Hepatocellular carcinoma review: current treatment, and evidence-based medicine. World J Gastroenterol. 2014;20:4115–27.
8. Chen CP. Role of radiotherapy in the treatment of hepatocellular carcinoma. J Clin Transl Hepatol. 2019;7:183–90.
9. Gallicchio R, Nardelli A, Mainenti P, Nappi A, Capaccione D, Simeon V, Sirignano C, Abbiruzi F, Barbagallo F, Landriscina M, Storto G. Therapeutic strategies in HCC: radiation modalities. Biomed Res Int. 2016;2016:1295329.
10. Lin Y-L, Li Y. Study on the hepatocellular carcinoma model with metastasis. Genes Dis. 2020;3:336–50.
11. Daher S, Massarwa M, Benson AA, Khoury T. Current and future treatment of hepatocellular carcinoma: an updated comprehensive review. J Clin Transl Hepatol. 2018;6:669–78.
12. Swamy SG, Kameshwar VH, Shubha PB, Looi CY, Shannumag MK, Arifuso F, Dharmarajan A, Sethi G, Shivananuj NS, Bishayee A. Targeting multiple oncogenic pathways for the treatment of hepatocellular carcinoma. Target Oncol. 2017;12:1–10.
13. Alqahtani A, Khan Z, Alloghabi A, Said Ahmed TS, Ashraf M, Hammouda DM. Hepatocellular carcinoma: molecular mechanisms and targeted therapies. Medicina (Kaunas). 2019;55:526.
14. Lachenmayer A, Alsinet C, Chang CY, Llovet JM. Molecular approaches to treatment of hepatocellular carcinoma. Dig Liver Dis. 2010;42(Suppl 3):S264–272.
15. Dimri M, Satyanaarayana A. Molecular signaling pathways and therapeutic targets in hepatocellular carcinoma. Cancers (Basel). 2020;12:491.
16. Ma Y-S, Liu J-B, Wu T-M, Fu D. New therapeutic options for advanced hepatocellular carcinoma. Hepatology. 2018;5:HEP08.
17. Ren T, Zhu L, Cheng M. CXCL10 accelerates EMT and metastasis by activating the glycogen synthase kinase-3 beta in hepatocellular carcinoma. Cell Physiol Biochem. 2020;68:153174.
18. Serova M, Tijeras-Raballand A, Dos Santos C, Albuquerque M, Paradis V, Neuzillet C, Benhadji KA, Raymond E, Faivre S, de Gramont A. Effects of TGF-beta signalling in hepatocellular carcinoma epithelial-to-mesenchymal transition reveals joint sonic hedgehog and Wnt pathway activation. Cancer Res. 2014;74:5963–77.
19. Toh TB, Lim JJ, Chow BK-H. Epigenetics of hepatocellular carcinoma. Clin Transl Med. 2019;8:13–13.
20. Shao J, Zhao S, Sun H. Dedifferentiation of hepatocellular carcinoma: molecular mechanisms and therapeutic implications. Am J Transl Res. 2020;12:2009–109.
21. Agarwal T, Subramanian B, Maiti TK. Liver tissue engineering: challenges and opportunities. ACS Biomater Sci Eng. 2019;5:4167–82.
22. Loh C-Y, Chai JY, Tang TT, Wong WF, Sethi G, Shannumag MK, Chong PP, Looi CY. The E-cadherin and N-cadherin switch in epithelial-to-mesenchymal transition: signaling, therapeutic implications, and challenges. Front Genet. 2019;8:1118.
23. Giannelli G, Koulidopoul P, Dituri F, Mikultits W. Role of epithelial to mesenchymal transition in hepatocellular carcinoma. J Hepatol. 2016;65:798–808.
24. Schep C, Baradau I, Costache R, Canutu C, Mihai GI, Didilescu AC, Constantin C, Neagu M. The role of matrix metalloproteinases in the epithelial-to-mesenchymal transition of hepatocellular carcinoma. Anal Cell Pathol (Amst). 2019;2019:942309.
