REVIEW

Translational Regulation in Hepatocellular Carcinogenesis

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Abstract: The mortality of hepatocellular carcinoma (HCC) is distributed unevenly worldwide. One of the major causes is hepatitis B or hepatitis C virus infection and the development and progression of liver cirrhosis. The carcinogenesis of HCC is among others regulated via the mTOR (mechanistic target of rapamycin) signaling pathway and represents a possible method of targeted treatment. The aim of our article was to address the most recent clinical advances and findings of basic studies on the mTOR signaling pathway and the involved factors. Risk factors play a key role in dysregulation of the signaling pathway, where both mTORCs are upregulated and protein synthesis is altered. eIFs and, to a lesser extent, eEFs play an essential role in this process. Whether the factor will be upregulated or downregulated, among others, depends on hepatitis B/C virus infection. The amount of a particular factor in a patient sample lets us know whether HCC recurrence will occur, what is the likelihood of chemoresistance, and what outcome is predicted for patients with an increased value. Our analysis shows that in addition to mTOR, eIF3, eIF4, and eIF5 play an important role, as they can serve as biomarkers for non- and virus-related HCC.

Keywords: mTOR, virus related HCC, non-virus related HCC, cancer, translation initiation, liver

Introduction

Hepatocellular carcinoma (HCC) is a major health problem, as it was the third most common cause of mortality worldwide in 2020.[1] Between 1990 and 2016 it ranked as the fifth most common cause of cancer in men and the seventh in women.[2]

Major risk factors include chronic infection with hepatitis B (HBV) or hepatitis C virus (HCV), exposure to aflatoxin B1, non-alcoholic fatty liver disease (NAFLD), non-alcoholic steatohepatitis (NASH), alcohol-related liver disease like alcoholic steatohepatitis (ASH), diabetes and obesity.[1,3,4]

Development of HCC begins with exposure to one or more risk factors, which trigger inflammation of liver parenchyma with fibrotic deposition. When cirrhosis and decreased activity occur, the likelihood of developing HCC increases. Changes in the genome, as well as epigenetic modifications, play an important role in further development. Although several authors have already dealt with the molecular profile of HCC and the associated progression or recurrence of the disease,[5,6] two main molecular groups were identified: proliferative and non-proliferative HCC.[7] A classification based on metabolism was presented.[8]

HCC classification, treatment, and survival follow the Barcelona Clinic Liver Cancer staging and treatment strategy (BCLC). BCLC presents five prognostic subclasses and specific treatments for each: surgical resection, liver transplantation,

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ablation, chemoembolization, and systemic therapy.[9] Despite the known risk factors and various options for monitoring and diagnosis, 40% of patients with an early stage (stage 0 and A) are suitable for treatment with resection, transplantation or local ablation. Stage B patients profit from chemoembolization and patients with advanced cancer (stage C) receive systemic therapy using sorafenib.[10,11] BCLC staging could be upgraded with additional molecular-based information as it is associated with patient recurrence and outcome. Dysregulation of the signaling pathways participating in HCC development could represent additional information for therapy decision making.[12]

Dysregulation in multiple signaling pathways such as phosphoinositide 3-kinase/protein kinase B/mechanistic target of rapamycin (PI3K/AKT/mTOR), Ras mitogen-activated protein kinase (Ras/Raf/MAPK), Hedgehog (HH), Wnt/β-catenin, Janus kinase-signal transducer activator of transcription factor (JAK/STAT) signaling pathway was found in HCC carcinogenesis.[13,14]

PI3K/AKT/mTOR signaling pathway has been extensively studied in the development of HCC with and without viral background. Dysregulation of this pathway is key in hepatocarcinogenesis.[15]

The present review article addresses advances in the mTOR signaling pathway and its factors, allowing us to better understand the treatment possibilities of patients with HCC.

PI3K/AKT/mTOR Pathway

The PI3K/AKT/mTOR pathway is an intracellular signaling pathway that is activated by insulin and various growth factors (Figure 1). PI3K activation phosphorylates and activates AKT, which, among others, activates mTOR, affecting the transcription of p70 or 4EBP1. mTOR has two complexes, mTORC1 and mTORC2. Activation of the signal pathway results in fatty acid synthesis, glycogen synthesis, cell survival, proliferation, autophagy, glycolysis and protein synthesis.[16]

The PI3K/AKT/mTOR signaling pathway is essential for protein synthesis, which is divided into four steps: initiation, elongation, termination, and ribosome recycling. Eukaryotic translation initiation factors (eIFs) thus enter the initiation phase followed by elongation, which is accompanied by eukaryotic translation elongation factors (eEFs). mTOR phosphorylation leads via additional phosphorylation steps to the phosphorylation of 4E-BP-1. 4E-BP1 dissociates and allows the eIF4F complex formation.

