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Review

Targeting the mTOR regulatory network in hepatocellular carcinoma: Are we making headway?

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ABSTRACT

The mechanistic target of rapamycin (mTOR) pathway coordinates organismal growth and homeostasis in response to growth factors, nutrients, and cellular energy stage. The pathway regulates several major cellular processes and is implicated in various pathological conditions, including hepatocellular carcinoma (HCC). This review summarizes recent advances of the mTOR pathway, highlights the potential of the mTOR pathway as a therapeutic target, and explores clinical trials targeting the mTOR pathway in HCC. Although the review focuses on the mTOR pathway involved in HCC, more comprehensive discussions (eg, developing a rational design for future trials targeting the mTOR pathway) are also applicable to other tumors.

1. Introduction

Hepatocellular carcinoma (HCC), accounting for 70–90% of all primary liver cancer, represents the sixth most frequent cancers, and ranks the fourth most prevalent cause of cancer-related mortality worldwide [1]. Hepatitis B and C virus infection, aflatoxin B1 exposure, excessive alcohol consumption, obesity, and some inherited metabolic disorders are generally considered to be major risk factors in HCC development [2]. Over the past decades, several families of signaling cascades, such as the EGFR (epidermal growth factor receptor) pathway, the c-Met/hepatocyte growth factor pathway, the Hedgehog pathway, the Ras/Raf/MAPK (mitogen-activated protein kinase)

Abbreviations: HCC, hepatocellular carcinoma; EGFR, epidermal growth factor receptor; MAPK, mitogen-activated protein kinase; PI3K, phosphoinositide 3-kinase; AKT, protein kinase B; mTOR, mechanistic target of rapamycin; TOR1, target of rapamycin 1; mTORC1, mTOR complex 1; mLST8, mammalian lethal with sec-13 protein 8; DEPTOR, DEP domain-containing mTOR-interacting protein; Raptor, regulatory-associated protein of mTOR; PRAS40, prolinerich Akt substrate 40 kDa; Rictor, rapamycin-insensitive companion of mTOR; mSin1, mammalian stress-activated map kinase-interacting protein 1; Protor1/2, protein observed with Rictor 1 and 2; S6K, S6 kinase; 4EBP1, eukaryotic translation initiation factor 4E binding protein 1; RHEB, ras homologue enriched in brain; TSC1/2, tuberous sclerosis 1 and 2; AMPK, AMP-activated protein kinase; PTEN, phosphatase and tensin homologue; SGK, serum/glucocorticoid regulated kinase; PKC, protein kinase C; TSC, tuberous sclerosis complex; LKB1, liver kinase B1; p-mTOR, phosphorylated mTOR; p-S6K1, phosphorylated ribosomal protein S6 kinase B1; p-4EBP1, phosphorylated 4EBP1; eIF4E, eukaryotic translation initiation factor 4E; PIK3CA, phosphoinositide 3-kinase catalytic subunit alpha; PIK3CB, phosphoinositide 3-kinase catalytic subunit beta isoform; RPS6KA3, ribosomal protein S6 kinase A3; IGF1R, insulin-like growth factor 1 receptor; HBX, hepatitis B virus X; HBV, hepatitis B virus; HCV, hepatitis C virus; p-TSC1, phosphorylated tuberous sclerosis 1; HPIP, hematopoietic pre–B-cell leukemia transcription factor-interacting protein; AFP, α-fetoprotein; VEGF, vascular endothelial growth factor; p-Akt, phosphorylated Akt; YAP1, yes-associated protein 1; TAZ, transcriptional coactivator with PDZ-binding domain; S6K1, ribosomal protein S6 kinase B1; RPS6, ribosomal protein S6; HIF-1α, hypoxia-inducible factor 1-alpha; Mcl-1, myeloid cell leukemia-1; MMP9, matrix metalloproteinase 9; ncRNAs, non-coding RNAs; miRNAs, microRNAs; lncRNA, long non-coding RNA; PIK3CD, phosphoinositide 3-kinase catalytic subunit delta; PIK3R3, phosphoinositide-3-kinase regulatory subunit 3; EpCAM, epithelial cell adhesion molecule; MALAT1, metastasis-associated lung adenocarcinoma transcript 1; SRSF1, serine/arginine-rich splicing factor 1; HULC, highly up-regulated in liver cancer; FDA, Food and Drug Administration; PFS, progression-free survival; EVOLVE-1, the first Everolimus for Liver Cancer Evaluation; HDAC, histone deacetylase; MTD, maximum tolerated dose; SHARP, Sorafenib Hepatocellular Carcinoma Assessment Randomized Protocol; TACE, transarterial chemoembolization; LT, liver transplantation; R, randomized; NR, non-randomized; TNM, tumor node metastasis; BCLC, Barcelona clinic liver cancer; PR, partial response; CR, complete response; DLTs, dose-limiting toxicities; DSMB, Data and Safety Monitoring Board; n.a., not available; vs., versus; RFS, recurrence-free survival; OS, overall survival.

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pathway, the Wnt pathway, and the PI3K (phosphoinositide 3-kinase)/Akt (protein kinase B)/mTOR (mechanistic target of rapamycin, originally known as mammalian target of rapamycin) pathway, have been identified aberrant activation in the development and progression of HCC [3].

mTOR responds to various environmental factors, and regulates diverse cellular functions, including proliferation, survival, growth, autophagy, metabolism, metastasis, and angiogenesis [4]. Growing evidence suggests that imbalanced mTOR expression is one of the most commonly observed pathological alterations in human malignancies including HCC, which highlights the potential of the mTOR pathway as an effective pharmacological target in anti-tumor therapy. To elucidate the significance of the pathway, excellent reviews have highlighted their role in any number of cancers [4–6].

Here we summarize current understanding of the fast-evolving field of the mTOR pathway in the context of molecular pathogenesis, biological effects, as well as crosstalk with non-coding RNAs and signaling pathway in HCC. We also explore therapeutic targets and clinical trials targeting the mTOR pathway for this deadly disease.

2. Discovery of the mTOR pathway and its regulation

Rapamycin, a lipophilic macrolide, is extracted from an organism (*Streptomyces hygroscopicus*) isolated from soil samples of Easter Island [7]. In the early 1990s, Heitman and his colleagues discovered target of rapamycin 1 (TOR1) and TOR2 genes functioning in rapamycin toxicity using genetic screening in budding yeast [8]. Soon afterwards, a 289-kDa protein was identified in mammalian cells and named mTOR [9–11]. The mTOR protein shares approximately 45% homology to both TOR1 and TOR2 of *Saccharomyces cerevisiae*, while mTOR proteins in human, rat and mouse share over 95% homology in the amino acid sequence [11,12], which indicates its evolutionary conservation across species.

mTOR belongs to a protein family termed PI3K-related kinase, and forms the core of two distinct multiprotein signaling complexes, mTOR complex 1 (mTORC1) and mTORC2, both of which contain shared and unique subunits (Fig. 1). Both mTOR complexes contain mTOR, mLST8 (mammalian lethal with sec-13 protein 8, also known as G-protein β-subunit−like protein (GβL)), and DEPTOR (DEP domain-containing mTOR-interacting protein) [6]. The scaffolding protein Raptor (protein regulatory-associated protein of mTOR) and PRAS40 (prolinerich Akt substrate 40 kDa) are unique to mTORC1 [6], while the scaffolding protein Rictor (rapamycin-insensitive companion of mTOR), mSin1 (mammalian stress-activated map kinase-interacting protein 1), and Protor1/2 (protein observed with Rictor 1 and 2) are specific to mTORC2 [6].

As diverse subunits exist, the mechanisms underlying activation of mTORC1 and mTORC2 are distinct (Fig. 1). mTORC1 is activated by growth factors, amino acids, oxygen status, and energy levels, while inhibited by stress and rapamycin in response to both intra- and extracellular stimuli [4]. Once activated, mTORC1 directly targets its downstream targets including SREBP (sterol regulatory element-binding protein), S6K (S6 kinase), 4EBP1 (4E binding protein 1), and autophagy components to stimulate energy metabolism, protein and lipid synthesis, as well as to block autophagy [4]. In addition, RHEB (ras homologue enriched in brain), Rag, TSC1/2 (tuberous sclerosis 1 and 2), and AMPK (AMP-activated protein kinase) also exert unique functions as mTORC1 upstream regulators [13]. RHEB and Rag positively regulate mTORC1 activation, while TSC1/2 and AMPK function as its upstream negative regulators [13]. It should be noted that PI3K, Akt, and PTEN (phosphatase and tensin homologue) as indirect upstream regulators of mTORC1, also play pivotal roles in the mTOR pathway. The PI3K/Akt pathway activated by growth factors or cytokines positively regulates the mTORC1 pathway through suppressing TSC1/2; while PTEN negatively modulates this pathway through inhibiting the PI3K pathway [4].

In contrast to mTORC1, mTORC2 is insensitive to nutrients but largely responsive to growth factors [14,15]. Insulin stimulates the association of mTORC2 with ribosome via the PI3K pathway [16]. Upon activation of mTORC2, its major downstream targets such as SGK (serum/glucocorticoid regulated kinase), Akt and PKC (protein kinase C), are phosphorylated to regulate multiple cellular processes including cell survival, metabolism and cytoskeleton organization [4,17]. However, the tangible mechanism on mTORC2 under physiological conditions is still less understood.

3. Alterations of the mTOR pathway in HCC

Alterations in the mTOR pathway are common and pivotal in the context of tumorigenesis. The canonical examples are tuberous sclerosis complex (TSC) and the Peutz-Jeghers syndrome, both of which are tumor-predisposing syndromes resulting from TSC1/2 mutation in the former and liver kinase B1 (LKB1) defective in the latter [18,19].

Dysregulation of the mTOR pathway is also a frequent case in HCC. mTOR mRNA level is upregulated in > 50% of HCC cases [20], and phosphorylated mTOR (p-mTOR) protein in 15–41% of HCC patients [21,22]. Activation of Rictor, a constituent of mTORC2, occurred in 25% of HCC cases [23]. Further, our previous studies and others showed that downstream targets of mTORC1, including phosphorylated ribosomal protein S6 kinase B1 (p-S6K1), phosphorylated 4EBP1 (p-4EBP1) and eukaryotic translation initiation factor 4E (eIF4E), increase in 37–49% of HCC cases [21,23,24].

A number of studies suggested that aberrant regulation of the mTOR pathway exerts oncogenic effects in hepatocytes, subsequently transforming them into cancer cells. These findings are supported by results from liver-specific TSC1 knockout mice in which chronic mTORC1 activation initiates sporadic development of pathological characteristics (including inflammation, necrosis, liver damage, and regeneration) [25], and from Raptor-deleted mice where mTORC1 inactivation impairs c-Myc − induced hepatocarcinogenesis [26]. The precise mechanisms whereby mTORC2 modulated hepatocarcinogenesis have remained poorly understood for a long time. However, recent studies demonstrated that mTORC2 promotes hepatocarcinogenesis through modulating lipid synthesis [27,28], and plays an oncogenic role in PTEN-knockout combined with c-Met − overexpression mice [29].

The underlying mechanism of the mTOR pathway dysfunction is still unknown in HCC. Generally, alterations in copy numbers or somatic mutations of PTEN and/or phosphoinositide 3-kinase catalytic subunit alpha (PIK3CA) are regarded as major factors in many cancers, such as gastric, breast, and colon cancers [5,30–33]. However, it's not the case in HCC. PTEN mutation was only found in 1–3.1% of HCC samples [23,34–36], as well as a low mutation rate of PIK3CA (0–1.6%) [23,35,37,38], phosphoinositide 3-kinase catalytic subunit beta isoform (PIK3CB; 3.1%) [34] and ribosomal protein S6 kinase A3 (RPS6KA3; also known as RSK2; 5.2–9.6%) was also found in HCC [34,38]. Currently, the most frequently mutated genes in the mTOR pathway of HCC cases were TSC1/2 (16.2%; Fig. 1) [34].

Increasing evidence supports a ligand-dependent mechanism for delineating activation of upstream receptor kinases in the mTOR pathway, such as EGFR in 68% of HCC cases [39], c-Met in 83% of HCC [40], insulin-like growth factor 1 receptor (IGF1R) in 68.7% of HCC [23]. In contrast, low or no expression of PTEN, another upstream regulator of mTOR, was found in 47% of HCC cases [21]. Significantly, positive correlations between upregulated EGFR or IGF1R and downregulated PTEN were recognized in the activation of the mTOR pathway in HCC [21,23].