25. Nault J-C, Ningarhari M, Rebouissou S, Zucman-Rossi J. The role of telomeres and telomerase in cirrhosis and liver cancer. Nat Rev Gastroenterol Hepatol. 2019;16:54–58.
26. Hong M, Almutairi MM, Li S, Li J. Wogonin inhibits cell cycle progression by activating the glycogen synthase kinase-3 beta in hepatocellular carcinoma. Phytomedicine. 2020;68:153174.
27. Llovet JM, Bruix J. Molecular targeted therapies in hepatocellular carcinoma. Hepatology. 2008;48:1312–27.
28. Cicchini C, Amicone L, Alonzi T, Marchetti A, Mancone C, Tripodi M. Molecular mechanisms controlling the phenotype and the EMT/MET dynamics of hepatocyte. Liver Int. 2013;35:302–10.
29. Gonzalez DM, Medici D. Signaling mechanisms of the epithelial-mesenchymal transition. Sci Signal. 2014;7:re8.
30. Zhang J, Gu C, Song Q, Zhu M, Xu Y, Xiao M, Zheng W. Identifying cancer-associated fibroblasts as emerging targets for hepatocellular carcinoma. Cell Biosci. 2020;10:127.
31. Hata A, Chen Y-G. TGF-β signaling from receptors to Smads. Cold Spring Harb Perspect Biol. 2016;8:a022061.
32. Fabregat I, Caballero-Diaz D. Transforming growth factor-beta-induced cell plasticity in liver fibrosis and hepatocarcinogenesis. Front Oncol. 2018;8:357.
33. Krstic J, Trivanovic D, Mesiovic S, Santibanez JF. Transforming growth factor-beta and oxidative stress interplay: implications in tumorigenesis and cancer progression. Oxid Med Cell Longev. 2015;2015:654594.
34. Mancarella S, Krol S, Crovace A, Leporatti S, Dituri F, Fruscante M, Gianelli G. Validation of hepatocellular carcinoma experimental models for TGF-β promoting tumor progression. Cancers (Basel). 2019;11:1510.
35. Steinway SN, Zanudo JD, Wing D, Rountree CB, Feith DJ, Loughran Albert TPR Jr. Network modeling of TGFbeta signaling in hepatocellular carcinoma epithelial-to-mesenchymal transition reveals joint sonic hedgehog and Wnt pathway activation. Cancer Res. 2018;78:1539–49.
36. Serova M, Tjeras-Raballand A, Dos Santos C, Albuquerque M, Paradis V, Neuzillet C, Benhadji KA, Raymond E, Faivre S, de Gramont A. Effects of TGF-beta signalling inhibition with galunisertib (LY2157299) in hepatocellular carcinoma models and in ex vivo whole tumor tissue samples from patients. Oncotarget. 2015;6:21614–27.
37. Fransvea E, Angelotti T, Antonacci S, Giannelli G. Blocking transforming growth factor-beta up-regulates E-cadherin and reduces migration and invasion of hepatocellular carcinoma cells. Hepatology. 2008;47:1557–66.
38. Kelley RK, Gane E, Assenat E, Siebler J, Galle PR, Merle P, Hourmand IO, Cleverly A, Zhao Y, Gueorguieva I, Lahn M, Faivre S, Benhadji KA, Raymond E, Faivre S, de Gramont A. Effects of TGF-beta signalling inhibition with galunisertib (LY2157299) in hepatocellular carcinoma models and in ex vivo whole tumor tissue samples from patients. Oncotarget. 2015;6:21614–27.
39. Serova M, Tjeras-Raballand A, Dos Santos C, Albuquerque M, Paradis V, Neuzillet C, Benhadji KA, Raymond E, Faivre S, de Gramont A. Effects of TGF-beta signalling inhibition with galunisertib (LY2157299) in hepatocellular carcinoma models and in ex vivo whole tumor tissue samples from patients. Oncotarget. 2015;6:21614–27.
signaling promotes M2-like macrophage polarization and reinforces tumor malignant behaviors. Cell Death Dis. 2018;9:793–793.