This results in a cap-dependent activation of translation initiation. The formation of a 43S pre-initiation complex consisting of a 40S small ribosomal unit, eIF3, eIF1, eIF1A, eIF2 and eIF5 starts. Later, the eIF4F complex recognizes the 5'(prime) end of mRNA and binds to the 43S pre-initiation complex. The scanning of the start codon begins. After recognition of the start codon, the recruitment of the 60S ribosome and finally the formation of the 80S ribosome follows. Subsequently, all eIFs are released.[17,18]

mRNA chain then enters the elongation phase, where amino acids are added into a growing polypeptide chain. There are binding sites for tRNA on the ribosome, namely P (peptidyl) and A (aminoacyl). Upon completion of initiation, Met-tRNAiMet is bound to the P site of the ribosome. The Met-tRNAiMet anticodon is paired with the initial messenger RNA (mRNA) codon, and the second open reading frame (ORF) codon is in place. Elongation begins with aminoacyl-tRNA (RNA transfer) to the A site of the ribosome. Following guanosine triphosphate (GTP) binding, the eukaryotic eEf1A translation elongation factor is activated, and a ternary complex is formed. The eEF1A/GTP/aminoacyl-tRNA complex binds to site A. A peptide bond is formed between amino acids during peptidyl transfer with the amino acid cleaving from the tRNA located at the site P. The mRNA shifts from the 5' to 3' end. tRNA that was previously at site A is moved to site P; from site P to exit point (site E), where it leaves the ribosome. This task is performed with an elongation factor 2, which hydrolyzes the GTP.[19–21]

PI3K/AKT/mTOR in HCC

HCC PI3K/AKT/mTOR signaling (Figure 2) is dysregulated, and both mTOR complexes are involved: mTORC1 regulates protein synthesis, autophagy, glucose and lipid metabolism, and mTORC2 stimulates liver tumorigenesis. Both mTOR complexes were reported to be upregulated in 40–50% of HCC patients.[22]