Other factors have also been reported to participate in alterations of the mTOR pathway in HCC, including hepatitis virus infection and the interaction between the mTOR pathway and the Hippo pathway (Fig. 1). Hepatitis B virus X (HBx) protein, a key player in the development of hepatitis B virus (HBV)-related HCC, inhibited phosphorylated tuberous sclerosis 1 (p-TSC1), upregulated p-S6K1 [41] and

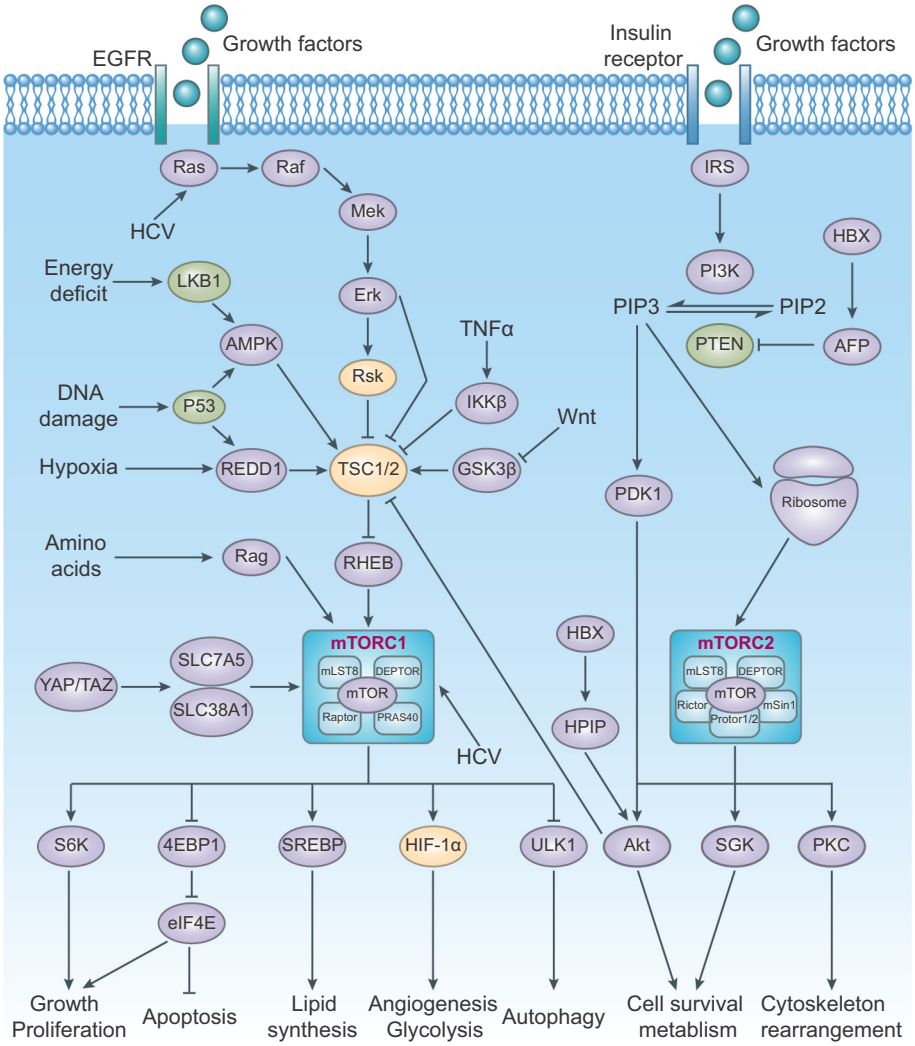

Fig. 1. Schematic illustration of major factors and actions of the mTOR pathway. The major mTORC1 upstream regulators include RHEB, Rag, TSC1/2, AMPK, PI3K, Akt, and PTEN. Among these, TSC1/2 is a key upstream regulator of mTORC1. Multiple growth factors, including Wnt, TNFα, and insulin and insulin-like growth factor-1 (IGF-1) that activate the PI3K and Ras pathways, stimulate mTORC1 through inhibiting TSC1/2. Intracellular and environmental stresses, including DNA damage, hypoxia and energy deficit, suppress mTORC1 through activating TSC1/2. mTORC1 acts on its downstream targets (including S6K, 4EBP1, SREBP, HIF-1α and autophagy components) to modulate cell growth, proliferation, apoptosis, lipid synthesis, glycolysis, angiogenesis and autophagy in HCC. Besides, HBV, HCV and YAP/TAZ can function as mTORC1 upstream positive regulators in HCC. In contrast to mTORC1, the only well-characterized knowledge about the upstream regulators of mTORC2 is that insulin stimulates the association of mTORC2 with ribosome via the PI3K pathway. Upon activation of mTORC2, its major downstream targets (such as SGK, Akt and PKC), are phosphorylated to regulate cell survival, metabolism, and cytoskeleton organization. Genetic mutation rate > 5% in mTOR pathway of HCC are labeled in orange, and tumor suppressors in green.

activated hematopoietic pre–B-cell leukemia transcription factor-interacting protein (HPIP)-mediated mTOR pathway [42]. HBx-stimulated α-fetoprotein (AFP) exerted its oncogenic function in hepatocytes via activating the PI3K/mTOR pathway [43]. HBV pre-S mutants could also activate the Akt/mTOR pathway and accelerate cell proliferation through upregulating vascular endothelial growth factor (VEGF) receptor-2 [44]. Furthermore, hepatitis C virus (HCV) has been reported to increase N-Ras, an activator of the PI3K/Akt pathway, contributing to a 4-fold increase of phosphorylated AKT (p-Akt) in HCC cells [45]. Another study provided strong evidence that HCV increases mTOR, p-mTOR and p-4EBP1 levels in HCC cells [46]. In addition, Yes-associated protein 1 (YAP1) and transcriptional coactivator with PDZ-binding domain (TAZ), central effectors of the Hippo pathway, mediated activation of mTOCR1 through overexpression of SLC38A1 and SLC7A5 [47].

Overall, alterations in the mTOR pathway are frequent events in HCC, yet the underlying mechanism remains to be an elusive goal. Hepatitis virus B or C infection, and the interaction between the mTOR pathway and the Hippo pathway are all implicated in contributing to the mTOR pathway activation, providing new clues for further exploring its mechanisms at molecular levels.

4. The mTOR pathway in cancer phenotypes

A growing number of studies suggest that proteins in the mTOR pathway are activated with high frequency during progression toward malignancy, and seem to have regulatory roles in a variety of cellular function in cancer cells, such as proliferation, survival, metastasis, autophagy, metabolism and drug resistance (Fig. 1). It is thus believed that understanding the mTOR pathway in tumor progression will help develop novel anti-tumor therapy strategies.

4.1. Growth and proliferation

Cell growth and proliferation are regulated by several signaling pathways in response to environmental stimuli, such as growth factors, nutrients, and hormones. However, dysregulations in these pathways result in the development of multiple cancers [48]. Several studies, including HCC, have revealed that mTORC1 promotes cell growth and proliferation largely by directly phosphorylating ribosomal protein S6 kinase B1 (S6K1) and 4EBP1 [49–52]. The activation of S6K1 positively regulates cell growth and proliferation via phosphorylating its substrate ribosomal protein S6 (RPS6), thereby contributing to increased mRNA translation characterized by a 5′ olygopyrimidine tract [13]. Phosphorylation of 4EBP1 promotes its dissociation from eIF4E, resulting in initiation of cap-dependent translation to affect cell growth and proliferation [4,53].

4.2. Autophagy

Autophagy participates in routine degradation of cell components and is strongly induced under stress conditions, such as nutrient

starvation [54]. A variety of malignant tumors, including HCC, often present defects in autophagy accompanied by activation of the PI3K/mTOR pathway [4]. Chronic activation of mTORC1 induces autophagy inhibition and unresolved endoplasmic reticulum stress which trigger hepatocyte damage and HCC progression in a liver-specific TSC1-knockout mice model [25]. Moreover, hydrogen sulfide accelerates autophagy of HCC cells through suppressing the expression of p-PI3K, p-Akt and mTOR, which mimics the effect of rapamycin [55].

However, how the mTOR pathway controls HCC autophagy and what kind of roles autophagy plays in HCC development remain mysteries. Autophagy induced by mTOR inhibitors may block the development and recurrence of HCC, especially in HCC patients with a background of hepatitis virus infection and constant DNA damage [56]. On the contrary, autophagy may induce the development and progression of HCC through assisting cancer cells in fighting against cellular stresses (such as DNA damage, nutrient deprivation and hypoxia) and chemotherapy drugs [57,58], suggesting that combining mTOR inhibitors with autophagy inhibitors may improve efficacy in HCC treatment.

4.3. Metabolism

Metabolic changes have been generally regarded as a hallmark of cancer [59]. Activation of the mTOR pathway in liver influences whole-body energy homeostasis, including glycolysis and lipid metabolism [60,61]. Multiple studies in HCC have demonstrated that mTORC1 enhances glucose uptake and glycolysis by promoting hypoxia-inducible factor-1 alpha (HIF-1α) expression through inhibition of 4EBP1 [60,62], and by increasing HIF-1α activity through RHEB-mediated mTORC1 activation during hypoxia [63]. Moreover, mTORC1 promotes lipid biosynthesis through phosphorylating either S6K1 or lipin 1 to increase SREBP expression [60,62,64–66]. As to mTORC2, it activates fatty acid and lipid synthesis, resulting in steatosis and hepatocarcinogenesis [27]. However, the precise mechanism on how mTORC2 promotes lipid synthesis in HCC remains to be defined.

4.4. Angiogenesis

Angiogenesis goes hand in hand with HCC progression [67]. Compared with non-HCC tissues, the number and immuostaining intensity of p-S6K1− and p-Akt−positive sinusoidal endothelial cells are markedly upregulated in HCC tissues, indicating an important role of the mTOR pathway in HCC angiogenesis [68]. Liver fatty acid-binding protein upregulates HIF-1α and VEGF (a downstream molecule of HIF-1α) to facilitate HCC angiogenesis via the mTOR pathway [69]. Conversely, accompanied with blockade of mTOR activity, expression of HIF-1α and VEGF is significantly reduced in HCC cells [70]. mTORC1-mediated activation of HIF-1α seems to be regarded as a mechanism in HCC angiogenesis [60,63].

4.5. Apoptosis

Apoptosis plays a critical role in the growth of cancers. The expression of antiapoptotic proteins, Bcl-2 and myeloid cell leukemia-1 (Mcl-1), is up-regulated in the PIK3CA/AKT/mTOR-activated HCC cells, which protect against c-Myc−induced apoptosis [71]. Bcl-2 is downregulated and the proapoptotic protein Bax is up-regulated in mTOR pathway-inhibited HCC cells, which induce hepatocyte apoptosis [72]. By promoting translation of these anti-apoptotic mRNAs, eIF4E-dependent translation is regarded as an underlying mechanism of the mTOR pathway in HCC apoptosis [73].

4.6. Metastasis

Alterations in the mTOR pathway contribute to invasion and metastasis, which is the main cause for treatment of failure and relapse in HCC. Up-regulation of p-mTOR and Akt, as well as loss of PTEN, are significantly associated with intrahepatic metastasis and vascular invasion in HCC patients [74]. Several studies further proved that mTORC1 enhances HCC metastasis through promoting the expression of matrix metalloproteinase 9 (MMP9) [74–76] and Golgi protein 73 [52]. Conversely, suppression of mTORC1 reduces HCC metastasis through suppressing the expression of MMP9 and HIF-1α [77,78]. In addition, mTORC2 is also involved in HCC metastasis via enhancing Akt phosphorylation at Ser473 [79]. How the mTOR pathway promotes the expression of these pro-metastatic proteins remains to be clarified.

4.7. Chemoresistance

Currently, there is no effective chemotherapy for HCC patients due to drug resistance. Sorafenib is an oral multi-kinase inhibitor with survival benefits in unresectable HCC. Activation of the mTOR pathway predicts poor response to sorafenib [57,80], as well as unresponsiveness of metformin [81] and aspirin [82] in HCC. Suppression of mTORC1 or mTORC2 could enhance sensitivity of HCC cells in response to chemotherapeutic drugs, including cisplatin [83] and doxorubicin [84,85]. Although molecular mechanisms of the mTOR pathway in chemotherapy insensitivity have not been well established, these findings encourage us to use the combination of mTOR inhibitors and other drugs in HCC treatment.

Taken together, the mTOR pathway participates in multiple cellular processes in HCC (Fig. 1). The roles of mTORC1 and its downstream targets have been relatively well-studied in HCC, however, some results need to be validated or further studied, such as autophagy, metastasis and drug resistance. Although it has been showed that the activation of mTORC2 is involved in the metastasis, metabolism and drug resistance of HCC [27,79,82,84], the precise mechanism of how mTORC2 participates in these processes is poorly understood. In addition, currently there is no report regarding the role of mTORC2 in other cellular processes in HCC.

5. Crosstalk with non-coding RNAs (ncRNAs) in HCC

Non-coding RNAs (ncRNAs) are important regulators of gene expression in a large variety of pathophysiological processes. In particular, several studies reported that ncRNAs participate in regulating critical factors in the mTOR pathway. Indeed, a large number of ncRNAs are not only part of genes coordinated by the mTOR pathway, but also required by the pathway to fine-tune its response and to fully accomplish its oncogenic and tumor suppressive roles.

5.1. Dysfunctions of microRNAs (miRNAs) and the mTOR pathway in HCC

miRNAs are a class of evolutionarily conserved, endogenous, small, noncoding RNA molecules of approximately 22 nucleotides in length that post-transcriptionally regulate gene expression via promoting mRNA degradation or inhibiting translation [86]. It is suggested that aberrant expression of miRNAs exerts pro-oncogenic or anti-oncogenic activity involved in multiple cancers, including HCC. Here those miRNAs that have direct and confirmed targets in the mTOR pathway are indicated (Table 1 and Fig. 2).

As a core component of mTORC1 and mTORC2 complexes, mTOR modulates diverse cellular processes and is targeted by several miRNAs in HCC. miR-199a-3p down-regulates in most HCC patients and inhibits proliferation, metastasis and drug resistance through targeting mTOR and c-Met [85,87,88]. mTOR has also been proved to be targeted by another four frequently downregulated miRNAs in HCC, including miR-99a, miR-100, miR-497 and miR-758-3p [89–93]. Among these miRNAs, miR-99a, miR-100 and miR-497 co-target mTOR and IGF1R (an upstream receptor kinase in the mTOR pathway) [89–92], suggesting a combination of these three miRNAs could be promising candidates for molecular therapy against HCC.

Table 1
Non-coding RNAs deregulated in HCC and with targets in the mTOR pathway.