48. Wang W, Smits R, Hao H, He C. Wnt/beta-catenin signaling in liver cancers. Cancers (Basel). 2019;11:926.

49. Xu W, Zhou W, Cheng M, Wang J, Liu Z, He S, Luo X, Huang W, Chen T, Yan W, Xiao J. Hypoxia activates Wnt/beta-catenin signaling by regulating the expression of BCL9 in human hepatocellular carcinoma. Sci Rep. 2017;7:40446.

50. Jung Y-S, Park J-H. Wnt signaling in cancer: therapeutic targeting of Wnt signaling beyond beta-catenin and the destruction complex. Exp Mol Med. 2020;52(2):183–91.

51. Stamos JL, Weis WI. The B-catenin destruction complex. Cold Spring Harb Perspect Biol. 2013;5:a007987–a007989.

52. Liu C, Takada K, Zhu D. Targeting Wnt/beta-catenin pathway for drug therapy. Med Drug Discov. 2020;8:100066.

53. Wang W, Smits R, Hao H, He C. Wnt/beta-catenin signaling in liver cancers. Cancers. 2019;11:926.

54. Zhan T, Rindtorff N, Boutros M. Wnt signaling in cancer. OncoGene. 2017;36:1461–73.

55. Ma Z, Guo D, Wang Q, Liu P, Xiao Y, Wu P, Wang Y, Chen B, Liu Z, Liu Q. Lgr5-mediated p53 Repression through PDCD3 leads to doxorubicin resistance in Hepatocellular Carcinomas. Theranostics. 2019;9:2967.

56. Koni M, Pinnarò V, Brizzi MF. The Wnt signalling pathway: a tailored target in cancer. Int J Mol Sci. 2016;18:585.

57. Effendi K, Yamazaki K, Fukuma M, Sakamoto M. Overexpression of leucine-rich repeat-containing G protein-coupled receptor S (LGRS) represents a typical Wnt/beta-catenin pathway-activated hepatocellular carcinoma. Liver Cancer. 2014;3:451–7.

58. Vilchez V, Turirol L, Marti F, Gedaly R. Targeting Wnt/beta-catenin pathway in hepatocellular carcinoma treatment. World J Gastroenterol. 2016;22:823–32.

59. Zheng J-J, Que Q-Y, Xu H-T. Hypoxia activates SOX5/Wnt/beta-catenin signaling by suppressing MiR-338-3p in gastric cancer. Technol Cancer Res Treat. 2020;19:1533038820905825.

60. Han Z, Li Y, Yang B, Tan R, Wang M, Zhang B, Dai C, Wei L, Chen D, Chen Z. Agmatine attenuates liver ischemia reperfusion injury by activating Wnt/beta-catenin signaling in mice. Transplantation. 2020;104(9):1906–16.

61. Yuan K, Xie K, Lan T, Xu L, Chen X, Li X, Liao M, Li J, Huang J, Zeng Y. TNXDC12 promotes EMT and metastasis of hepatocellular carcinoma cells via activation of beta-catenin. Cell Death Differ. 2020;27:1355–68.

62. Hou J, Zhao N, Zhu R, Pang J, Du Y, Shen W. Irradiated esenchymal stem cells support stemness maintenance of hepatocellular carcinoma stem cells through Wnt/beta-catenin signaling pathway. Cell Biosci. 2020;10:1–7.

63. Seto K, Sakabe T, Itaba N, Azumi J, Oka H, Morimoto M, Umeyita Y, Shiota G. A novel small-molecule WNT inhibitor, IC-2, has the potential to suppress liver cancer stem cells. Anticancer Res. 2013;33:3659–79.

64. Shen X, Peng Y, Li H. The injury-related activation of hedgehog signaling pathway modulates the repair-associated inflammation in liver fibrosis. Front Immunol. 2017;8:1450–1450.

65. Jeng KS, Jeng CJ, Jeng WJ, Sheen I, Li SY, Leu CM, Tsay YG, Chang CF. Sonic Hedgehog signaling pathway as a potential target to inhibit the progression of hepatocellular carcinoma. Oncol Lett. 2019;18:4377–84.

66. Li Y, Jiang M, Li M, Chen Y, Wei C, Peng J, Liu C, Xu Z, Tong G, Zhou D, He J. Compound phyllanthus urinaria L inhibits HBV-related HCC via inhibition of matrix metalloproteinase-2 and -9 and vascular endothelial growth factor. Oncol Rep. 2012;28:874–82.

67. Peng Q, Deng Z, Pan H, Gu L, Liu Q, Tang Z. Mitogen-activated protein kinase signaling pathway in oral cancer. Oncol Lett. 2018;15:1379–88.

