MINIREVIEWS

Mechanisms of hepatocellular carcinoma and challenges and opportunities for molecular targeted therapy

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Abstract

The incidence and mortality of hepatocellular carcinoma (HCC) have fallen dramatically in China and elsewhere over the past several decades. Nonetheless, HCC remains a major public health issue as one of the most common malignant tumors worldwide and one of the leading causes of death caused by cancer in China. Hepatocarcinogenesis is a very complex biological process associated with many environmental risk factors and factors in heredity, including abnormal activation of cellular and molecular signaling pathways such as Wnt/β-catenin, hedgehog, MAPK, AKT, and ERK signaling pathways, and the balance between the activation and inactivation of the proto-oncogenes and anti-oncogenes, and the differentiation of liver cancer stem cells. Molecule-targeted therapy, a new approach for the treatment of liver cancer, blocks the growth of cancer cells by interfering with the molecules required for carcinogenesis and tumor growth, making it both specific and selective. However, there is no one drug completely designed for liver cancer, and further development in the research of liver cancer targeted drugs is now almost stagnant. The purpose of this review is to discuss recent advances in our understanding of the molecular mechanisms underlying the development of HCC and in the development of novel strategies for cancer therapeutics.

Key words: Hepatocellular carcinoma; Oncogene; Signal pathway; Cancer stem cell; Molecular targeted therapy

Core tip: The molecular mechanism of hepatocarcinogenesis is complex and is associated with the regulation function of a variety of signal transduction pathways and key molecules. Presently, there are many drugs that target the molecules that are involved in tumor development (molecule-targeted drugs), but the specificity of such drugs is lacking. This paper summarizes the targeted molecular drugs which may be useful for the clinical treatment of liver cancer, and lays the theoretical foundation for the further study of more specific and effective drugs that target the molecules involved in liver cancer.

INTRODUCTION

Hepatocellular carcinoma (HCC) is one of the most common malignant tumors worldwide and severely harms public health. In China, mortality caused by liver cancer accounts for about 50% of the total mortality due to liver cancer worldwide, making China one of the countries most affected by this disease. Surgical treatment is still the most effective way to treat liver cancer. However, a low curative resection ratio and high recurrent metastasis ratio make this treatment less than ideal[1,2]. Molecule-targeted therapy is a new approach in liver cancer treatment and is based upon the study of HCC carcinogenic mechanisms and the molecular biology of liver cancer. The key point in the study of molecule-targeting drugs for the treatment of liver cancer is to clarify the molecular mechanism of hepatocarcinogenesis and identify the important target molecules.

This paper examines the molecular mechanisms that control hepatocarcinogenesis and discusses the challenges and potential new approaches to studying molecule-targeting drugs for the treatment of HCC.

THE MOLECULAR MECHANISM OF HEPATOCARCINOGENESIS

In recent years, research on the molecular mechanisms of tumor development has been advancing very rapidly, and many new theories have been proposed. However, progress in the research of hepatocarcinogenesis mechanisms is relatively slow, and practical studies that fully examine the interplay between these mechanisms are few. Therefore, we still do not fully understand the mechanisms of hepatocarcinogenesis which is closely related to the specialized functions of the liver.

The competition between proto-oncogene and anti-oncogene

An important contributing factor to the development of a tumor is the balance between the activation and inactivation of the proto-oncogenes and anti-oncogenes. Proto-oncogenes activate cells to enter the proliferation cycle, prevent apoptosis and inhibit differentiation. In healthy cells, proto-oncogenes are expressed at very low levels are not expressed at all. However, environmental influences such as ionizing radiation, physical damage, and specific chemicals can cause genetic mutations to occur in these genes, activating the proto-oncogenes into oncogenes. In liver cancer, N-ras and hepatitis B virus (HBV) X protein are the most common proto-oncogenes[3].

A strong correlation between chronic HBV infection and HCC has been identified, among HBV proteins, HBx has been termed "viral oncoprotein" because of its multifunctional activities on cellular signal transduction pathways, transcriptional regulations, cell cycle progress, DNA repair, apoptosis, and genetic stability by interacting with different host factors[4]. HBx is often expressed from integrated fragments of the HBV genome in HCC tissues, and mice expressing HBx in their liver either develop HCC spontaneously or display increased susceptibility to hepatocarcinogens. Moreover, HBx also interacts with various signaling pathways that are linked to cell proliferation and survival, such as RAS/RAF/MAPK, MEKK1/JNK and PI3K/AKT/mTOR. Additionally, HBx can modulate apoptosis and immune response by direct or indirect interaction with host factors.