Type of ncRNAs	ncRNAs name	Dysregulation in HCC	Target genes in mTOR pathway	Sample types	Characteristics	References
miRNA	miR-99a	Down	mTOR, IGF1R	HCC tissues, cell lines and mouse models	Growth and prognosis	[89,92]
	miR-100	Down	mTOR, IGF1R	HCC tissues, cell lines and mouse models	Autophagy, growth, metastasis and apoptosis	[90,91]
	miR-497	Down	mTOR, IGF1R	HCC tissues, cell lines and mouse models	Growth and metastasis	[92]
	miR-199a-3p	Down	mTOR, c-Met	HCC tissues, cell lines and mouse models	Cell cycle progression, invasion and apoptosis	[85,87,88]
	miR-758-3p	Down	mTOR	HCC tissues and cell lines	Proliferation, migration and invasion	[93]
	miR-494	Up	PTEN	HCC tissues and cell lines	Proliferation, metastasis and drug resistance	[94]
	miR-7	Down	PIK3CD	HCC tissues, cell lines and mouse models	Growth, metastasis	[95]
	miR-124	Down	PIK3CA	Cell lines and mouse models	Proliferation	[96]
	miR-132	Down	PIK3R3	HCC tissues, cell lines and mouse models	Proliferation and metastasis	[97]
	miR-511	Down	PIK3R3	HCC tissues and cell lines	Proliferation and metastasis	[98]
	miR-149	Down	AKT1	HCC tissues and cell lines	Proliferation, migration and invasion	[99]
	miR-137	Down	AKT2	HCC tissues, cell lines and mouse models	Growth, metastasis and prognosis	[100]
lncRNA	HULC	Up	PTEN	HCC tissues, cell lines and mouse models	Growth and autophagy	[104]
	LINC00152	U	EpCAM	HCC tissues, cell lines and mouse models	Proliferation	[102]
	MALAT1	Up	S6K1	HCC tissues, cell lines and mouse models	Proliferation and survival	[103]

PTEN is an essential upstream regulator of mTORC1. Upregulated miR-494 promotes HCC cell cycle progression and metastasis by suppressing PTEN, and the activated mTOR pathway is also involved in sorafenib resistance [94]. PI3K complexes are a family of enzymes playing positive roles in activation of the mTOR pathway. miR-7 targets phosphoinositide 3-kinase catalytic subunit delta (PIK3CD) to downregulate Akt, mTOR, and S6K1, as well as to upregulate 4EBP1, thus inhibiting HCC growth and metastasis [95]. PIK3CA, another component of PI3K(s), is targeted by miR-124 to regulate HCC cell proliferation through the PI3KCA/Akt/mTOR pathway [96]. Both miR-132 and miR-511 target phosphoinositide-3-kinase regulatory subunit 3 (PIK3R3) to regulate cell proliferation and metastasis via the PIK3R3/AKT/mTOR pathway in HCC [97,98].

AKTs are the core components of the PI3K/AKT pathway due to its phosphorylation following by activation of mTOR and various downstream signals. miR-149 inhibits proliferation, migration, and invasion of HCC cells via blockade of the AKT1/mTOR pathway [99]. miR-137 targets AKT2 in the AKT2/mTOR pathway to inhibit HCC growth and metastasis [100].

5.2. Long non-coding RNA (lncRNA) and the mTOR pathway

lncRNA often referred to the 'dark matter' of the genome, is an RNA molecule consisting of > 200 nucleotides with no or little protein-encoding function [101]. It has attracted much attention in cancer research field due to its essential role in regulating gene expression and transcription, processing of post-transcriptional mRNA, protein activity or localization, and intercellular signaling, as well as serving as structural RNAs [101]. Recent studies revealed that several lncRNAs participate in HCC development through the mTOR pathway (Table 1, Fig. 2).

The lncRNA, LINC00152, binds to EpCAM (epithelial cell adhesion molecule) promoter leading to activation of the mTOR pathway and plays a positive role in cell proliferation and tumor growth of HCC [102]. The lncRNA MALAT1 (metastasis-associated lung adenocarcinoma transcript 1) modulates the alternative splicing of S6K1 through induction of SRSF1 (serine/arginine-rich splicing factor 1), contributing to activation of the mTOR pathway [103]. Moreover, a recent study showed that another lncRNA HULC (highly up-regulated in liver cancer) activates the mTOR pathway via PTEN suppression by the ubiquitin-proteasome system [104].

Taken together, deregulation of all these ncRNAs plays an emerging role in activation or inactivation of the mTOR pathway, suggesting a potential therapy for HCC. However, more work is needed to further confirm possible applications in clinical trials, and to determine whether they represent driving events in HCC.

6. The mTOR pathway as therapeutic targets for HCC

Considering the crucial role of the mTOR pathway, it is not surprising that targeting the mTOR pathway including mTOR inhibitors, metformin and ncRNAs, has become increasingly attractive for HCC treatment. Moreover, several mTOR inhibitors and metformin are thus tested in clinical trials in recent years (Tables 2–5; available on http://www.clinicaltrials.gov).

6.1. mTOR inhibitors in HCC

Rapamycin (sirolimus) is a specific mTOR inhibitor, which forms a complex with its intracellular receptor FKBP12 to exert immunosuppressive and anti-tumor effects [8,9]. The mTORC1 and mTORC2 complexes, however, show different sensitivities to rapamycin, the former is sensitive; whereas the latter generally resistant [4]. Several rapamycin derivative compounds ("rapalogs"), known as first-generation mTOR inhibitors, have also been developed (Fig. 3), including everolimus (RAD001), temsirolimus (torisel, CCI-779), and

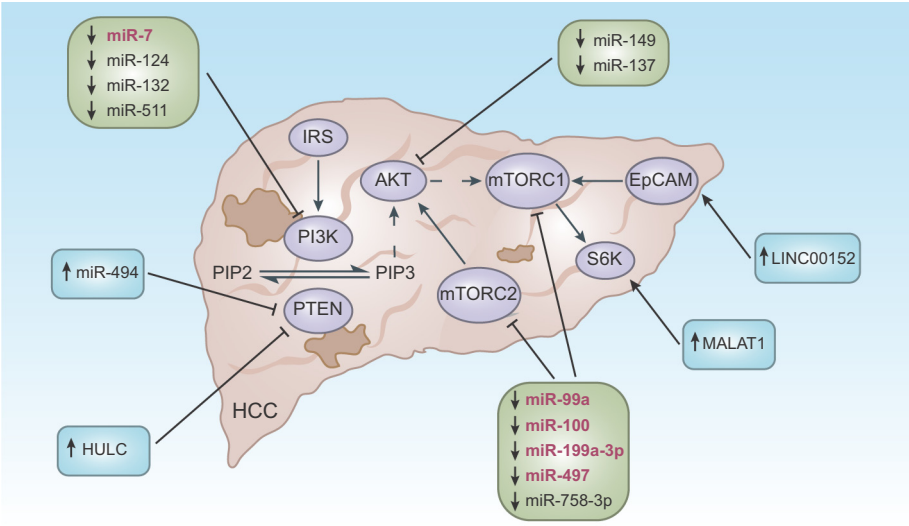

Fig. 2. Interaction between non-coding RNAs and the mTOR pathway in hepatocellular carcinoma. The appearance of red color reflects that the non-coding RNAs are promising to be applied to clinical trials. HCC, hepatocellular carcinoma.

deforolimus (MK-8669, AP23573, or ridaforolimus). However, the therapeutic potential of rapamycin and rapalogs has been limited in cancer treatment. One possible reason is that these inhibitors only inhibit mTORC1, contributing to an escaping mechanism via the PI3K-mTORC2-AKT pathway [56]. Second, these inhibitors partially block phosphorylation of 4EBP1 and selectively suppress phosphorylation of mTOR Ser2481 in HCC [4,105]. Furthermore, negative feedback loops may be activated when mTORC1 is suppressed, such as activation of the mTORC1-MAPK feedback loop [106].

To overcome the shortcomings mentioned above, several groups have developed small molecules, generally called second-generation mTOR inhibitors (Fig. 3) [4]. Compared to rapalogs, a new generation of mTOR inhibitors, functioning as ATP-competitive inhibitors of mTOR, hinder phosphorylation of all downstream targets of mTORC1 and mTORC2. In addition, dual mTOR/PI3K inhibitors have been developed by understanding the close interaction of mTOR with the PI3K pathway [4]. Moreover, the inhibitors of PI3K and AKT have been developed to block the upstream components of the mTOR pathway.

Several first-generation mTOR inhibitors have been approved by the US Food and Drug Administration (FDA) in the management of cancers, such as temsirolimus for advanced renal cell carcinoma, along with everolimus for advanced renal cell carcinoma, HER2negative breast cancer in combination with exemestane, renal angiomyolipoma, and other diseases. Despite no mTOR inhibitors are clinically available for HCC, a number of preclinical studies underscore the potentially favorable anti-tumor effects of mTOR inhibitors, and clinical trials are underway or have recently been completed for HCC treatment (Tables 2–5).

6.2. Single mTOR inhibitor in HCC treatment

Rapamycin and rapalogs have been proved to suppress HCC development in many preclinical studies [56], which encourages clinical trials in HCC patients (Table 2). Three earlier clinical trials using sirolimus in advanced HCC patients could not draw any firm conclusions due to different therapy schedules and fewer participants [107–109]. Two phase I/II studies using everolimus also reported disappointing results. Only one patient achieved a partial response in each study, while other 65 patients enrolled in these two studies had produced no response with the maximum tolerated dose ranging from 7.5–10 mg per day [110,111]. Although one of the studies was not a randomized control trial, encouraging anti-tumor activity was observed, with a

Table 2
Summary of completed and ongoing clinical trials of the single mTOR inhibitor in HCC.

Drug	Study phase and design	Patient number	HCC stage	Child-Pugh score	Results	References or trial number
Sirolimus	Pilot. NR	21	I to IV (TNM)	A, B, C	PR: n = 1.	[107]
	Pilot. NR	18	B, C, D (BCLC)	A, B, C	No objective response.	[109]
	II. NR	25	B or C (BCLC)	A, B	CR: n = 1 and PR: n = 1.	[108]
Everolimus	I/II. NR	28	B or C (BCLC)	A, B	PR: n = 1. MTD = 10 mg/d PFS: 3.8 months	[110]
	I/II. R	39	C (BCLC)	A, B	PR: n = 1. MTD = 7.5 mg/d or 70 mg/wk. DLTs not reached for weekly schedule.	[111]
	III. R	546	Advanced	A	Failed to indicate efficacy compared to placebo.	[112]
Temsirolimus	I/II. NA	Phase I: 19 Phase II: 36	Advanced	A	MTD = 30 mg/wk. PFS endpoint not reached.	[113]
	II. NR	6	Advanced	B	Terminated early according to DSMB recommendations.	NCT01079767
	I/II. NR	46	Advanced	A	Completed. Not yet published.	NCT01251458
	II. NR	25	Advanced	A, B (≤9)	Unknown.	NCT01567930
AZD8055	I. NR	26	Advanced	NA	Completed. Not yet published.	NCT00999882
MK2206	II. NR	15	Advanced	A	Early termination for discouraging results.	NCT01239355
MLN0128	I/II. R	11	Advanced	A	Active, not recruiting.	NCT02575339

R, randomized; NR, non-randomized; TNM, tumor node metastasis; BCLC, Barcelona clinic liver cancer; PR, partial response; CR, complete response; DLTs, dose-limiting toxicities; DSMB, Data and Safety Monitoring Board; MTD, maximum tolerated dose; PFS, progression-free survival; NA, not available.

Table 3
Summary of completed and ongoing clinical trials of the combination of mTOR inhibitors with other drugs in HCC.

Drug	Study phase and design	Patient number	HCC stage	Child-Pugh score	Results	References or trial number
Rapamycin + bevacizumab	I. NR	24	B or C (BCLC)	A, B	CR: n = 1. PR: *n* = 2. MTD = 4 mg/d.	[120]
Rapamycin + vorinostat	I. NR	6	Advanced	NA	Main drug-related toxic effect: thrombocytopenia.	[145]
Everolimus + bevacizumab	I/II. NR	33	B or C (BCLC)	A, B	Completed. Not yet published.	NCT00775073
Everolimus ± sorafenib	I/II. NR	130	B or C (BCLC)	A	Terminated: everolimus MTD (2.5 mg/d) too low.	[123]
Sorafenib ± everolimus	II. R	106	B or C (BCLC)	A, B (≤7)	Failed to indicate efficacy compared to sorafenib.	[124]
Everolimus + pasireotide	II. R	24	C (BCLC)	A	Failed to indicate efficacy.	[146]
Everolimus ± leuprolide + letrozole	II. R	84	Advanced	NA	Active, not recruiting.	NCT01642186
Temsirolimus + sorafenib	I. NR	25	III, IV (TNM)	A, B (≤7)	PR: n = 2; MTD = temsirolimus 10 mg/week + sorafenib 200 twice daily.	[121]
	I. NR	25	III, IV (TNM)	A, B (≤7)	Completed. Not yet published.	NCT01008917
	I/II. NR	0	Advanced	A, B	Withdrawn.	NCT01335074
	II. NR	27	II, III, IV (TNM)	A, B (≤7)	Active, not recruiting.	NCT01687673

R, randomized; NR, non-randomized; TNM, tumor node metastasis; BCLC, Barcelona clinic liver cancer; PR, partial response; CR, complete response; MTD, maximum tolerated dose; PFS, progression-free survival; NA, not available.

progression-free survival (PFS) rate at 24 weeks of 28.6% [110]. However, the Everolimus for Liver Cancer Evaluation (EVOLVE-1) [112] and another study [113] fired the hope to use everolimus or temsirolimus for the treatment of advanced HCC patients, ascribing the failures partly to conducting the studies in an unselected population. Although largely yielding negative results using rapamycin/rapalogs, these clinical trials suggest that further studies should pay attention to suitable patient selection based on molecular classification and predictive biomarkers.

The second-generation inhibitors including mTORC1/2 inhibitors, AZD8055 (NCT00999882) and MLN0128 (NCT02575339), as well as AKT inhibitor, MK-2206 (NCT01239355) are also used in clinical trials for the treatment of HCC (Table 2). However, these clinical trials are ongoing or have been completed with results not published yet. Therefore, it is too early to draw any conclusions from current trials. The ongoing clinical studies are expected to provide further evidence whether the second-generation mTOR inhibitors is a safe therapeutic agent for effectively treating HCC.

6.3. Combination of mTOR inhibitors with other drugs in HCC treatment

Drug resistance and compensatory activation of other signaling pathways have been attributed to be the cause for blunted efficacy of mTOR inhibitors. Therefore, the combinations of mTOR inhibitors with other drugs hold great promise for HCC treatment.

The combination of everolimus and BEZ235 (a PI3K/mTOR inhibitor) synergistically induces tumor mitophagy (a tumor-suppressor process in the liver) in a diethylnitrosamine-induced HCC model in mice [114]. Moreover, several preclinical studies reported synergistic effects of mTOR inhibitors in combination with others, such as sorafenib [115], bevacizumab (a vascular endothelial growth factor inhibitor) [116], histone deacetylase (HDAC) inhibitor [117], autophagy inhibitors [118,119].