Under physiological conditions, the liver is an organ highly involved in lipid metabolism. The liver transforms glucose into fatty acids, which is regulated by the PI3K/AKT/mTOR pathway with insulin and enzymes. It was shown that upregulation of lipid synthesis causes hepatosteatosis and consequently NASH and HCC.[23] mTORC2 stimulates the uptake of fatty acids directly from the blood or is involved in de novo synthesis of fatty acids.[24]
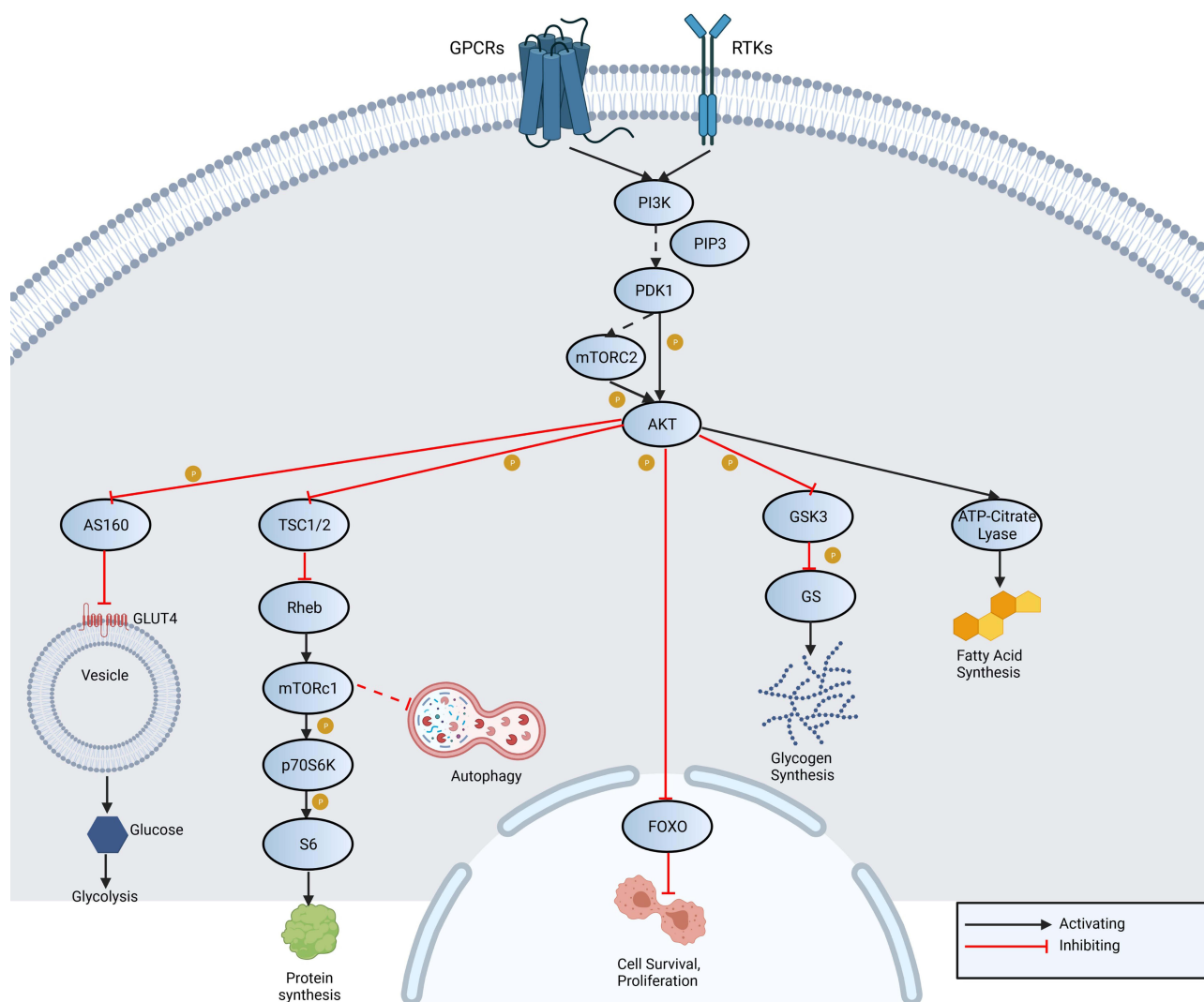
Figure 1 An overview of PI3K/AKT/mTOR signaling pathway shows activation of PI3K through receptor tyrosine kinases (RTK) and G-protein coupled receptors (GPCR). It acts directly on AKT or indirectly via mTORC2. Activation of inhibition of adenosine triphosphate (ATP)-citrate lyase, glycogen synthase kinase 3 (GSK3), forkhead box protein O (FOXO), Rab GTPase-activating protein (AS160) and tuberous sclerosis 1/2. As a result fatty acid synthesis, glycogen synthesis, cell survival, proliferation, autophagy, glycolysis and protein synthesis occur.
Abbreviations: PIP3, phosphatidylinositol (3,4,5)-trisphosphate; GS, glycogen synthase; GLUT4, glucose transporter type 4; Rheb, Ras homolog enriched in brain; p70S6K, ribosomal protein S6 kinase beta-1; S6, ribosomal protein S6.

NAFLD is caused by free fatty acids and their accumulation in hepatocytes. 30–40% of patients who later develop NASH[25] Center for pathogenesis of NASH are free fatty acids produced by lipolysis in adipose tissue. In the case of over elevated levels of free fatty acids, lipotoxic elements can be formed, which leads to endoplasmic reticulum stress, oxidant stress and activation of inflammation cells.[26] Elevated levels of fatty acids can induce NAFLD, which might progress to NASH and lead to HCC development.[25,27] It was also shown that increased resistance to insulin was connected to decreased mTORC2-activity.[25]

Chronic alcohol consumption can lead to liver cirrhosis due to oxidative stress and the formation of steatosis by promoting hepatic lipogenesis. Increased phosphorylation of mTOR, activation of mTORC1 and downstream kinase S6K1 in hepatocytes has to take place.[28] Occasional alcohol consumption promotes autophagy as a protective mechanism against hepatocyte damage from alcohol.[28,29] Chronic alcohol consumption is frequently associated with oxidative stress, inhibition of hepatocellular autophagy and apoptosis through inhibition of AMPK and activation of mTOR.[30]