Anti-oncogenes (antioncogenes) are recessive and act as susceptibility genes for cancer. They are expressed in normal cells and regulate the proliferation and differentiation of cells. These genes can inhibit cells from entering the proliferation cycle, induce terminal differentiation and cell apoptosis, are essential for the maintenance of organism genome integrity and inhibit tumor growth. The *p53*, *Rb*, *p21* and *PTEN* genes are the most common anti-oncogenes[5]. The formation of malignant tumors is a multi-step process involving many kinds of oncogene activation and anti-oncogene inactivation. Moreover, apart from the competition between proto-oncogenes and anti-oncogenes, some anti-oncogenes may participate in gene regulation pathways that have normal anticancer functions but have not been completely proven to be anti-oncogenes. They have been proven to play an important role in the initiation and progression of the tumor. For example, B cell translocation gene-2 (*BTG2*) is a member of the anti-proliferative gene family located on human chromosome locus 1q32 and encodes a protein of 158 amino acids with a molecular weight of about 17 kDa. BTG2 contains the response element of the wild-type *p53* gene at position-74 to -122. Many experiments have shown that BTG2 activation requires the assistance of p53 activation[6]. BTG2 is a member of the family of early response genes, connecting the upstream p53 and downstream cell cycle proteins, inhibiting cell proliferation, and is expressed at low levels in a variety of tumors. Our previous studies have shown that the expression of BTG2 decreased significantly in HCC, but expression correlated positively relative to the expression of p53 and negatively relative to the expression of cyclin E. However, in rat models of liver cancer, BTG2 expression was significantly increased in primary cancers of the liver, but dramatically decreased in advanced tumors[7]. BTG2 functions as a tumor suppressor, but due to a lack of evidence of mutations in tumors, BTG2 cannot be confirmed to be an anti-oncogene. However, numerous studies have suggested BTG2 to be a potential anti-oncogene. Of course, the function of many proto-oncogenes and anti-oncogenes is unknown,

and the balanced expression of these genes is currently considered to be a central regulator of homeostasis. Once this balance is upset, tumor formation may occur.

Abnormal activation of molecules signaling pathway

Research of tumor signal transduction has been a hot topic in the field of tumor basic research and has served as the theoretical basis of a variety of molecule-targeted drugs. It is thought that abnormal activation of many molecules in various signaling pathways contributes to the progression of liver cancer, and the foundation of molecule-targeted drugs is to selectively block these signal transduction pathways and disrupt that regulatory mechanism, including the following several kinds of classic signal transduction pathways.

Ras/Raf/MAPK signaling pathway

Among the investigated signaling pathways involved in HCC, the Ras/Raf/MAPK is the most critical pathway in the development of HCC. Signals from membrane-bound tyrosine kinase receptors, such as endothelial growth factor receptor (EGFR), insulin-like growth factor receptor (IGFR), vascular EGFR, c-Met and platelet derived growth factor receptor (PDGFR), are transduced to the cell nucleus through Ras/Raf/MAPK pathway in order to regulate multiple cellular functions including cell growth and survival, and differentiation. Dysregulation of this pathway leads to inappropriate cellular activities including enhanced cell growth, differentiation, and survival, and ultimately to cancer[8]. Up-regulated activation of the Ras/Raf/MAPK signaling pathway has been well studied in HCC and correlates with advanced stage. Mechanisms for the increased activity of the Ras/Raf/MAPK signaling pathway in HCC include aberrant upstream signals (EGFR signaling, IGF signaling), inactivation of Raf kinase inhibitor protein (a suppressor of the Ras/Raf/MAPK pathway) and induction by hepatitis viral proteins (such as the hepatitis B x protein and the hepatitis C core protein)[9]. Potent drugs blocking Ras/Raf/MAPK signaling are still at exploratory phase, except for sorafenib that has activity inhibiting B-Raf.