68. Cargnello M, Roux PP. Activation and function of the MAPKs and their substrates, the MAPK-activated protein kinases. Microbiol Mol Biol Rev. 2011;75:50–83.

69. Zeng Z, Xiong W. Function of the c-Met receptor tyrosine kinase in carcinogenesis and associated therapeutic opportunities. Mol Cancer. 2020;8:1–5.

70. Wang Y, Han C, Lu L, Magliato S, Wu T. Hedgehog signaling pathway regulates autophagy in human hepatocellular carcinoma cells. Hepatology. 2013;58:995–1010.

71. Wang Y, Chen X, Qin S, Cheng AL, Stammberger U, Locatelli G, Faivre S. Recent developments of c-Met as a therapeutic target in hepatocellular carcinoma. Hepatology. 2018;67:1132–49.
93. You H, Ding W, Dang H, Jiang Y, Routen ee CB. c-Met represents a potential therapeutic target for personalized treatment in hepatocellular carcinoma. Hepatology. 2011;54:879–89.

94. Luo T, Zhang SC, Zhu LF, Zhang FX, Li W, Zhao K, Wen XX, Yu M, Zhan YQ, Chen H, Ge CH, Gao HY, Wang L, Yang XM, Li CY. A selective c-Met and Trks inhibitor Indos suppresses hepatocellular carcinoma growth. J Exp Clin Cancer Res. 2019;38:130.

95. D u Z, Caenepael S, Shen Y, Rex K, Zhang Y, He Y, Tang ET, Wang O, Zhong W, Zhou H, Huang J, Huang E, Hu L, Coxon A, Zhang M. Preclinical Evaluation of AMG 337, a highly selective small molecule MET inhibitor, in hepatocellular carcinoma. Mol Cancer Ther. 2016;15:1227–37.

96. Korh an P, Erdal E, Atabey N. miR-181a-5p is downregulated in hepatocellular carcinoma and suppresses motility, invasion and branching-morphogenesis by directly targeting c-Met. Biochem Biophys Res Commun. 2014;450:1304–12.

97. Lugano R, Ramachandran M, Dimberg A. Tumor angiogenesis: causes, consequences, challenges and opportunities. Cell Mol Life Sci. 2020;77:1745–70.

98. Semela D, Dufour JF. Angiogenesis and hepatocellular carcinoma. J Hepatol. 2004;41:864–80.

99. Mahonnet M, Descottes B, Vallex D, Labrousse F, Denizot Y. VEGF in hepatocellular carcinoma and surrounding cirrhotic liver tissues. World J Gastroenterol. 2006;12:830–1.

100. Hamdy M, Shaheen K, Awad MA, Barakat EM, Shalaby S, Gupta N, Gupta V. Vascular endothelial growth factor (VEGF) as a biochemical marker for the diagnosis of hepatocellular carcinoma (HCC). Clin Pract. 2020;7:1441–53.

101. More MA, Sun W, Kim R, He AR, Abada PB, Mynderse M, Finn RS. The role of angiogenesis in hepatocellular carcinoma. Clin Cancer Res. 2019;25:921–20.

102. Tang W, Chen Z, Zhang W, Cheng Y, Zhang B, Wu F, Wang Q, Wang S, Rong D, Reiter FP, De Toni EN, Wang X. The mechanisms of sorafenib resistance in hepatocellular carcinoma: theoretical basis and therapeutics. World J Gastroenterol. 2006;12:830–1.

103. Mazzocca A, Francesca E, Lavezzi G, Antonaci S, Giannelli G. Inhibition of transforming growth factor beta receptor I kinase blocks hepatocellular carcinoma growth through neo-angiogenesis regulation. Hepatology. 2009;50:1140–51.

104. Kudo M. Signaling pathway/molecular targets and new targeted agents under development in hepatocellular carcinoma. World J Gastroenterol. 2012;18:6005–17.

105. Moeini A, Cornellà H, Villanueva A. Emerging signaling pathways in hepatocellular carcinoma. J Hepatol. 2019;70:141–51.

106. Liu Y, Wang X, Yang Y. Hepatic Hippo signaling inhibits development of hepatocellular carcinoma. Clin Mol Hepatol. 2020;26:472–50.

107. Luo T, Wang J, Jiang C, Wu J. The role of hypoxia inducible factor-1 in hepatocellular carcinoma. Biomed Res Int. 2014;2014:490272.