Such a combinatorial approach has been examined in 11 clinical trials (Table 3). A phase I study in 24 advanced HCC patients showed that combination of rapamycin (4 mg/day) and bevacizumab (5 mg/kg every 14 days) is tolerable, three reached complete or partial response and 14 reached stable disease in 20 evaluable cases [120]. However, there are no phase II clinical trials to further evaluate the efficacy of this combination for HCC treatment. In addition, several clinical trials using a combination of rapalogs with other drugs are performed. A phase I study combined temsirolimus with sorafenib, reached 8% partial response and 60% stable disease in 25 patients at maximum tolerated dose (MTD; temsirolimus 10 mg/week and sorafenib 200 mg twice daily) [121]; however, the PFS (5.65 months) was similar to the results from single-agent sorafenib in the Sorafenib Hepatocellular Carcinoma Assessment Randomized Protocol (SHARP) trial [122]. A phase I study combined everolimus with sorafenib had to be terminated as the MTD for everolimus (2.5 mg/day) was too low to achieve a biologically effective concentration [123], suggesting to use an adjusted dose for this combination in future studies. Moreover, a phase II study combined everolimus (5 mg/day) and sorafenib (400 mg twice daily) in advanced HCC patients also revealed disappointing results, reminding further studies to attach importance to dose adjustment and patient selection [124].

Although no conclusive trials support the combined use of second-generation mTOR inhibitors with other drugs in HCC, these results provide some encouraging information and favorable suggestions for future studies. Also, a number of clinical trials have provided some encouraging results for the treatment of other types of advanced solid tumors [5,56]. Combining mTOR inhibitors with other drugs should consider drug toxicity and metabolism burden, especially in patients with severe hepatic impairment. Therefore, it is important to find ways to circumvent these conditions and to find a comfortable dose and frequency of administration.

6.4. mTOR inhibitors in HCC patients after liver transplantation

Immunosuppressive drugs, such as calcineurin inhibitors (cyclosporine A, tacrolimus, or pimecrolimus), are required for the prevention of graft rejection after liver transplantation, however, enhanced tumor growth is a frequent feature in preclinical investigations [125,126]. On the contrary, mTOR inhibitors with both immunosuppressive and antitumor activity have received increasing attention. Compared to other reagents, everolimus significantly reduced expression of donor-specific antibodies in patients after liver transplantation [127]. A meta-analysis of five retrospective studies demonstrated that tumor recurrence was decreased and overall survival was obviously improved in rapamycin-treated HCC patients compared with rapamycin-free controls after liver transplantation [128]. However, this study has several limitations, with the most significant being not randomized and relatively small sample size. A randomized, open-label, phase III multicenter trial (SiLVER) found that, although rapamycin did not improve recurrence-free and overall survival outcomes beyond 5 years, the outcomes were improved in the first 3 to 5 years in 525 HCC patients after liver transplantation [129].

There are also clinical studies using the combination of mTOR inhibitors and other drugs in HCC patients after liver transplantation. In a

Table 4
Summary of the completed and ongoing clinical trials of mTOR inhibitors in HCC after liver transplantation.

Drug	Study phase and design	Patient number	HCC stage	Child-Pugh score	Results	References or trial number
Sirolimus	II/III. NR	70	NA	NA	Completed. Not yet published.	NCT00328770
Sirolimus vs. mTOR inhibitor free	III. R	525	NA	NA	RFS and OS benefit in the first 3 to 5 years.	[129]
Sirolimus vs. FK506	III. R	220	Exceeding Milan criteria	NA	Unknown.	NCT00554125
Sirolimus vs. mTOR inhibitor free	II. R	45	Exceeding Milan criteria	NA	Completed. Not yet published.	NCT01374750
Sirolimus + tacrolimus + MMF vs. tacrolimus + MMF	II/III. R	130	NA	NA	Not yet recruiting.	NCT03500848
Everolimus + tacrolimus vs. tacrolimus	IV. R	333	NA	NA	Completed. Not yet published.	[147]
Everolimus + reduced tacrolimus vs. standard tacrolimus	III. R	284	NA	NA	Less HCC recurrence and better renal function on everolimus + reduced tacrolimus group.	[132,133]
Everolimus vs. standard tacrolimus	II. RII. R	50	NA	NA	Recruiting.	NCT01998789
Everolimus vs. tacrolimus vs. myfortic vs. cellcept vs. imuran	IV. R	336	Exceeding Milan criteria	NA	Recruiting.	NCT02081755
Everolimus vs. everolimus + tacrolimus	III. R	78	NA	NA	Completed. Not yet published.	NCT02115113

R, randomized; NR, non-randomized; TNM, tumor node metastasis; BCLC, Barcelona clinic liver cancer; NA, not available; vs., versus; RFS, recurrence-free survival; OS, overall survival.

retrospective study, the combination of rapamycin and metformin significantly prolonged survival by suppressing tumor growth [130]. Furthermore, the combination of an mTOR inhibitor (sirolimus or everolimus) with sorafenib has been proved to be effective in a multicenter, uncontrolled, retrospective study of 31 patients with HCC recurrence after liver transplantation [131]. A recently published phase III study demonstrated that less HCC recurrence and better renal function was observed in HCC patients with everolimus plus reduced tacrolimus treatment compared with standard tacrolimus [132,133]. Currently, another eight clinical trials are performed to evaluate the efficacy of mTOR inhibitors in HCC patients after liver transplantation (Table 4). Two phase IV trials (NCT01551212 and NCT02081755) are ongoing, and the results are pending. Taken together, current clinical trials using mTOR inhibitors have provided some promising results in HCC patients after liver transplantation. Furthermore, large-scale, randomized clinical trials and selected patient populations are still needed to further validate these results.

6.5. *mTOR inhibitors in HCC patients after transarterial chemoembolization (TACE)*

TACE is an effective therapy for unresectable HCC by its dual effects of chemotherapy and ischemic hypoxia. However, in the TACE-induced hypoxic condition, increased expression of HIF-1α contributes to VEGF up-regulation, which enhances angiogenesis and is associated with poor prognosis [134]. A preclinical study suggested that anti-tumor effect of TACE was enhanced when combined with everolimus [134]. Furthermore, a combination of mTOR inhibitors with TACE is conducted in three clinical trials (Table 5). One trial has completed, and the results are pending (NCT02724332); whereas two were terminated due to slow patient recruitment (NCT01009801 and NCT01379521).

6.6. *Challenges for future treatment strategies*

Although previous clinical trials using mTOR inhibitors did not provide sufficient information on how to use them in HCC treatment, they remain an attractive and promising therapeutic option for HCC patients, especially for those undergoing liver transplantation (Fig. 4). It is common that mTOR inhibitors contribute to drug resistance and compensatory activation of other signaling pathways when used as monotherapy, dual-specificity inhibitors that simultaneously affect mTOR and other agents targeting known resistance pathways that interact with mTOR are thus interesting candidates for developing innovative anti-HCC agents. However, current clinical trials do not provide satisfying results. Effective drug combinations and sufficient dose administration will have to be considered in future studies. Additionally, critical lessons from completed clinical trials including the EVOLVE-1 and SiLVER studies, should be applied to identify patient populations based on molecular classification and predictive biomarkers, providing a new direction for using mTOR inhibitors in HCC.

There have been multiple clinical trials of mTOR inhibitors for biomarker-based selection in patients with solid tumors, including sirolimus for advanced-stage solid cancer patients with PIK3CA mutation and/or amplification (NCT02449564) and INK128 (a mTORC1/2 inhibitor) for advanced-stage bladder cancer patients with TSC1 or TSC2 mutations (NCT03047213) [5]. These personalized treatments require the availability of predictive biomarkers for treatment response. Although no clinical trial with biomarker-based selection in HCC so far, several studies indicated that some biomarkers may predict response to mTOR inhibitors treatment in HCC [113,135–139]. For example, TSC2 loss is predictive of a response and prolonged overall survival in HCC patients treated with everolimus [137,138]. Another study suggested that disease stabilization is more likely to be achieved in patients who received temsirolimus and whose tumors have high p-mTOR protein levels [113]. Furthermore, preclinical evidence indicated that CD44 is a predictive biomarker for the response to INK128 [136] and CTNNB1

Table 5
Summary of the completed and ongoing clinical trials of mTOR inhibitors in HCC with TACE or metformin in the treatment for HCC.

Drug	Study phase and design	Patient number	HCC stage	Child-Pugh score	Results	Trial numbers
TACE + rapamycin	I. R	300	I (TNM)	A, B	Completed. Not yet published.	NCT02724332
TACE ± everolimus	I/II. R	27	B (BCLC)	A, B (≤7)	Terminated: slow patient recruitment.	NCT01009801
	II. R	65	B (BCLC)	A, early B	Terminated: low enrollment.	NCT01379521
Metformin vs. placebo	III. R	11	NA	A, B (≤7)	Terminated.	NCT02319200
Sorafenib ± metformin	II. RII. R	82	C (BCLC)	A	Unknown.	NCT02672488
Metformin + statin vs. placebo	II. R	3	NA	NA	Terminated: insufficient for the fund.	NCT02819869
Metformin vs. metformin + celebrex vs. celebrex	III. NR	200	NA	A, B	Recruiting.	NCT03184493

R, randomized; NR, non-randomized; TNM, tumor node metastasis; BCLC, Barcelona clinic liver cancer; NA, not available; vs., versus.

mutations make liver tumors susceptible to rapamycin [139]. However, further work still needs to develop biomarkers to guide clinical decisions, including factors predicting treatment response or resistance. Therefore, identifying predictive biomarkers that truly respond to mTOR inhibitors will be important for future studies.

6.7. The role of metformin in HCC treatment

Despite being a widely used drug for the treatment of type 2 diabetes, many studies show that metformin decreases cancer cell viability through inactivation of mTOR in HCC [140,141]. In a meta-analysis of 5 studies involving > 105,000 type 2 diabetes patients, the risk of liver cancer was reduced by approximately 62% in patients who received metformin treatment instead of non-metformin treatment [142]. Similarly, a multivariate stratified analysis revealed that metformin reduces the risk of HCC among diabetics [143]. While two ongoing clinical trials are expected to confirm the effectiveness of metformin for HCC, another two clinical trials were halted because of the decision of investigator (NCT02319200) and insufficiency for the fund (NCT02819869; Table 5). Therefore, the safety and efficacy of metformin for HCC treatment still need to be further investigated in future clinical trials.

6.8. The role of ncRNAs in HCC treatment

Another promising approach for HCC treatment is to target ncRNAs that affect mTOR pathway. The sensitivity to rapamycin was restored when suppression of the pro-oncogenic miR-17-92 cluster or delivery of tumor suppressor miRNAs including let-7, miR-22, and miR-143, in rapamycin resistance cancer cells, indicating miRNAs participated in rapamycin resistance [144]. Several miRNAs (including miR-99a, miR-100 and miR-497) that co-target mTOR and IGF1R and have a strong anti-tumor effect in preclinical studies, are likely to be applied for HCC treatment [89,90,92]. Although no ncRNAs that regulate mTOR pathway are ready for clinical trials, these findings demonstrate that targeting these miRNAs may be a potential treatment for HCC.

7. Conclusions

The deregulation of the mTOR pathway core components, upstream regulators, along with crosstalk with ncRNAs and other pathways have been correlated with tumorigenesis of HCC. However, a number of questions remain to be answered in order to determine clinical relevance of these treatments.

Future directions and challenges targeting the mTOR pathway in HCC include, a) elucidation of carcinogenic mechanism responsible for alterations of this pathway; b) identification and validation of candidate biomarkers for improved patient selection, prognostication, response evaluation and individualized tailoring; c) safety, efficacy and tolerability profile in large-scale clinical trials, especially in those with impaired liver function, either as a monotherapy or in combination with

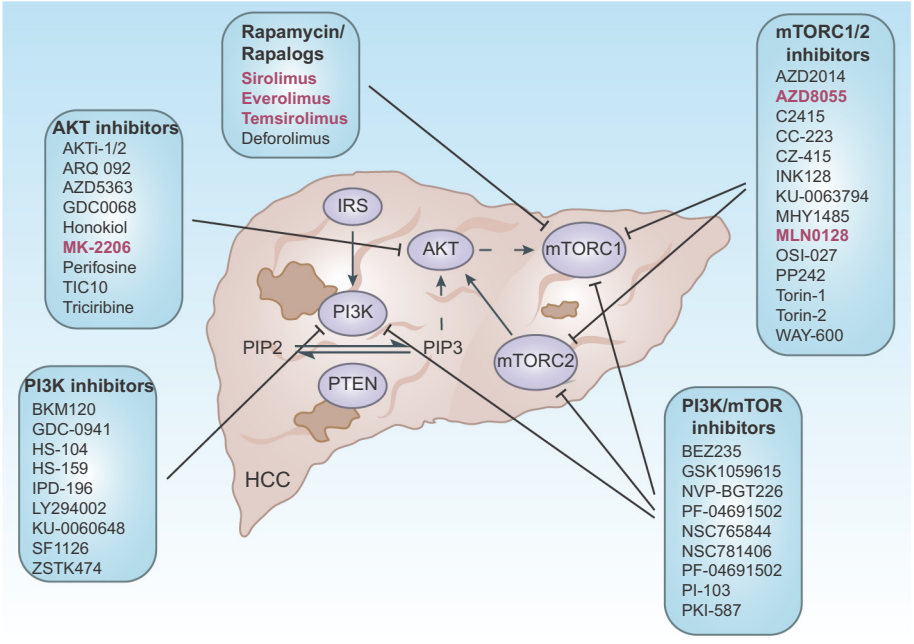

Fig. 3. Schematic diagram illustrating mTOR inhibitors reported in hepatocellular carcinoma. The appearance of red color reflects the drug used in clinical trials. HCC, hepatocellular carcinoma. → = activation; ⊣ = inhibition.