Dysregulated bile acids are associated with liver diseases as increased cell proliferation and autophagy are mediated by AMPK/mTOR pathway. Due to a protective mechanism enhanced cancer cell viability, and consequently, metastases could be formed.[31]
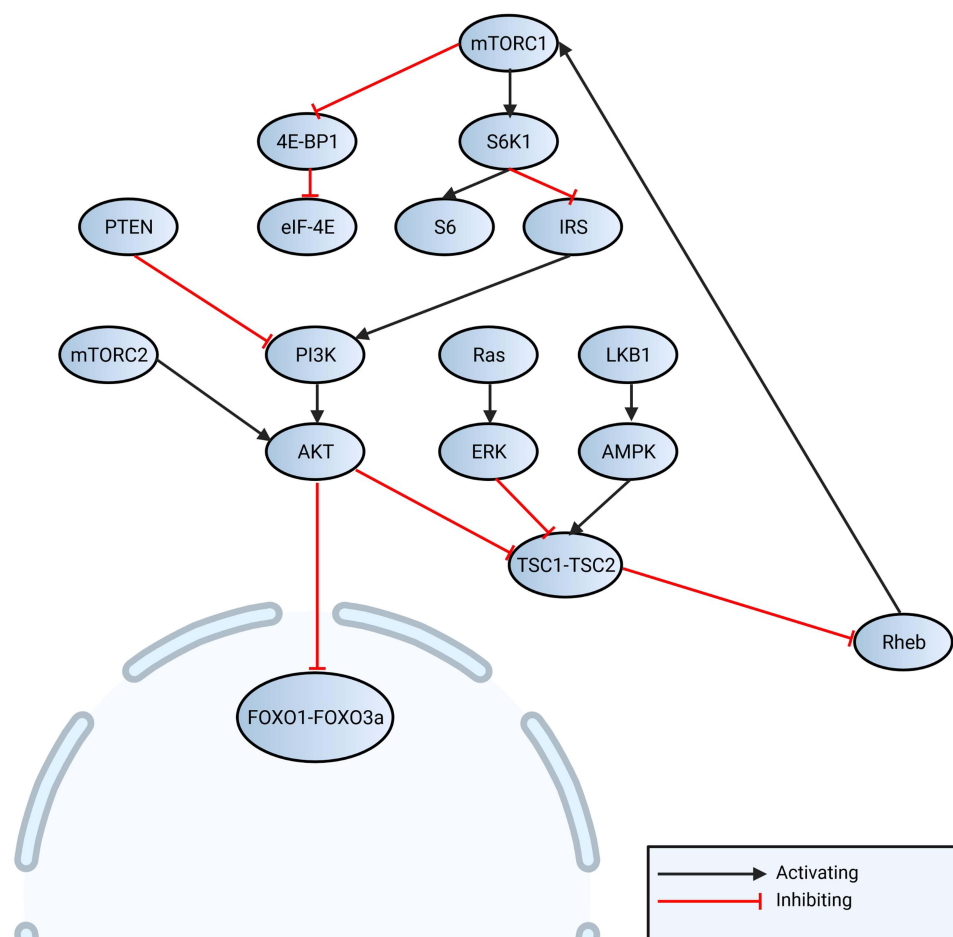
Figure 2 Non-virus associated regulation of mTOR is regulated by PI3K-AKT, which regulates FOXO (1 and 3a) as also protein translation by S6 and eIF4E. Liver kinase B1 (LKB1) - adenosine monophosphate-activated protein kinase (AMPK) pathway can activate TSC1-TSC2 complex, which regulates Ras (small GTPase) homolog enriched in brain (Rheb) and activates mTORC1. Activation of mTORC1 can be regulated by mitogen-activated protein kinases/extracellular signal-regulated kinases (MAPK/ERK) pathway.

One of the risk factors for HCC is a chronic infection with HBV or HCV. It is typical for viruses that require their own translation of the host's proteins, for which they use several different mechanisms, PI3K/AKT/mTOR, for example. As mentioned, this signal pathway pertains to the cap-dependent translation, where 4E-BP is needed for the regulation of the creation of the eIF4F complex. The interaction between eIF4E and 4E-BP is regulated, on the one hand, by phosphorylation and, on the other hand, by viruses, both in negative and positive ways.[32] The activation of this pathway, caused by viruses, inhibits apoptosis and promotes the survival of infected cells for further viral replication.[33]

HCV uses the PI3K/AKT signal pathway to enter a cell. It has been evidenced that the HCV NS5A protein binds itself to PI3K and activates the PI3K/AKT signaling pathway (Figure 3).[34,35]