108. Jia J, Qiao Y, Pilo MG, Cigliano A, Liu X, Shao Z, Calvisi DF, Chen X. The role of hypoxia inducible factor (HIF) in hepatocellular carcinoma. Biomed Res Int. 2014;2014:409272.

109. Li W, Zhang Q, Chen K, Sima Z, Liu J, Yu Q. 2-Ethoxystyrpxandrone, a novel small-molecule STAT3 signaling inhibitor from Polygonum cuspidatum, inhibits cell growth and induces apoptosis of HCC cells and HCC cancer stem cells. BMC Complement Altern Med. 2019;19:38.

110. Shi J, Farzaneh M, Khoshnam SE. Yes-associated protein and PDZ-binding motif: a critical signaling pathway in the control of human pluripotent stem cells self-renewal and differentiation. Cell Reprogram. 2020;22:55–61.

111. Warren JSA, Xiao Y, Lamarr JM. YAP/TAZ Activation as a Target for Treat- ing Metastatic Cancer. Cancers (Basel). 2018;10:1115.

112. Pobabi AV, Hong W. A combat with the YAP/TAZ-TEAD oncoproteins for cancer therapy. Theranostics. 2020;10:3622–35.

113. Liu Y, Wang X, Yang Y. Hepatic Hippo signaling inhibits development of hepatocellular carcinoma. Clin Mol Hepatol. 2020;26:472–50.

114. Perra A, Kovalik MA, Ghiso E, Ledda-Columbano GM, Di Tommaso L, Angioni MM, Raschioni C, Testore E, Roncalli M, Giordano S, Colombano A. YAP activation is an early event and a potential therapeutic target in liver cancer development. J Hepatol. 2014;61:1088–96.

115. Chen C, Lou T. Hypoxia inducible factors in hepatocellular carcinoma. Oncotarget. 2017;8:46691–703.

116. Lee JW, Ko J, Cu C, Eltzschig HK. Hypoxia signaling in human diseases and therapeutic targets. Exp Mol Med. 2019;51:1–13.

117. Lin D, Wu J. Hypoxia inducible factor in hepatocellular carcinoma: a therapeutic target. World J Gastroenterol. 2015;21:12171–8.

118. Mu H, Yu G, L-H, Wang M, Cu Y, Zhang T, Song T, Liu C. Mild chronic hypoxia-induced HIF-2α interacts with c-MYC through competition with HIF-1α in hepatocellular carcinoma proliferation. 2020;33:1–13.

119. Guo Y, Xiao Z, Yang L, Gao Y, Zhou H, Hu L, Huang D, Xu Q. Hypoxiainducible factors in hepatocellular carcinoma (Review). Oncol Rep. 2019;8(28):46691.

120. Liu X, Chen S, Tu J, Cai W, Yu Q. HSP90 inhibits apoptosis and promotes growth by regulating HIF-1α abundance in hepatocellular carcinoma. Int J Mol Med. 2016;37:825–35.

121. Koziel A, Jarmuszkiewicz W. Hypoxia and aerobic metabolism adaptation of human endothelial cells. Pflugers Arch. 2017;469:815–27.

122. Jia YY, Zhao JF, Li BL, Gao K, Song Y, Liu ML, Yang XJ, Xue Y, Wen AD, Shi L. miR-92/WS1/HIF-1α axis inhibits glycolytic metabolism to decrease hepatocellular carcinoma growth. Oncotarget. 2016;7:35257–69.

123. Masoud GN, Li W. HIF-1α pathway: role, regulation and intervention for cancer therapy. Acta Pharm Sin B. 2015;5:378–89.

124. Zhang X, Li Y, Ma H, Yang L, Wang T, Meng X, Zong Z, Sun X, Hua X, Li H. Yes-associated protein (YAP) binds to HIF-1α and sustains HIF-1α protein stability to promote hepatocellular carcinoma cell glycolysis under hypoxic stress. J Exp Clin Cancer Res. 2018;37:216.

125. Xu J, Zheng L, Chen J, Sun Y, Lin H, Jin RA, Tang M, Liang X, Cai X. Increasing AR by HIF-2α/parp inhibitor (PT2385) overcomes the side-effects of sorafenib by suppressing hepatocellular carcinoma invasion via alteration of pSTAT3, pAKT and pERK signals. Cell Death Dis. 2018;9:e3095.