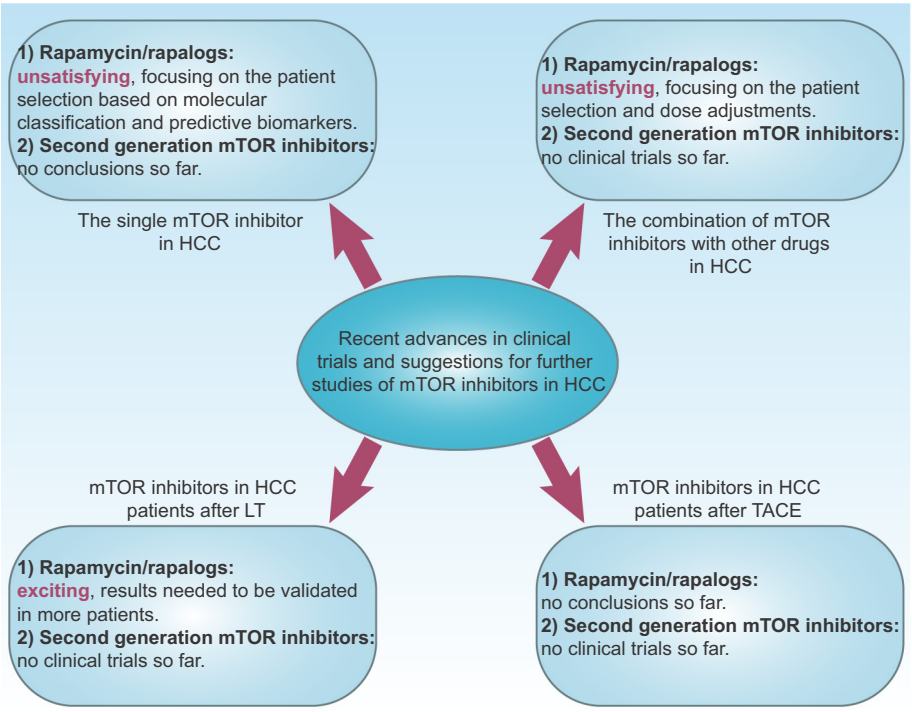

Fig. 4. Recent advances in clinical trials and suggestions for further studies of mTOR inhibitors in hepatocellular carcinoma. HCC, hepatocellular carcinoma; LT, liver transplantation; TACE, transarterial chemoembolization.

other therapies; and d) change of tumor microenvironment after using mTOR inhibitors.

In spite of these unresolved issues, the use of the mTOR inhibitors as a monotherapy or in combination with other therapies is still an attractive and promising therapeutic strategy for HCC treatment. The efforts in resolving these obstacles combined with the work of other clinical and basic researchers will reshape our views of the mTOR pathway in HCC.

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Declaration of conflict of interest

None.

References

[1] F. Bray, J. Ferlay, I. Soerjomataram, R.L. Siegel, L.A. Torre, A. Jemal, Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries, CA Cancer J. Clin. 68 (2018) 394–424.
[2] A. Forner, M. Reig, J. Bruix, Hepatocellular carcinoma, Lancet (London, England) 391 (2018) 1301–1314.
[3] X. Shi, H.R. Zhu, T.T. Liu, X.Z. Shen, J.M. Zhu, The Hippo pathway in hepatocellular carcinoma: non-coding RNAs in action, Cancer Lett. 400 (2017) 175–182.
[4] M. Laplante, D.M. Sabatini, mTOR signaling in growth control and disease, Cell 149 (2012) 274–293.
[5] F. Janku, T.A. Yap, F. Meric-Bernstam, Targeting the PI3K pathway in cancer: are we making headway? Nat. Rev. Clin. Oncol. 15 (2018) 273–291.
[6] R.A. Saxton, D.M. Sabatini, mTOR Signaling in growth, metabolism, and disease, Cell 168 (2017) 960–976.
[7] C. Vezina, A. Kudelski, S.N. Sehgal, Rapamycin (AY-22,989), a new antifungal antibiotic. I. Taxonomy of the producing streptomycete and isolation of the active principle, J. Antibiot. 28 (1975) 721–726.
[8] J. Heitman, N.R. Movva, M.N. Hall, Targets for cell cycle arrest by the immunosuppressant rapamycin in yeast, Science (New York, N.Y.) 253 (1991) 905–909.
[9] E.J. Brown, M.W. Albers, T.B. Shin, K. Ichikawa, C.T. Keith, W.S. Lane, S.L. Schreiber, A mammalian protein targeted by G1-arresting rapamycin-receptor complex, Nature 369 (1994) 756–758.
[10] D.M. Sabatini, H. Erdjument-Bromage, M. Lui, P. Tempst, S.H. Snyder, RAFT1: a mammalian protein that binds to FKBP12 in a rapamycin-dependent fashion and is homologous to yeast TORs, Cell 78 (1994) 35–43.
[11] C.J. Sabers, M.M. Martin, G.J. Brunn, J.M. Williams, F.J. Dumont, G. Wiederrecht, R.T. Abraham, Isolation of a protein target of the FKBP12-rapamycin complex in mammalian cells, J. Biol. Chem. 270 (1995) 815–822.
[12] R.T. Abraham, G.J. Wiederrecht, Immunopharmacology of rapamycin, Annu. Rev. Immunol. 14 (1996) 483–510.
[13] K. Xu, P. Liu, W. Wei, mTOR signaling in tumorigenesis, Biochim. Biophys. Acta 1846 (2014) 638–654.
[14] E. Jacinto, R. Loewith, A. Schmidt, S. Lin, M.A. Ruegg, A. Hall, M.N. Hall, Mammalian TOR complex 2 controls the actin cytoskeleton and is rapamycin insensitive, Nat. Cell Biol. 6 (2004) 1122–1128.
[15] D.D. Sarbassov, S.M. Ali, D.H. Kim, D.A. Guertin, R.R. Latek, H. Erdjument-Bromage, P. Tempst, D.M. Sabatini, Rictor, a novel binding partner of mTOR, defines a rapamycin-insensitive and raptor-independent pathway that regulates the cytoskeleton, Curr. Biol. 14 (2004) 1296–1302.
[16] V. Zinzalla, D. Stracka, W. Oppliger, M.N. Hall, Activation of mTORC2 by association with the ribosome, Cell 144 (2011) 757–768.
[17] L.R. Pearce, D. Komander, D.R. Alessi, The nuts and bolts of AGC protein kinases, Nat. Rev. Mol. Cell Biol. 11 (2010) 9–22.
[18] P.B. Crino, K.L. Nathanson, E.P. Henske, The tuberous sclerosis complex, N. Engl. J. Med. 355 (2006) 1345–1356.
[19] A. Hemminki, D. Markie, I. Tomlinson, E. Avizienyte, S. Roth, A. Loukola, G. Bignell, W. Warren, M. Aminoff, P. Hoglund, H. Jarvinen, P. Kristo, K. Pelin, M. Ridanpaa, R. Salovaara, T. Toro, W. Bodmer, S. Olschwang, A.S. Olsen, M.R. Stratton, A. de la Chapelle, L.A. Aaltonen, A serine/threonine kinase gene defective in Peutz-Jeghers syndrome, Nature 391 (1998) 184–187.
[20] J.H. Kwon, N. Lee, J.Y. Park, Y.S. Yu, J.P. Kim, J.H. Shin, D.S. Kim, J.W. Joh, D.S. Kim, K.Y. Choi, K.J. Kang, G. Kim, Y.H. Moon, H.J. Wang, Actionable gene expression-based patient stratification for molecular targeted therapy in hepatocellular carcinoma, PLoS One 8 (2013) e64260.
[21] W. Sieghart, T. Fuereder, K. Schmid, D. Cejka, J. Werzowa, F. Wrba, X. Wang, D. Gruber, S. Rasoul-Rockenschaub, M. Peck-Radosavljevic, V. Wacheck, Mammalian target of rapamycin pathway activity in hepatocellular carcinomas of patients undergoing liver transplantation, Transplantation 83 (2007) 425–432.
[22] F. Sahin, R. Kannangai, O. Adegbola, J. Wang, G. Su, M. Torbenson, mTOR and P70 S6 kinase expression in primary liver neoplasms, Clin. Cancer Res. 10 (2004) 8421–8425.
[23] A. Villanueva, D.Y. Chiang, P. Newell, J. Peix, S. Thung, C. Alsinet, V. Tovar,

S. Roayaie, B. Minguez, M. Sole, C. Battiston, S. Van Laarhoven, M.I. Fiel, A. Di Feo, Y. Hoshida, S. Yea, S. Toffanin, A. Ramos, J.A. Martignetti, V. Mazzaferro, J. Bruix, S. Waxman, M. Schwartz, M. Meyerson, S.L. Friedman, J.M. Llovet, Pivotal role of mTOR signaling in hepatocellular carcinoma, Gastroenterology 135 (2008) 1972–1983 1983.e1971-1911.

[24] X.M. Jiang, X.N. Yu, R.Z. Huang, H.R. Zhu, X.P. Chen, J. Xiong, Z.Y. Chen, X.X. Huang, X.Z. Shen, J.M. Zhu, Prognostic significance of eukaryotic initiation factor 4E in hepatocellular carcinoma, J. Cancer Res. Clin. Oncol. 142 (2016) 2309–2317.

[25] S. Menon, J.L. Yecies, H.H. Zhang, J.J. Howell, J. Nicholatos, E. Harputlugil, R.T. Bronson, D.J. Kwiatkowski, B.D. Manning, Chronic activation of mTOR complex 1 is sufficient to cause hepatocellular carcinoma in mice, Sci. Signal. 5 (2012) ra24.

[26] P. Liu, M. Ge, J. Hu, X. Li, L. Che, K. Sun, L. Cheng, Y. Huang, M.G. Pilo, A. Cigliano, G.M. Pes, R.M. Pascale, S. Brozzetti, G. Vidili, A. Porcu, A. Cossu, G. Palmieri, M.C. Sini, S. Ribback, F. Dombrowski, J. Tao, D.F. Calvisi, L. Chen, X. Chen, A functional mammalian target of rapamycin complex 1 signaling is indispensable for c-Myc-driven hepatocarcinogenesis, Hepatology (Baltimore, Md.) 66 (2017) 167–181.

[27] Y. Guri, M. Colombi, E. Dazert, S.K. Hindupur, J. Roszik, S. Moes, P. Jenoe, M.H. Heim, I. Riezman, H. Riezman, M.N. Hall, mTORC2 promotes tumorigenesis via lipid synthesis, Cancer Cell 32 (2017) 807-823.e812.

[28] L. Li, G.M. Pilo, X. Li, A. Cigliano, G. Latte, L. Che, C. Joseph, M. Mela, C. Wang, L. Jiang, S. Ribback, M.M. Simile, R.M. Pascale, F. Dombrowski, M. Evert, C.F. Semenkovich, X. Chen, D.F. Calvisi, Inactivation of fatty acid synthase impairs hepatocarcinogenesis driven by AKT in mice and humans, J. Hepatol. 64 (2016) 333–341.

[29] Z. Xu, J. Hu, H. Cao, M.G. Pilo, A. Cigliano, Z. Shao, M. Xu, S. Ribback, F. Dombrowski, D.F. Calvisi, X. Chen, Loss of Pten synergizes with c-Met to promote hepatocellular carcinoma development via mTORC2 pathway, Exp. Mol. Med. 50 (2018) e417.

[30] Y. Samuels, Z. Wang, A. Bardelli, N. Silliman, J. Ptak, S. Szabo, H. Yan, A. Gazdar, S.M. Powell, G.J. Riggins, J.K. Willson, S. Markowitz, K.W. Kinzler, B. Vogelstein, V.E. Velculescu, High frequency of mutations of the PIK3CA gene in human cancers, Science (New York, N.Y.) 304 (2004) 554.

[31] S. Velho, C. Oliveira, A. Ferreira, A.C. Ferreira, G. Suriano, S. Schwartz Jr., A. Duval, F. Carneiro, J.C. Machado, R. Hamelin, R. Seruca, The prevalence of PIK3CA mutations in gastric and colon cancer, Eur. J. Cancer 41 (2005) 1649–1654.

[32] D. Zardavas, W.A. Phillips, S. Loi, PIK3CA mutations in breast cancer: reconciling findings from preclinical and clinical data, Breast Cancer Res. 16 (2014) 201.

[33] S.A. Danielsen, P.W. Eide, A. Nesbakken, T. Guren, E. Leithe, R.A. Lothe, Portrait of the PI3K/AKT pathway in colorectal cancer, Biochim. Biophys. Acta 1855 (2015) 104–121.

[34] D.W.H. Ho, L.K. Chan, Y.T. Chiu, I.M.J. Xu, R.T.P. Poon, T.T. Cheung, C.N. Tang, V.W.L. Tang, I.L.O. Lo, P.W.Y. Lam, D.T.W. Yau, M.X. Li, C.M. Wong, I.O.L. Ng, TSC1/2 mutations define a molecular subset of HCC with aggressive behaviour and treatment implication, Gut 66 (2017) 1496–1506.

[35] Z. Kan, H. Zheng, X. Liu, S. Li, T.D. Barber, Z. Gong, H. Gao, K. Hao, M.D. Willard, J. Xu, R. Hauptschein, P.A. Rejto, J. Fernandez, G. Wang, Q. Zhang, B. Wang, R. Chen, J. Wang, N.P. Lee, W. Zhou, Z. Lin, Z. Peng, K. Yi, S. Chen, L. Li, X. Fan, J. Yang, R. Ye, J. Ju, K. Wang, H. Estrella, S. Deng, P. Wei, M. Qiu, I.H. Wulur, J. Liu, M.E. Ehsani, C. Zhang, A. Loboda, W.K. Sung, A. Aggarwal, R.T. Poon, S.T. Fan, J. Wang, J. Hardwick, C. Reinhard, H. Dai, Y. Li, J.M. Luk, M. Mao, Whole-genome sequencing identifies recurrent mutations in hepatocellular carcinoma, Genome Res. 23 (2013) 1422–1433.

[36] Y.J. Yao, X.L. Ping, H. Zhang, F.F. Chen, P.K. Lee, H. Ahsan, C.J. Chen, P.H. Lee, M. Peacocke, R.M. Santella, H.C. Tsou, PTEN/MMAC1 mutations in hepatocellular carcinomas, Oncogene 18 (1999) 3181–3185.