Even though the use of the PI3K/AKT/mTOR is useful to the viruses, it at the same time limits the procreation of HBV,[36] because the lessened RNA-transcription and lessened the HBV X (HBx) protein plays an essential role in pathogenesis since it encourages cell cycle progression, deactivates negative growth factors and has an effect on the tumor-suppressing genes.[37] Upon HBx-transfection, autophagy is heightened by activating the PI3K/AKT/mTOR signal pathway.[38] In cases of HCV-coinfection, the HBV-replication is suppressed.[39]

Translation Control Upon Viral Infection and as Response to Non-Virally Triggered Stress
eIFs

eIFs are part of the translation process and together with other PI3K/AKT/mTOR pathway members are dysregulated
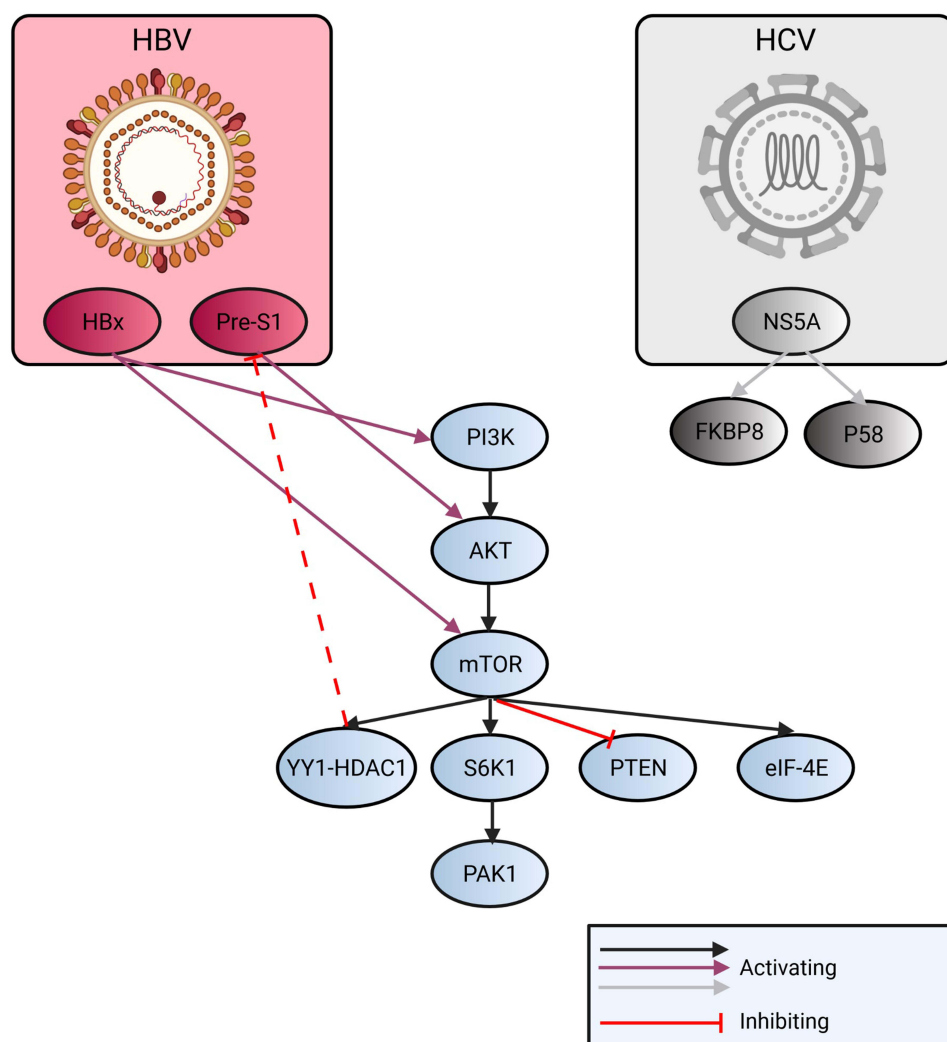
Figure 3 Overview of the regulation of mTOR related to HBV and HCV infection. HCV infection activates NS5A (nonstructural protein 5A). Its P85 form modulates with endoplasmic reticulum membrane or dysregulates association of FKBP5 (FK506 binding protein 5) with mTOR and PTEN is downregulated. HBV upregulates VEGFR-2 (vascular endothelial growth factor) by pre-S1 promoter and the AKT/mTOR pathway is activated. As a negative feedback, YY1-HDAC1 (mammalian zinc-finger transcription factor - Histone deacetylase 1) can inhibit pre-S1 transcription. HBx can activate PI3K and downstream pathway.

in malignancies,[40] but the studies involving eIFs and eEFs in HCC are scarce.