126. Nault JC, Ningarhari M, Rebouissou S, Zucman-Rossi J. The role of telomeres and telomerase in cirrhosis and liver cancer. Nat Rev Gastroenterol Hepatol. 2019;16(9):544–58.
138. Bisteau X, Caldez MJ, Kaldis P. The complex relationship between liver cancer and the cell cycle: a story of multiple regulations. Cancers (Basel). 2014;6:79–111.

139. Sonntag R, Giebler N, Nevozorova YA, Bangen J-M, Fahrenkamp D, Lambertz D, Haas U, Hu W, Cassler N, Guberl FJ, Müller-Newen G, Abdallah AT, Weiskirchen R, Ticconi F, Costa KG, Barbacid M, Trautwein C, Liedtke C. Cyclin E1 and cyclin-dependent kinase 2 are critical for initiation, but not for progression of hepatocellular carcinoma. Proc Natl Acad Sci U S A. 2018;115:9282–7.

140. Wang TJ, Huang MS, Hong CY, Tse V, Silverberg GD, Hsiao M. Comparisons of tumor suppressor p53, p21, and p16 gene therapy effects on glioblastoma tumorigenicity in situ. Biochem Biophys Res Commun. 2001;287:173–80.

141. Shamloo B, Usluer S. p21 in cancer research. Cancers (Basel).

142. Yang Z, Zhang J, Lin X, Wu D, Li G, Zhong C, Li M, Fang L, Jiang P, Yin L, Zhang L. Inhibition of neddylation modification by MLN4924 sensitizes hepatocellular carcinoma cells to sorafenib. OncoRep. 2019;41:3257–69.

143. Shao YY, Li YS, Hsu HW, Lin H, Wang HY, Wo RR, Cheng AL, Hsu CH. Apoptosis: a target for anticancer therapy. Int J Mol Sci. 2018;19:448.

144. Fayyaz S, Yaylim I, Turan S, Kanwal S, Farooqi AA. Hepatocellular carcinoma: targeting of oncogenic signaling networks in TRAIL resistant cancer cells with autophagy involvement. Biochem Biophys Res Commun. 2020;521:232–7.

145. Liu F, Wang F, Dong X, Xiu P, Sun P, Li Z, Shi X, Zhong J. T7 peptide cytotoxicity in human hepatocellular carcinoma cells is mediated by suppression of apoptosis. Int J Mol Med. 2019;44:523–34.

146. Stiuso P, Potenza N, Lombardi A, Ferrandino I, Monaco A, Zappavigna S, Vanacore D, Mosca N, Castiello F, Porto S, Addeo R, Peres SD, De Vita F, Russo A, Caraglia M. MicroRNA-423-5p promotes autophagy in cancer cells and is increased in serum from hepatocarcinoma patients treated with sorafenib. Mol Ther Nucleic Acids. 2015;4:e233.

147. Simioni C, Cani A, Martelli AM, Zauli G, Alimena AA, Ultimo S, Tabelini G, McCubrey JA, Capitan S, Neri LM. The novel dual P38/mTOR inhibitor NVP-BGT226 displays cytotoxic activity in both normoxic and hypoxic hepatocarcinoma cells. Oncotarget. 2016;7:1325–37.

148. Aguilar-V, Tuli HS, Varol A, Thakral F, Yerer MB, Sak K, Varol M, Jain A, Khar MA, Sethi G. Role of reactive oxygen species in cancer progression: molecular mechanisms and recent advancements. Biomolecules. 2019;9(1):735.

149. Takekawa A, Yamamoto K. Control of oxidative stress in hepatocellular carcinoma: helpful or harmful? World J Hepatol. 2015;7:968–79.

150. Cardin R, Piciocchi M, Bortolami M, Kotsas A, Barzon L, Lavezzo E, Siniaglia A, Rodriguez-Castro KI, Rugge M, Farinati F. Oxidative damage in the progression of chronic liver disease to hepatocellular carcinoma: an intricate pathway. World J Gastroenterol. 2014;20:3078–86.