[37] Y. Totoki, K. Tatsuno, K.R. Covington, H. Ueda, C.J. Creighton, M. Kato, S. Tsuji, L.A. Donehower, B.L. Slagle, H. Nakamura, S. Yamamoto, E. Shinbrot, N. Hama, M. Lehmkuhl, F. Hosoda, Y. Arai, K. Walker, M. Dahdouli, K. Gotoh, G. Nagae, M.C. Gingras, D.M. Muzny, H. Ojima, K. Shimada, Y. Midorikawa, J.A. Goss, R. Cotton, A. Hayashi, J. Shibahara, S. Ishikawa, J. Guiteau, M. Tanaka, T. Urushidate, S. Ohashi, N. Okada, H. Doddapaneni, M. Wang, Y. Zhu, H. Dinh, T. Okusaka, N. Kokudo, T. Kosuge, T. Takayama, M. Fukayama, R.A. Gibbs, D.A. Wheeler, H. Aburatani, T. Shibata, Trans-ancestry mutational landscape of hepatocellular carcinoma genomes, Nat. Genet. 46 (2014) 1267–1273.

[38] C. Guichard, G. Amaddeo, S. Imbeaud, Y. Ladeiro, L. Pelletier, I.B. Maad, J. Calderaro, P. Bioulac-Sage, M. Letexier, F. Degos, B. Clement, C. Balabaud, E. Chevet, A. Laurent, G. Couchy, E. Letouze, F. Calvo, J. Zucman-Rossi, Integrated analysis of somatic mutations and focal copy-number changes identifies key genes and pathways in hepatocellular carcinoma, Nat. Genet. 44 (2012) 694–698.

[39] Y. Ito, T. Takeda, M. Sakon, M. Tsujimoto, S. Higashiyama, K. Noda, E. Miyoshi, M. Monden, N. Matsuura, Expression and clinical significance of erb-B receptor family in hepatocellular carcinoma, Br. J. Cancer 84 (2001) 1377–1383.

[40] D. Tavian, G. De Petro, A. Benetti, N. Portolani, S.M. Giulini, S. Barlati, u-PA and c-MET mRNA expression is co-ordinately enhanced while hepatocyte growth factor mRNA is down-regulated in human hepatocellular carcinoma, International journal of cancer, J. Int. Cancer 87 (2000) 644–649.

[41] C.J. Yen, Y.J. Lin, C.S. Yen, H.W. Tsai, T.F. Tsai, K.Y. Chang, W.C. Huang, P.W. Lin, C.W. Chiang, T.T. Chang, Hepatitis B virus X protein upregulates mTOR signaling through IKKbeta to increase cell proliferation and VEGF production in hepatocellular carcinoma, PLoS One 7 (2012) e41931.

[42] X. Xu, Z. Fan, L. Kang, J. Han, C. Jiang, X. Zheng, Z. Zhu, H. Jiao, J. Lin, K. Jiang, L. Ding, H. Zhang, L. Cheng, H. Fu, Y. Song, Y. Jiang, J. Liu, R. Wang, N. Du, Q. Ye, Hepatitis B virus X protein represses miRNA-148a to enhance tumorigenesis, J. Clin. Invest. 123 (2013) 630–645.

[43] M. Zhu, J. Guo, W. Li, Y. Lu, S. Fu, X. Xie, H. Xia, X. Dong, Y. Chen, M. Quan, S. Zheng, K. Xie, M. Li, Hepatitis B virus X protein induces expression of alpha-fetoprotein and activates PI3K/mTOR signaling pathway in liver cells, Oncotarget 6 (2015) 12196–12208.

[44] J.C. Yang, C.F. Teng, H.C. Wu, H.W. Tsai, H.C. Chuang, T.F. Tsai, Y.H. Hsu, W. Huang, L.W. Wu, I.J. Su, Enhanced expression of vascular endothelial growth factor-A in ground glass hepatocytes and its implication in hepatitis B virus hepatocarcinogenesis, Hepatology (Baltimore, Md.) 49 (2009) 1962–1971.

[45] P. Mannova, L. Beretta, Activation of the N-Ras-PI3K-Akt-mTOR pathway by hepatitis C virus: control of cell survival and viral replication, J. Virol. 79 (2005) 8742–8749.

[46] S. Shrivastava, J. Bhanja Chowdhury, R. Steele, R. Ray, R.B. Ray, Hepatitis C virus upregulates Beclin1 for induction of autophagy and activates mTOR signaling, J. Virol. 86 (2012) 8705–8712.

[47] Y.Y. Park, B.H. Sohn, R.L. Johnson, M.H. Kang, S.B. Kim, J.J. Shim, L.S. Mangala, J.H. Kim, J.E. Yoo, C. Rodriguez-Aguayo, S. Pradeep, J.E. Hwang, H.J. Jang, H.S. Lee, R. Rupaimoole, G. Lopez-Berestein, W. Jeong, I.S. Park, Y.N. Park, A.K. Sood, G.B. Mills, J.S. Lee, Yes-associated protein 1 and transcriptional coactivator with PDZ-binding motif activate the mammalian target of rapamycin complex 1 pathway by regulating amino acid transporters in hepatocellular carcinoma, Hepatology (Baltimore, Md.) 63 (2016) 159–172.

[48] E. Jacinto, V. Facchinetti, D. Liu, N. Soto, S. Wei, S.Y. Jung, Q. Huang, J. Qin, B. Su, SIN1/MIP1 maintains rictor-mTOR complex integrity and regulates Akt phosphorylation and substrate specificity, Cell 127 (2006) 125–137.

[49] D.A. Guertin, D.M. Sabatini, Defining the role of mTOR in cancer, Cancer Cell 12 (2007) 9–22.

[50] N. Hay, N. Sonenberg, Upstream and downstream of mTOR, Genes Dev. 18 (2004) 1926–1945.

[51] J. Samarin, V. Laketa, M. Malz, S. Roessler, I. Stein, E. Horwitz, S. Singer, E. Dimou, A. Cigliano, M. Bissinger, C.S. Falk, X. Chen, S. Dooley, E. Pikarsky, D.F. Calvisi, C. Schultz, P. Schirmacher, K. Breuhahn, PI3K/AKT/mTOR-dependent stabilization of oncogenic far-upstream element binding proteins in hepatocellular carcinoma cells, Hepatology (Baltimore, Md.) 63 (2016) 813–826.

[52] X. Chen, Y. Wang, J. Tao, Y. Shi, X. Gai, F. Huang, Q. Ma, Z. Zhou, H. Chen, H. Zhang, Z. Liu, Q. Sun, H. Peng, R. Chen, Y. Jing, H. Yang, Y. Mao, H. Zhang, mTORC1 up-regulates GP73 to promote proliferation and migration of hepatocellular carcinoma cells and growth of xenograft tumors in mice, Gastroenterology 149 (2015) 741-752.e714.

[53] J.D. Richter, N. Sonenberg, Regulation of cap-dependent translation by eIF4E inhibitory proteins, Nature 433 (2005) 477–480.

[54] B. Ravikumar, M. Futter, L. Jahreiss, V.I. Korolchuk, M. Lichtenberg, S. Luo, D.C. Massey, F.M. Menzies, U. Narayanan, M. Renna, M. Jimenez-Sanchez, S. Sarkar, B. Underwood, A. Winslow, D.C. Rubinsztein, Mammalian macroautophagy at a glance, J. Cell Sci. 122 (2009) 1707–1711.

[55] S.S. Wang, Y.H. Chen, N. Chen, L.J. Wang, D.X. Chen, H.L. Weng, S. Dooley, H.G. Ding, Hydrogen sulfide promotes autophagy of hepatocellular carcinoma cells through the PI3K/Akt/mTOR signaling pathway, Cell Death Dis. 8 (2017) e2688.

[56] M.S. Matter, T. Decaens, J.B. Andersen, S.S. Thorgeirsson, Targeting the mTOR pathway in hepatocellular carcinoma: current state and future trends, J. Hepatol. 60 (2014) 855–865.

[57] S. Lu, Y. Yao, G. Xu, C. Zhou, Y. Zhang, J. Sun, R. Jiang, Q. Shao, Y. Chen, CD24 regulates sorafenib resistance via activating autophagy in hepatocellular carcinoma, Cell Death Dis. 9 (2018) 646.

[58] J. Cui, Z. Gong, H.M. Shen, The role of autophagy in liver cancer: molecular mechanisms and potential therapeutic targets, Biochim. Biophys. Acta 1836 (2013) 15–26.

[59] J.R. Cantor, D.M. Sabatini, Cancer cell metabolism: one hallmark, many faces, Cancer Discov. 2 (2012) 881–898.

[60] K. Duvel, J.L. Yecies, S. Menon, P. Raman, A.I. Lipovsky, A.L. Souza, E. Triantafellow, Q. Ma, R. Gorski, S. Cleaver, M.G. Vander Heiden, J.P. MacKeigan, P.M. Finan, C.B. Clish, L.O. Murphy, B.D. Manning, Activation of a metabolic gene regulatory network downstream of mTOR complex 1, Mol. Cell 39 (2010) 171–183.

[61] M. Cornu, V. Albert, M.N. Hall, mTOR in aging, metabolism, and cancer, Curr. Opin. Genet. Dev. 23 (2013) 53–62.

[62] M. Evert, D.F. Calvisi, K. Evert, V. De Murtas, G. Gasparetti, S. Mattu, G. Destefanis, S. Ladu, A. Zimmermann, S. Delogu, S. Thiel, A. Thiele, S. Ribback, F. Dombrowski, V-AKT murine thymoma viral oncogene homolog/mammalian target of rapamycin activation induces a module of metabolic changes contributing to growth in insulin-induced hepatocarcinogenesis, Hepatology (Baltimore, Md.) 55 (2012) 1473–1484.

[63] S.C. Land, A.R. Tee, Hypoxia-inducible factor 1alpha is regulated by the mammalian target of rapamycin (mTOR) via an mTOR signaling motif, J. Biol. Chem. 282 (2007) 20534–20543.

[64] J. Li, Q. Huang, X. Long, J. Zhang, X. Huang, J. Aa, H. Yang, Z. Chen, J. Xing, CD147 reprograms fatty acid metabolism in hepatocellular carcinoma cells through Akt/mTOR/SREBP1c and P38/PPARalpha pathways, J. Hepatol. 63 (2015) 1378–1389.

[65] T.R. Peterson, S.S. Sengupta, T.E. Harris, A.E. Carmack, S.A. Kang, E. Balderas, D.A. Guertin, K.L. Madden, A.E. Carpenter, B.N. Finck, D.M. Sabatini, mTOR complex 1 regulates lipin 1 localization to control the SREBP pathway, Cell 146 (2011) 408–420.

[66] D.F. Calvisi, C. Wang, C. Ho, S. Ladu, S.A. Lee, S. Mattu, G. Destefanis, S. Delogu,