As already published by our group, eIF4E and eIF3I are involved in non-virus related HCC formation. Additionally, eIF4E and eIF3I were downregulated for HBV- and HCV-related HCC, as well as for chronic hepatitis B and C. eIF5 was downregulated in non-virus related HCC, HCV-related HCC and chronic hepatitis C, but was overexpressed in HBV-related HCC. The eIF subunits p-2a, 2a, 3B, 3C, 3D, 3J, p-4B, 4G and 6 were upregulated in HCV-associated HCC. With the help of eIF3 subunits B, C, I, eIF4E, eIF4G and eIF5, a reduction in protein expression in HCV was observed. In HBV-associated HCC and chronic hepatitis B, overexpression of p-eIF2a, p-eIF4B and eIF4G was seen. Downregulation of eIF2a, eIF3D,

eIF3H, eIF3I, eIF3J, eIF4E and eIF6 in HBV-associated HCC and chronic hepatitis B was observed.[41]

Another component of the translation, eIF3, is a protein complex that can act as a tumor suppressor or tumor promoting factor.[42] In HCC, overexpression of eIF3h and eIF3i was observed. Interaction of overexpressed eIF3i with AKT1 prevents PP2A-mediated dephosphorylation and consequently activation of AKT1 signaling. If eIF3i specific inhibitors are applied, tumorigenesis is suppressed.[43]

Overexpression of eIF4E is associated with a higher risk of HCC recurrence, but the factor is overexpressed in other liver cancers as well.[40] Another factor, namely eIF4AIII, was found to be overexpressed in HCC and promoted tumor cell proliferation and migration.[44] eIF4AII was identified as a host factor supporting HCV

replication in different replication systems, in contrast to eIF4AI where an opposite function was observed.[45] A study on HepG2 cells showed that eIF4E phosphorylation with Mnk inhibitor blockage could be a new therapeutic option against chemoresistant HCC.[46] It has also been suggested that patients with HCC who have elevated eIF4E factor in tumor samples have an increased likelihood of tumor recurrence.[40] In a case where sorafenib, a kinase inhibitor, was used as a line of therapy, drug resistance can occur. It was shown that the eIF4E-eIF4G complex reduces the effectiveness of therapy.[47]

The eIF5 factor was shown to be increased in HCC.[48,49] In patients with elevated expression level of eIF5A1, multiple tumor nodules were associated, and in eIF5A2, increased venous infiltration was associated. Inhibition of both isoforms was manifested by reduced HCC proliferation and invasiveness.[48] In patients receiving chemotherapy, HCC cells go through epithelial–mesenchymal transition (EMT) and, as a result, chemotherapy is less effective, but a link to eIF5A2 has been found in chemoresistant samples. If eIF5A2 is inhibited, EMT occurs to a lesser extent, and chemotherapy is more effective.[50] As suggested by Tang et al, eIF5A2 in the case of chemoresistance suppresses HCC autophagic cell death.[51] It was also shown that eIF5A2 could promote cell proliferation and trigger metabolic pathways for glucose metabolism and fatty acid biosynthesis in HCC. The factor could thus not only be a new target for therapy but could also serve as a new prognostic and predictive marker.[52] eIF5A2 is considered to be a factor that promotes metastasis. Inhibition of the eIF5A2 results in a minor invasion and metastasis, for which the reactive oxygen species pathway is thought to be responsible.[53,54] For another factor from eIF5 family, namely eIF5B, it was shown that its expression was increased in HCC tissue samples. The factor is associated with a poorer outcome. The proliferation and invasion of cancer cells is accelerated by ASAP1 expression.[55] In general, eIF5 is believed to serve as a biomarker for non-virus-related and virus-related HCC.[41]

To our knowledge, no study can be found to directly indicate the expression profile of eIF6 in HCC. However, it was shown that through competitively binding with miR-588 eIF6 expression is promoted and PI3K/AKT/mTOR signaling pathway activated by p38-inhibited cutaneous squamous cell carcinoma-associated lincRNA (PICSAR) in HCC.[56]

eEFs

Recently, more attention has been paid to eEF1 and eEF2 and their subunits and complexes, as they are involved in the elongation step.