151. Kim EK, Jang M, Song MJ, Kim D, Kim Y, Jang HH. Redox-mediated mechanism of chemoresistance in cancer cells. Antioxidants (Basel). 2019;8(10):471.
180. Huang G, Pan S-T. ROS-mediated therapeutic strategy in chemo-/ radiotherapy of head and neck cancer. Oxid Med Cell Longev. 2020;2020:5047987.
181. Perillo B, Di Donato M, Pezone A, Di Zazzo E, Giovannelli P, Galasso G, Castoria G, Migliaccio A. ROS in cancer therapy: the bright side of the moon. Exp Mol Med. 2020;52:192–203.
182. Liou G-Y, Storz P. Reactive oxygen species in cancer. Free Radic Res. 2010;44:479–96.
183. Lv H, Wang C, Fang T, Li T, Lv G, Han Q, Yang W, Wang H. Vitamin C preferentially kills cancer stem cells in hepatocellular carcinoma via SVCT-2. NPJ Precis Oncol. 2018;2:1.
184. Hwang-Bo H, Jeong JW, Han MH, Park C, Hong SH, Kim GY, Moon SK, Cheong J, Kim WJ, Yoo YH, Choi YH. Auranofoxin, an inhibitor of thioredoxin reductase, induces apoptosis in hepatocellular carcinoma Hep3B cells by generation of reactive oxygen species. Gen Physiol Biophys. 2017;36:117–28.
185. Hwang-Bo H, Lee WS, Nagappan A, Kim HJ, Panchanathan R, Park C, Chang SH, Kim ND, Lee SH, Chang YC, Kwon TK, Cheong JH, Kim GS, Jung JM, Shin SC, Hong SC, Choi YH. Morin enhances auranofoxin anticancer activity by up-regulation of DR4 and DR5 and modulation of Bcl-2 through reactive oxygen species generation in Hep3B human hepatocellular carcinoma cells. Phytother Res. 2019;33:1384–93.
186. Wei PL, Huang CY, Chang YJ. Propyl gallate inhibits hepatocellular carcinoma cell growth through the induction of ROS and the activation of autophagy. PLoS ONE. 2019;14:e0210513.
187. Yuan Z, Liang Z, Yi J, Chen X, Li R, Wu J, Sun Z. Koumine promotes ROS production to suppress hepatocellular carcinoma cell proliferation via NF-kappaB and ERK/p38 MAPK signaling. Biomolecules. 2019;9(10):559.
188. Li Y, Lu J, Chen Q, Han S, Shao H, Chen P, Jin Q, Yang M, Shangguan F, Fei M, Wang L, Liu Y, Liu N, Lu B. Artemisinin suppresses hepatocellular carcinoma cell growth, migration and invasion by targeting cellular bioenergetics and Hippo-YAP signaling. Arch Toxicol. 2019;93:3367–83.
189. Liu C, Yang S, Wang K, Bao X, Liu Y, Zhou S, Liu H, Qiu Y, Wang T, Yu H. Alkaloids from Traditional Chinese Medicine against hepatocellular carcinoma. Biomed Pharmacother. 2019;120:109543.
190. Singh A, Shafi S, Upadhyay T, Najmi AK, Kohli K, Pottore FH. Insights into nanotherapeutic strategies as an impending approach to liver cancer treatment. Curr Top Med Chem. 2020;20:1839–54.
191. Wei W, Chua MS, Grepper S, So S. Small molecule antagonists of Tcf4/ beta-catenin complex inhibit the growth of HCC cells in vitro and in vivo. Int J Cancer. 2010;126:2426–36.
192. Jeng KS, Sheen IS, Jeng WJ, Yu MC, Tsai HH, Chang FY, Su JC. Blockade of the sonic hedgehog pathway effectively inhibits the growth of hepatoma in mice: An in vivo study. Oncol Lett. 2012;4:1158–62.
193. Tsang CM, Cheung KC, Cheung YC, Man K, Lui VW, Tiao SW, Feng Y. Berberine suppresses Id-1 expression and inhibits the growth and development of lung metastases in hepatocellular carcinoma. Biochim Biophys Acta. 2015;1852:541–51.
194. Wang H, Zhang C, Chi H, Meng Z. Synergistic anti-hepatoma effect of bufalin combined with sorafenib via mediating the tumor vascular microenvironment by targeting mTOR/VEGF signaling. Int J Oncol. 2018;52:2051–60.

Publisher’s Note
Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.