A. Zimmermann, J. Ericsson, S. Brozzetti, T. Staniscia, X. Chen, F. Dombrowski, M. Evert, Increased lipogenesis, induced by AKT-mTORC1-RPS6 signaling, promotes development of human hepatocellular carcinoma, Gastroenterology 140 (2011) 1071–1083.
[67] K. Sugimachi, S. Tanaka, T. Terashi, K. Taguchi, T. Rikimaru, K. Sugimachi, The mechanisms of angiogenesis in hepatocellular carcinoma: angiogenic switch during tumor progression, Surgery 131 (2002) S135–S141.
[68] W. Li, D. Tan, Z. Zhang, J.J. Liang, R.E. Brown, Activation of Akt-mTOR-p70S6K pathway in angiogenesis in hepatocellular carcinoma, Oncol. Rep. 20 (2008) 713–719.
[69] C.Y. Ku, Y.H. Liu, H.Y. Lin, S.C. Lu, J.Y. Lin, Liver fatty acid-binding protein (L-FABP) promotes cellular angiogenesis and migration in hepatocellular carcinoma, Oncotarget 7 (2016) 18229–18246.
[70] X. Zhao, J. Li, J. Zhuo, L. Cai, Reexpression of ARHI inhibits tumor growth and angiogenesis and impairs the mTOR/VEGF pathway in hepatocellular carcinoma, Biochem. Biophys. Res. Commun. 403 (2010) 417–421.
[71] S. Ladu, D.F. Calvisi, E.A. Conner, M. Farina, V.M. Factor, S.S. Thorgeirsson, E2F1 inhibits c-Myc-driven apoptosis via PIK3CA/Akt/mTOR and COX-2 in a mouse model of human liver cancer, Gastroenterology 135 (2008) 1322–1332.
[72] K.H. Jung, M.J. Choi, S. Hong, H. Lee, S.W. Hong, H.M. Zheng, H.S. Lee, S. Hong, S.S. Hong, HS-116, a novel phosphatidylinositol 3-kinase inhibitor induces apoptosis and suppresses angiogenesis of hepatocellular carcinoma through inhibition of the PI3K/AKT/mTOR pathway, Cancer Lett. 316 (2012) 187–195.
[73] M. Bhat, N. Sonenberg, G.J. Gores, The mTOR pathway in hepatic malignancies, Hepatology (Baltimore, Md.) 58 (2013) 810–818.
[74] J.S. Chen, Q. Wang, X.H. Fu, X.H. Huang, X.L. Chen, L.Q. Cao, L.Z. Chen, H.X. Tan, W. Li, J. Bi, L.J. Zhang, Involvement of PI3K/PTEN/AKT/mTOR pathway in invasion and metastasis in hepatocellular carcinoma: association with MMP-9, Hepatol. Res. 39 (2009) 177–186.
[75] J.C. Cheng, C.H. Chou, M.L. Kuo, C.Y. Hsieh, Radiation-enhanced hepatocellular carcinoma cell invasion with MMP-9 expression through PI3K/Akt/NF-kappaB signal transduction pathway, Oncogene 25 (2006) 7009–7018.
[76] T.W. Chung, Y.C. Lee, C.H. Kim, Hepatitis B viral HBx induces matrix metalloproteinase-9 gene expression through activation of ERK and PI-3K/AKT pathways: involvement of invasive potential, FASEB J. 18 (2004) 1123–1125.
[77] H. Wang, C. Zhang, L. Xu, K. Zang, Z. Ning, F. Jiang, H. Chi, X. Zhu, Z. Meng, Bufalin suppresses hepatocellular carcinoma invasion and metastasis by targeting HIF-1alpha via the PI3K/AKT/mTOR pathway, Oncotarget 7 (2016) 20193–20208.
[78] J.J. Lin, J.H. Su, C.C. Tsai, Y.J. Chen, M.H. Liao, Y.J. Wu, 11-epi-Sinulariolide acetate reduces cell migration and invasion of human hepatocellular carcinoma by reducing the activation of ERK1/2, p38MAPK and FAK/PI3K/AKT/mTOR signaling pathways, Mar. Drugs 12 (2014) 4783–4798.
[79] F. Jiang, L. Chen, Y.C. Yang, X.M. Wang, R.Y. Wang, L. Li, W. Wen, Y.X. Chang, C.Y. Chen, J. Tang, G.M. Liu, W.T. Huang, L. Xu, H.Y. Wang, CYP3A5 functions as a tumor suppressor in hepatocellular carcinoma by regulating mTORC2/Akt signaling, Cancer Res. 75 (2015) 1470–1481.
[80] M. Masuda, W.Y. Chen, A. Miyanaga, Y. Nakamura, K. Kawasaki, T. Sakuma, M. Ono, C.L. Chen, K. Honda, T. Yamada, Alternative mammalian target of rapamycin (mTOR) signal activation in sorafenib-resistant hepatocellular carcinoma cells revealed by array-based pathway profiling, Mol. Cell. Proteomics 13 (2014) 1429–1438.
[81] H. Yang, Y.F. Peng, H.M. Ni, Y. Li, Y.H. Shi, W.X. Ding, J. Fan, Basal autophagy and feedback activation of Akt are associated with resistance to metformin-induced inhibition of hepatic tumor cell growth, PLoS One 10 (2015) e0130953.
[82] M. Gao, Q. Kong, H. Hua, Y. Yin, J. Wang, T. Luo, Y. Jiang, AMPK-mediated up-regulation of mTORC2 and MCL-1 compromises the anti-cancer effects of aspirin, Oncotarget 7 (2016) 16349–16361.
[83] K.H. Tam, Z.F. Yang, C.K. Lau, C.T. Lam, R.W. Pang, R.T. Poon, Inhibition of mTOR enhances chemosensitivity in hepatocellular carcinoma, Cancer Lett. 273 (2009) 201–209.
[84] B.W. Chen, W. Chen, H. Liang, H. Liu, C. Liang, X. Zhi, L.Q. Hu, X.Z. Yu, T. Wei, T. Ma, F. Xue, L. Zheng, B. Zhao, X.H. Feng, X.L. Bai, T.B. Liang, Inhibition of mTORC2 induces cell-cycle arrest and enhances the cytotoxicity of doxorubicin by suppressing MDR1 expression in HCC cells, Mol. Cancer Ther. 14 (2015) 1805–1815.
[85] F. Fornari, M. Milazzo, P. Chieco, M. Negrini, G.A. Calin, G.L. Grazi, D. Pollutri, C.M. Croce, L. Bolondi, L. Gramantieri, MiR-199a-3p regulates mTOR and c-Met to influence the doxorubicin sensitivity of human hepatocarcinoma cells, Cancer Res. 70 (2010) 5184–5193.
[86] R. Rupaimoole, F.J. Slack, MicroRNA therapeutics: towards a new era for the management of cancer and other diseases, Nat. Rev. Drug Discov. 16 (2017) 203–222.
[87] A. Varshney, J.J. Panda, A.K. Singh, N. Yadav, C. Bihari, S. Biswas, S.K. Sarin, V.S. Chauhan, Targeted delivery of microRNA-199a-3p using self-assembled dipeptide nanoparticles efficiently reduces hepatocellular carcinoma in mice, Hepatology (Baltimore, Md.) 67 (2018) 1392–1407.
[88] E. Callegari, L. D'Abundo, P. Guerriero, C. Simioni, B.K. Elamin, M. Russo, A. Cani, C. Bassi, B. Zagatti, L. Giacomelli, S. Blandamura, F. Moshiri, S. Ultimo, A. Frassoldati, G. Altavilla, L. Gramantieri, L.M. Neri, S. Sabbioni, M. Negrini, miR-199a-3p modulates MTOR and PAK4 pathways and inhibits tumor growth in a hepatocellular carcinoma transgenic mouse model, molecular therapy, Nucleic Acids 11 (2018) 485–493.
[89] D. Li, X. Liu, L. Lin, J. Hou, N. Li, C. Wang, P. Wang, Q. Zhang, P. Zhang, W. Zhou, Z. Wang, G. Ding, S.M. Zhuang, L. Zheng, W. Tao, X. Cao, MicroRNA-99a inhibits hepatocellular carcinoma growth and correlates with prognosis of patients with hepatocellular carcinoma, J. Biol. Chem. 286 (2011) 36677–36685.
[90] Y.Y. Ge, Q. Shi, Z.Y. Zheng, J. Gong, C. Zeng, J. Yang, S.M. Zhuang, MicroRNA-100 promotes the autophagy of hepatocellular carcinoma cells by inhibiting the expression of mTOR and IGF-1R, Oncotarget 5 (2014) 6218–6228.
[91] H.C. Zhou, J.H. Fang, L.R. Shang, Z.J. Zhang, Y. Sang, L. Xu, Y. Yuan, M.S. Chen, L. Zheng, Y. Zhang, S.M. Zhuang, MicroRNAs miR-125b and miR-100 suppress metastasis of hepatocellular carcinoma by disrupting the formation of vessels that encapsulate tumour clusters, J. Pathol. 240 (2016) 450–460.
[92] H. Cheng, J. Xue, S. Yang, Y. Chen, Y. Wang, Y. Zhu, X. Wang, D. Kuang, Q. Ruan, Y. Duan, G. Wang, Co-targeting of IGF1R/mTOR pathway by miR-497 and miR-99a impairs hepatocellular carcinoma development, Oncotarget 8 (2017) 47984–47997.
[93] D. Jiang, W. Cho, Z. Li, X. Xu, Y. Qu, Z. Jiang, L. Guo, G. Xu, MiR-758-3p suppresses proliferation, migration and invasion of hepatocellular carcinoma cells via targeting MDM2 and mTOR, Biomed. Pharmacother. 96 (2017) 535–544.
[94] D. Pollutri, C. Patrizi, S. Marinelli, C. Giovannini, E. Trombetta, F.A. Giannone, M. Baldassarre, S. Quarta, Y.P. Vandewynckel, A. Vandierendonck, H. Van Vlierberghe, L. Porretti, M. Negrini, L. Bolondi, L. Gramantieri, F. Fornari, The epigenetically regulated miR-494 associates with stem-cell phenotype and induces sorafenib resistance in hepatocellular carcinoma, Cell Death Dis. 9 (2018) 4.
[95] Y. Fang, J.L. Xue, Q. Shen, J. Chen, L. Tian, MicroRNA-7 inhibits tumor growth and metastasis by targeting the phosphoinositide 3-kinase/Akt pathway in hepatocellular carcinoma, Hepatology (Baltimore, Md.) 55 (2012) 1852–1862.
[96] Q. Lang, C. Ling, MiR-124 suppresses cell proliferation in hepatocellular carcinoma by targeting PIK3CA, Biochem. Biophys. Res. Commun. 426 (2012) 247–252.
[97] K. Liu, X. Li, Y. Cao, Y. Ge, J. Wang, B. Shi, MiR-132 inhibits cell proliferation, invasion and migration of hepatocellular carcinoma by targeting PIK3R3, Int. J. Oncol. 47 (2015) 1585–1593.
[98] G. Cao, W. Dong, X. Meng, H. Liu, H. Liao, S. Liu, MiR-511 inhibits growth and metastasis of human hepatocellular carcinoma cells by targeting PIK3R3, Tumour Biol. 36 (2015) 4453–4459.
[99] Y. Zhang, X. Guo, L. Xiong, L. Yu, Z. Li, Q. Guo, Z. Li, B. Li, N. Lin, Comprehensive analysis of microRNA-regulated protein interaction network reveals the tumor suppressive role of microRNA-149 in human hepatocellular carcinoma via targeting AKT-mTOR pathway, Mol. Cancer 13 (2014) 253.
[100] L.L. Liu, S.X. Lu, M. Li, L.Z. Li, J. Fu, W. Hu, Y.Z. Yang, R.Z. Luo, C.Z. Zhang, J.P. Yun, FoxD3-regulated microRNA-137 suppresses tumour growth and metastasis in human hepatocellular carcinoma by targeting AKT2, Oncotarget 5 (2014) 5113–5124.
[101] R. Spizzo, M.I. Almeida, A. Colombatti, G.A. Calin, Long non-coding RNAs and cancer: a new frontier of translational research? Oncogene 31 (2012) 4577–4587.
[102] J. Ji, J. Tang, L. Deng, Y. Xie, R. Jiang, G. Li, B. Sun, LINC00152 promotes proliferation in hepatocellular carcinoma by targeting EpCAM via the mTOR signaling pathway, Oncotarget 6 (2015) 42813–42824.
[103] P. Malakar, A. Shilo, A. Mogilevsky, I. Stein, E. Pikarsky, Y. Nevo, H. Benyamini, S. Elgavish, X. Zong, K.V. Prasanth, R. Karni, Long noncoding RNA MALAT1 promotes hepatocellular carcinoma development by SRSF1 Upregulation and mTOR activation, Cancer Res. 77 (2017) 1155–1167.
[104] X. Xin, M. Wu, Q. Meng, C. Wang, Y. Lu, Y. Yang, X. Li, Q. Zheng, H. Pu, X. Gui, T. Li, J. Li, S. Jia, D. Lu, Long noncoding RNA HULC accelerates liver cancer by inhibiting PTEN via autophagy cooperation to miR15a, Mol. Cancer 17 (2018) 94.
[105] K. Watari, A. Nishitani, T. Shibata, M. Noda, A. Kawahara, J. Akiba, Y. Murakami, H. Yano, M. Kuwano, M. Ono, Phosphorylation of mTOR Ser2481 is a key target limiting the efficacy of rapalogs for treating hepatocellular carcinoma, Oncotarget 7 (2016) 47403–47417.
[106] A. Carracedo, L. Ma, J. Teruya-Feldstein, F. Rojo, L. Salmena, A. Alimonti, A. Egia, A.T. Sasaki, G. Thomas, S.C. Kozma, A. Papa, C. Nardella, L.C. Cantley, J. Baselga, P.P. Pandolfi, Inhibition of mTORC1 leads to MAPK pathway activation through a PI3K-dependent feedback loop in human cancer, J. Clin. Invest. 118 (2008) 3065–3074.
[107] M. Rizell, M. Andersson, C. Cahlin, L. Hafstrom, M. Olausson, P. Lindner, Effects of the mTOR inhibitor sirolimus in patients with hepatocellular and cholangiocellular cancer, Int. J. Clin. Oncol. 13 (2008) 66–70.
[108] T. Decaens, A. Luciani, E. Itti, A. Hulin, F. Roudot-Thoraval, A. Laurent, E.S. Zafrani, A. Mallat, C. Duvoux, Phase II study of sirolimus in treatment-naive patients with advanced hepatocellular carcinoma, Digest. Liver Dis. 44 (2012) 610–616.
[109] M. Schoniger-Hekele, C. Muller, Pilot study: rapamycin in advanced hepatocellular carcinoma, Aliment. Pharmacol. Ther. 32 (2010) 763–768.
[110] A.X. Zhu, T.A. Abrams, R. Miksad, L.S. Blaszkowsky, J.A. Meyerhardt, H. Zheng, A. Muzikansky, J.W. Clark, E.L. Kwak, D. Schrag, K.R. Jors, C.S. Fuchs, A.J. Iafrate, D.R. Borger, D.P. Ryan, Phase 1/2 study of everolimus in advanced hepatocellular carcinoma, Cancer 117 (2011) 5094–5102.
[111] H.S. Shiah, C.Y. Chen, C.Y. Dai, C.F. Hsiao, Y.J. Lin, W.C. Su, J.Y. Chang, J. Whang-Peng, P.W. Lin, J.D. Huang, L.T. Chen, Randomised clinical trial: comparison of two everolimus dosing schedules in patients with advanced hepatocellular carcinoma, Aliment. Pharmacol. Ther. 37 (2013) 62–73.
[112] A.X. Zhu, M. Kudo, E. Assenat, S. Cattan, Y.K. Kang, H.Y. Lim, R.T. Poon, J.F. Blanc, A. Vogel, C.L. Chen, E. Dorval, M. Peck-Radosavljevic, A. Santoro, B. Daniele, J. Furuse, A. Jappe, K. Perraud, O. Anak, D.B. Sellami, L.T. Chen, Effect of everolimus on survival in advanced hepatocellular carcinoma after failure of sorafenib: the EVOLVE-1 randomized clinical trial, Jama 312 (2014) 57–67.
[113] W. Yeo, S.L. Chan, F.K. Mo, C.M. Chu, J.W. Hui, J.H. Tong, A.W. Chan, J. Koh, E.P. Hui, H. Loong, K. Lee, L. Li, B. Ma, K.F. To, S.C. Yu, Phase I/II study of temsirolimus for patients with unresectable Hepatocellular Carcinoma (HCC)- a