eEF1A1 regulates cell cycle and promotes malignant cells proliferation. In the case of HCC, downregulation of

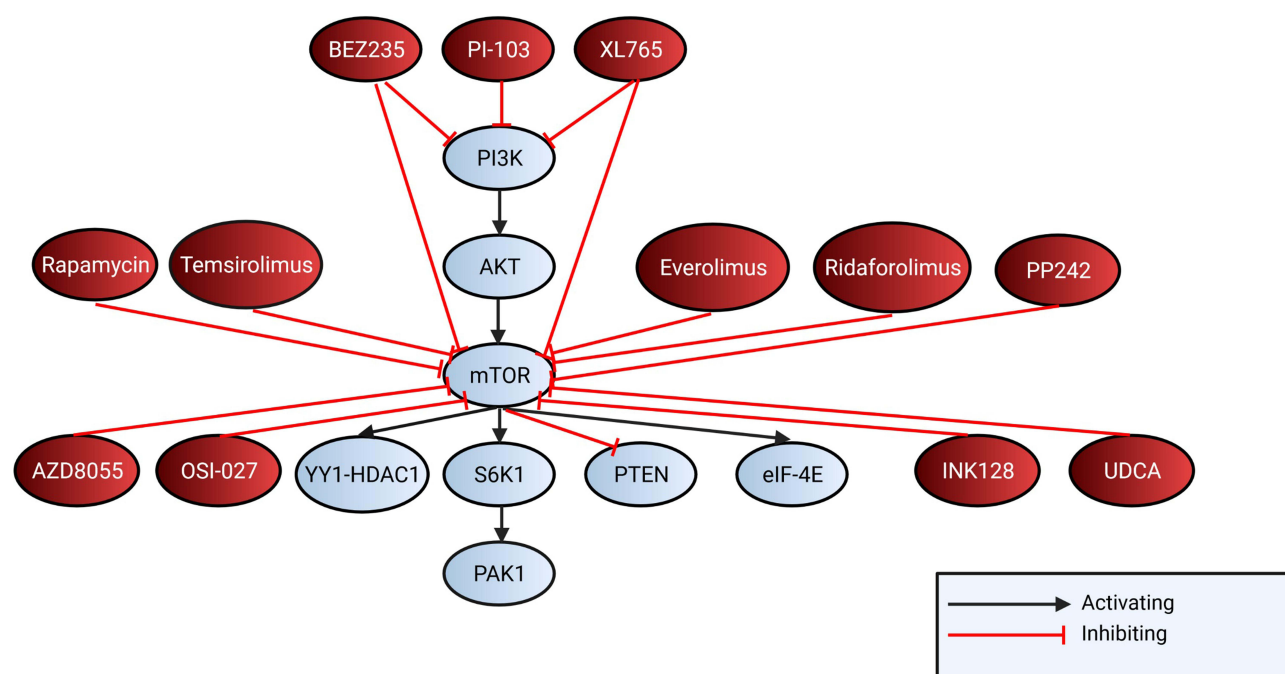

Figure 4 Shows mTOR and PI3 inhibitors targeting the mTOR pathway. BEZ235 and XL765 both target mTOR and PI3K and PI-103 targets PI3K. Rapamycin, temsirolimus, everolimus, ridaforolimus, PP242, AZD8055, OSI-027, INK128 and UDCA inhibit mTOR.

eEF1A1 inhibited tumor cell proliferation.[57] Based on mRNA study, it was shown that eEF1A was increased in HCC compared to non-tumor liver. In cell lines, eEF1A1 and eEF1A2 are correlated with cell growth and differentiation stage.[58]

It was shown that eEF2 promotes angiogenesis in tumor progression of HCC[59] and is a potential drug target.[60] Additionally, it might serve as a prognostic marker.[60]

Ongoing Clinical Trials

Regarding the extent and nature of HCC atezolizumab, bevacizumab (first line) or sorafenib, lenvatinib (first line), regorafenib (second line), pembrolizumab or nivolumab, hormonal compounds (tamoxifen, antiandrogens, and seocalcitiol) and systemic chemotherapy are standard therapy to treat HCC[61] (Figure 4).

Current ongoing clinical trials (*clinicaltrials.gov) investigating the role of different mTOR inhibitors are summarized in Tables 1 and 2.