correlative study to explore potential biomarkers for response, BMC Cancer 15 (2015) 395.
[114] H.E. Thomas, C.A. Mercer, L.S. Carnevalli, J. Park, J.B. Andersen, E.A. Conner, K. Tanaka, T. Matsutani, A. Iwanami, B.J. Aronow, L. Manway, S.M. Maira, S.S. Thorgeirsson, P.S. Mischel, G. Thomas, S.C. Kozma, mTOR inhibitors synergize on regression, reversal of gene expression, and autophagy in hepatocellular carcinoma, Sci. Transl. Med. 4 (2012) 139ra184.
[115] P. Newell, S. Toffanin, A. Villanueva, D.Y. Chiang, B. Minguez, L. Cabellos, R. Savic, Y. Hoshida, K.H. Lim, P. Melgar-Lesmes, S. Yea, J. Peix, K. Deniz, M.I. Fiel, S. Thung, C. Alsinet, V. Tovar, V. Mazzaferro, J. Bruix, S. Roayaie, M. Schwartz, S.L. Friedman, J.M. Llovet, Ras pathway activation in hepatocellular carcinoma and anti-tumoral effect of combined sorafenib and rapamycin in vivo, J. Hepatol. 51 (2009) 725–733.
[116] H. Huynh, P.K. Chow, N. Palanisamy, M. Salto-Tellez, B.C. Goh, C.K. Lee, A. Somani, H.S. Lee, R. Kalpana, K. Yu, P.H. Tan, J. Wu, R. Soong, M.H. Lee, H. Hor, K.C. Soo, H.C. Toh, P. Tan, Bevacizumab and rapamycin induce growth suppression in mouse models of hepatocellular carcinoma, J. Hepatol. 49 (2008) 52–60.
[117] H. Shao, C. Gao, H. Tang, H. Zhang, L.R. Roberts, B.L. Hylander, E.A. Repasky, W.W. Ma, J. Qiu, A.A. Adjei, G.K. Dy, C. Yu, Dual targeting of mTORC1/C2 complexes enhances histone deacetylase inhibitor-mediated anti-tumor efficacy in primary HCC cancer in vitro and in vivo, J. Hepatol. 56 (2012) 176–183.
[118] R. Wu, R. Murali, Y. Kabe, S.W. French, Y.M. Chiang, S. Liu, L. Sher, C.C. Wang, S. Louie, H. Tsukamoto, Baicalein targets GTPase-mediated autophagy to eliminate liver tumor-initiating stem cell-like cells resistant to mTORC1 inhibition, Hepatology (Baltimore, Md.) 68 (2018) 1726–1740.
[119] T. Yongxi, H. Haijun, Z. Jiaping, S. Guoliang, P. Hongying, Autophagy inhibition sensitizes KU-0063794-mediated anti-HepG2 hepatocellular carcinoma cell activity in vitro and in vivo, Biochem. Biophys. Res. Commun. 465 (2015) 494–500.
[120] S.P. Choo, B. Chowbay, Q.S. Ng, C.H. Thng, C. Lim, S. Hartono, T.S. Koh, H. Huynh, D. Poon, M.K. Ang, S. Chang, H.C. Toh, A Phase 1 dose-finding and pharmacodynamic study of rapamycin in combination with bevacizumab in patients with unresectable hepatocellular carcinoma, Eur. J. Cancer 49 (2013) 999–1008.
[121] R.K. Kelley, H.S. Nimeiri, P.N. Munster, M.T. Vergo, Y. Huang, C.M. Li, J. Hwang, M.F. Mulcahy, B.M. Yeh, P. Kuhn, M.S. Luttgen, J.A. Grabowsky, L. Stucky-Marshall, W.M. Korn, A.H. Ko, E.K. Bergsland, A.B. Benson 3rd, A.P. Venook, Temsirolimus combined with sorafenib in hepatocellular carcinoma: a phase I dose-finding trial with pharmacokinetic and biomarker correlates, Ann. Oncol. 24 (2013) 1900–1907.
[122] J.M. Llovet, S. Ricci, V. Mazzaferro, P. Hilgard, E. Gane, J.F. Blanc, A.C. de Oliveira, A. Santoro, J.L. Raoul, A. Forner, M. Schwartz, C. Porta, S. Zeuzem, L. Bolondi, T.F. Greten, P.R. Galle, J.F. Seitz, I. Borbath, D. Haussinger, T. Giannaris, M. Shan, M. Moscovici, D. Voliotis, J. Bruix, Sorafenib in advanced hepatocellular carcinoma, N. Engl. J. Med. 359 (2008) 378–390.
[123] R.S. Finn, R.T. Poon, T. Yau, H.J. Klumpen, L.T. Chen, Y.K. Kang, T.Y. Kim, C. Gomez-Martin, C. Rodriguez-Lope, T. Kunz, T. Paquet, U. Brandt, D. Sellami, J. Bruix, Phase I study investigating everolimus combined with sorafenib in patients with advanced hepatocellular carcinoma, J. Hepatol. 59 (2013) 1271–1277.
[124] D. Koeberle, J.F. Dufour, G. Demeter, Q. Li, K. Ribi, P. Samaras, P. Saletti, A.D. Roth, D. Horber, M. Buehlmann, A.D. Wagner, M. Montemurro, G. Lakatos, J. Feilchenfeldt, M. Peck-Radosavljevic, D. Rauch, B. Tschanz, G. Bodoky, Sorafenib with or without everolimus in patients with advanced hepatocellular carcinoma (HCC): a randomized multicenter, multinational phase II trial (SAKK 77/08 and SASL 29), Ann. Oncol. 27 (2016) 856–861.
[125] M. Hojo, T. Morimoto, M. Maluccio, T. Asano, K. Morimoto, M. Lagman, T. Shimbo, M. Suthanthiran, Cyclosporine induces cancer progression by a cell-autonomous mechanism, Nature 397 (1999) 530–534.
[126] C.E. Freise, L. Ferrell, T. Liu, N.L. Ascher, J.P. Roberts, Effect of systemic cyclosporine on tumor recurrence after liver transplantation in a model of hepatocellular carcinoma, Transplantation 67 (1999) 510–513.
[127] K. Willuweit, A. Heinold, J. Rashidi-Alavijeh, F.M. Heinemann, P.A. Horn, A. Paul, G. Gerken, K. Herzer, Immunosuppression with mTOR inhibitors prevents the development of donor-specific antibodies after liver transplant, Clin. Transpl. 31 (2017).
[128] W. Liang, D. Wang, X. Ling, A.A. Kao, Y. Kong, Y. Shang, Z. Guo, X. He, Sirolimus-based immunosuppression in liver transplantation for hepatocellular carcinoma: a meta-analysis, Liver Transplant. 18 (2012) 62–69.
[129] E.K. Geissler, A.A. Schnitzbauer, C. Zulke, P.E. Lamby, A. Proneth, C. Duvoux, P. Burra, K.W. Jauch, M. Rentsch, T.M. Ganten, J. Schmidt, U. Settmacher, M. Heise, G. Rossi, U. Cillo, N. Kneteman, R. Adam, B. van Hoek, P. Bachellier, P. Wolf, L. Rostaing, W.O. Bechstein, M. Rizell, J. Powell, E. Hidalgo, J. Gugenheim, H. Wolters, J. Brockmann, A. Roy, I. Mutzbauer, A. Schlitt, S. Beckebaum, C. Graeb, S. Nadalin, U. Valente, V.S. Turrion, N. Jamieson, T. Scholz, M. Colledan, F. Fandrich, T. Becker, G. Soderdahl, O. Chazouilleres, H. Makisalo, G.P. Pageaux, R. Steininger, T. Soliman, K.P. de Jong, J. Pirenne, R. Margreiter, J. Pratschke, A.D. Pinna, J. Hauss, S. Schreiber, S. Strasser, J. Klempnauer, R.I. Troisi, S. Bhoori, J. Lerut, I. Bilbao, C.G. Klein, A. Konigsrainer, D.F. Mirza, G. Otto, V. Mazzaferro, P. Neuhaus, H.J. Schlitt, Sirolimus use in liver transplant recipients with hepatocellular carcinoma: a randomized, multicenter, open-label phase 3 trial, Transplantation 100 (2016) 116–125.
[130] C. Shen, C. Peng, B. Shen, Z. Zhu, N. Xu, T. Li, J. Xie, Sirolimus and metformin synergistically inhibit hepatocellular carcinoma cell proliferation and improve long-term survival in patients with HCC related to hepatitis B virus induced cirrhosis after liver transplantation, Oncotarget 7 (2016) 62647–62656.
[131] C. Gomez-Martin, J. Bustamante, J.F. Castroagudin, M. Salcedo, E. Garralda, M. Testillano, I. Herrero, A. Matilla, B. Sangro, Efficacy and safety of sorafenib in combination with mammalian target of rapamycin inhibitors for recurrent hepatocellular carcinoma after liver transplantation, Liver Transplant. 18 (2012) 45–52.
[132] K.S. Suh, L.-B. Jeng, A.S. Soin, W.-C. Lee, S.G. Lee, D. Grant, T. Yoshizumi, M. El Meteini, M. Meier, J. Kochuparampil, C. Sips, S. Kaneko, G. Levy, Renal function outcomes with everolimus plus reduced-exposure tacrolimus in de novo living donor liver transplantation: 24-month results from the H2307 study, Transplantation 102 (2018) S21–S22.
[133] L.B. Jeng, S.G. Lee, A.S. Soin, W.C. Lee, K.S. Suh, D.J. Joo, S. Uemoto, J. Joh, T. Yoshizumi, H.R. Yang, G.W. Song, P. Lopez, J. Kochuparampil, C. Sips, S. Kaneko, G. Levy, Efficacy and safety of everolimus with reduced tacrolimus in living-donor liver transplant recipients: 12-month results of a randomized multicenter study, Am. J. Transplant. Off. J. Am. Soc. Transplant. Am. Soc. Transplant Surg. 18 (2018) 1435–1446.
[134] A.K. Chow, T.C. Yau, L. Ng, A.C. Chu, W.L. Law, R.T. Poon, R.W. Pang, A preclinical study on the combination therapy of everolimus and transarterial chemoembolization in hepatocellular carcinoma, Am. J. Cancer Res. 5 (2015) 2376–2386.
[135] Y. Zhang, Q.A. Jia, D. Kadel, X.F. Zhang, Q.B. Zhang, Targeting mTORC1/2 complexes inhibit tumorigenesis and enhance sensitivity to 5-flourouracil (5-FU) in hepatocellular carcinoma: a preclinical study of mTORC1/2-targeted therapy in hepatocellular carcinoma (HCC), Med. Sci. Monit. 24 (2018) 2735–2743.
[136] M. Badawi, J. Kim, A. Dauki, D. Sutaria, T. Motiwala, R. Reyes, N. Wani, S. Kolli, J. Jiang, C.C. Coss, S.T. Jacob, M.A. Phelps, T.D. Schmittgen, CD44 positive and sorafenib insensitive hepatocellular carcinomas respond to the ATP-competitive mTOR inhibitor INK128, Oncotarget 9 (2018) 26032–26045.
[137] J. Cho, J. Lee, J. Kim, S.T. Kim, S. Lee, S.Y. Kim, S.Y. Ha, C.K. Park, H.Y. Lim, Loss of tuberous sclerosis complex 2 (TSC2) as a predictive biomarker of response to mTOR inhibitor treatment in patients with hepatocellular carcinoma, Transl. Oncol. 9 (2016) 466–471.
[138] H. Huynh, H.X. Hao, S.L. Chan, D. Chen, R. Ong, K.C. Soo, P. Pochanard, D. Yang, D. Ruddy, M. Liu, A. Derti, M.N. Balak, M.R. Palmer, Y. Wang, B.H. Lee, D. Sellami, A.X. Zhu, R. Schlegel, A. Huang, Loss of tuberous sclerosis complex 2 (TSC2) is frequent in hepatocellular carcinoma and predicts response to mTORC1 inhibitor everolimus, Mol. Cancer Ther. 14 (2015) 1224–1235.
[139] A.O. Adebayo Michael, S. Ko, J. Tao, A. Moghe, H. Yang, M. Xu, J.O. Russell, T. Pradhan-Sundd, S. Liu, S. Singh, M. Poddar, J.S. Monga, P. Liu, M. Oertel, S. Ranganathan, A. Singhi, S. Rebouissou, J. Zucman-Rossi, S. Ribback, D. Calvisi, N. Qvartskhava, B. Gorg, D. Haussinger, X. Chen, S.P. Monga, Inhibiting glutamine-dependent mTORC1 activation ameliorates liver cancers driven by beta-catenin mutations, Cell Metab. (2019) Epub ahead of print.
[140] Z.Y. Zhang, D. Hong, S.H. Nam, J.M. Kim, Y.H. Paik, J.W. Joh, C.H. Kwon, J.B. Park, G.S. Choi, K.Y. Jang, C.K. Park, S.J. Kim, SIRT1 regulates oncogenesis via a mutant p53-dependent pathway in hepatocellular carcinoma, J. Hepatol. 62 (2015) 121–130.
[141] F. Cauchy, M. Mebarki, B. Leporq, S. Laouirem, M. Albuquerque, S. Lambert, P. Bourgoin, O. Soubrane, B.E. Van Beers, S. Faivre, P. Bedossa, V. Paradis, Strong antineoplastic effects of metformin in preclinical models of liver carcinogenesis, Clinical Sci. 131 (2017) 27–36.
[142] Z.J. Zhang, Z.J. Zheng, R. Shi, Q. Su, Q. Jiang, K.E. Kip, Metformin for liver cancer prevention in patients with type 2 diabetes: a systematic review and meta-analysis, J. Clin. Endocrinol. Metab. 97 (2012) 2347–2353.
[143] H.P. Chen, J.J. Shieh, C.C. Chang, T.T. Chen, J.T. Lin, M.S. Wu, J.H. Lin, C.Y. Wu, Metformin decreases hepatocellular carcinoma risk in a dose-dependent manner: population-based and in vitro studies, Gut 62 (2013) 606–615.
[144] H. Totary-Jain, D. Sanoudou, I.Z. Ben-Dov, C.N. Dautriche, P. Guarnieri, S.O. Marx, T. Tuschl, A.R. Marks, Reprogramming of the microRNA transcriptome mediates resistance to rapamycin, J. Biol. Chem. 288 (2013) 6034–6044.
[145] H. Park, I. Garrido-Laguna, A. Naing, S. Fu, G.S. Falchook, S.A. Piha-Paul, J.J. Wheler, D.S. Hong, A.M. Tsimberidou, V. Subbiah, R.G. Zinner, A.O. Kaseb, S. Patel, M.A. Fanale, V.M. Velez-Bravo, F. Meric-Bernstam, R. Kurzrock, F. Janku, Phase I dose-escalation study of the mTOR inhibitor sirolimus and the HDAC inhibitor vorinostat in patients with advanced malignancy, Oncotarget 7 (2016) 67521–67531.
[146] H.K. Sanoff, R. Kim, A. Ivanova, A. Alistar, A.J. McRee, B.H. O'Neil, Everolimus and pasireotide for advanced and metastatic hepatocellular carcinoma, Investig. New Drugs 33 (2015) 505–509.
[147] B. Nashan, P. Schemmer, F. Braun, M. Dworak, P. Wimmer, H. Schlitt, Evaluating the efficacy, safety and evolution of renal function with early initiation of everolimus-facilitated tacrolimus reduction in de novo liver transplant recipients: study protocol for a randomized controlled trial, Trials 16 (2015) 118.