Table 1 Drugs and Its Targets

Target	Group	Drug
VEGFR, PDFGRA, c-KIT, c-RAF and B-RAF	Multikinase inhibitor	Sorafenib[65] Lenvatinib[61]
mTOR kinase domain	mTOR inhibitor	Everolimus[66]
mTOR pathway	Bile acid	Ursodeoxycholic Acid (UDCA)[67]

In a prospective randomized trial, the effect of sirolimus (rapamycin, an mTOR inhibitor) on the HCC recurrence after liver transplantation was studied (Clinicaltrials.gov: NCT00355862). Results showed that treatment with mTOR-inhibitor for ≥3 months improved the outcome. A subgroup of patients with elevated alpha-fetoprotein where better outcome was observed, indicating the usefulness of mTOR-inhibitors in this group of patients.[62]

Another prospective-randomized open-label international trial (ClinicalTrials.gov NCT00355862) investigated the effect of sirolimus in liver transplanted patients. It was

Table 2 Clinical Trials for HCC and mTOR

ClinicalTrials.gov Identifier	Phase and Status	Sample Size	Drug	Country
NCT00355862	Phase 3 Completed	525	CNI, MMF, Steroids, Aza etc. (mTOR inhibitor free) Sirolimus	Germany
NCT01374750	Phase 2 Completed	45	Sirolimus m-TOR inhibitor free	South Korea
NCT00390195	Phase 1 and 2 Completed	134	Everolimus	China
NCT04803318	Phase 2 Recruiting	100	Combination of three inhibitors Trametinib, Everolimus and Lenvatinib	China
NCT01335074	Phase 1 and 2 Withdrawn	0	Temsirolimus and Sorafenib	Germany
NCT01281943	Phase 2 Withdrawn	0	Temsirolimus doxorubicin	USA
NCT01265810	Phase 3 Completed	64	Supersaturated calcium-phosphate	Netherlands
NCT01177397	Phase 1 and 2 Completed	173	CC-223	USA, France, Spain, UK
NCT03190174	Phase 1 and 2 Recruiting	40	Nab-Rapamycin Nivolumab	USA
NCT01522820	Phase 1 Completed	18	DEC-205/NY-ESO-1 Fusion Protein CDX-1401 Sirolimus	USA
NCT00999882	Phase 1 Completed	26	AZD8055	China, South Korea

(*Continued*)

Table 2 (Continued).

ClinicalTrials.gov Identifier	Phase and Status	Sample Size	Drug	Country
NCT02465060	Phase 2 Recruiting	6452	Adavosertib Afatinib Dimaleate Binimetinib Capivasertib Copanlisib Hydrochloride Crizotinib Dabrafenib Dabrafenib Mesylate Dasatinib Defactinib Defactinib Hydrochloride Erdafitinib FGFR Inhibitor AZD4547 Ipatasertib Larotrectinib Larotrectinib Sulfate Biological: Nivolumab Osimertinib Palbociclib Pertuzumab PI3K-beta Inhibitor GSK2636771 Sapanisertib Sunitinib Malate Taselisib Trametinib Trastuzumab Trastuzumab Emtansine Ulixertinib Vismodegib	USA

shown that patients could benefit from sirolimus as recurrence-free survival and overall survival were better in the first 3 to 5 years.[63]

An open-label, parallel-group, noncomparative, randomised study investigated the maximum tolerated dose of the mTOR-inhibitor everolimus for patients with advanced HCC. It was recommended that 7.5 mg of everolimus should be taken daily with acceptable tolerability and evidence of efficiency (ClinicalTrials.gov NCT00390195).[64]

Future Consideration

Although the risk factors for hepatocellular carcinogenesis are known, treatment is successful to a low extent as HCC-based mortality is still high. As described, different signaling pathways play an important role in HCC development, also the PI3K/AKT/mTOR pathway with and without viral interplay.

Recently, new techniques have opened up additional possibilities for studying the carcinogenesis of HCC. One of them is liquid biopsy, which is one of the least studied in HCC.[68,69] Another promising area is molecular profiling of the tumor, which would allow us an individual approach to treatment. However, there is a problem with inter- and intraheterogeneity of the tumor, as well as the heterogeneity of the tumor in its development. Studies of molecular mechanisms as well as clinical studies suggest that much

remains unclear in the field of hepatocellular carcinogenesis as well as targeted therapy and offers us opportunities to research and develop new target drugs in the future.[70,71]

Disclosure

The authors declare no conflicts of interest.

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