PI3K/AKT/mTOR signaling pathway

The PI3K/AKT/mTOR signaling pathway, which plays a significant function in cell growth, survival regulation, metabolism, and antiapoptosis also plays an important role in HCC and is activated in 30%-50% of HCC. In normal tissue, this pathway is negatively regulated by the tumor suppressor phosphatase on chromosome 10 [phosphatase and tensin homolog (PTEN)], which targets the lipid products of PI3K for dephosphorylation. Anomalies in PTEN function may lead to overactivation of the PI3K/AKT/mTOR pathway in HCC. PTEN expression is reduced in nearly half of all HCC tumors, resulting in constitutive activation of the PI3K/AKT/mTOR pathway[10]. A tissue microarray analysis of HCC samples revealed that the loss of PTEN and overexpression of pAkt and p-mTOR were correlated with tumor grade, intrahepatic metastasis, vascular invasion, TNM stage,

Ki-67 labeling index, and matrix metalloproteinase (MMP)-2 and (MMP)-9 upregulation[11].

Wnt/β-catenin signaling pathways

The Wnt/β-catenin signaling pathway, often called the Wnt classic signaling pathway, is composed of the Wnt protein, Wnt protein ligand (frizzled protein), and related regulator proteins such as GSK-3β and β-catenin. A study found that the Wnt/β-catenin signaling pathway is an important signaling pathway in the process of growth and development, and its abnormal activation is closely related to the occurrence of cancer. When the pathway is activated by upstream stimulation, the Wnt protein binds to its ligand and β-catenin accumulates in cells, where it is activated and transferred into nucleus. In the nucleus, β-catenin dimerizes with the downstream specific transcription factor LEF/TCF, which regulates the transcription of key genes such as cyclin D[12,13]. The abnormal activation of Wnt/β-catenin is an important signaling pathway in the carcinogenesis of hepatoma, and aberrant β-catenin could be detected in 90% liver cancer[14]. Calvisi *et al*[15] reported that transgenic c-myc/TGF-P mice developed liver cancer, and this was associated with a gene mutation in β-catenin, suggesting that the activation of the β-catenin gene may increase the growth and metastasis of cancer. Infection with HBV and HCV can induce high levels of β-catenin, and promote the occurrence of liver cancer[16,17].

Hedgehog signaling pathways

Currently, the hedgehog (Hh) signaling pathway is a key regulation pathway in the formation of liver cancer. In mammals, the Hh signaling pathway is mainly composed of the Hedgehog ligand, two transmembrane protein receptors (Ptch and Smo), and the nuclear transcription factor Glib and downstream genes. When the Hh signaling pathway is activated, the Hh ligands bind to the Ptch receptors, and block the inhibitory effect on Ptch on Smo. Smo enters into the cytoplasm to activate downstream transcription factor Gli, inducing the expression of specific genes, thereby regulating cell growth, proliferation and differentiation. The Hh signaling pathway in liver cancer is abnormally activated, but in mature normal liver tissue, the Hh signaling pathway is not initiated[18-20]. Studies by Sicklick *et al*[21] suggested a dysfunction of Hh signaling in human liver, and found a high expression of Hh signaling as demonstrated by elevated expression of Shh, Ptch, Smo and Gli1, all of which regulate c-myc gene expression mediated by Smo. Kim *et al*[22] found that in liver cancer tissues, the inhibition of the *Gli2* gene can decrease the expression of c-myc and Bcl-2 and increase the expression of p27, which regulates the cell cycle, inhibits proliferation and abrogates the growth of liver cancer cells.

Other signaling pathways

There are many other cell signaling pathways involved in liver cancer, including Notch signaling pathway[23-25],

IGF/IGFR signaling pathway[26,27], HGF/c-Met signaling pathway[28,29] and EGFR signaling pathway[30]. They are the important regulatory pathways of liver cancer and are important for initiation and development of metastasis. Many gene regulatory points in these pathways have been used as targets for targeted therapy of cancer in clinical trials, and these molecular targeted drugs designed for these pathways are expected to become the new direction for the treatment of liver cancer.

Liver stem cells and liver cancer stem cells

The tumor stem cell theory postulates that there are cancer stem cells in the human body that give rise to cancerous tissues. There are two main liver cancer stem cell theories[31,32]. One theory states that liver cancer stem cells are derived from mature hepatocytes. The other theory argues that liver cancer cells are derived from intrahepatic undifferentiated stem cells or abnormal differentiated cells of oval cells. Of course, the latter theory is supported by the greatest number of studies. Liver cancer can be initiated by stem cells and their daughter cells. This may occur in depolarized mature hepatocytes and bile duct epithelial cells. Baumann *et al*[33] found that the occurrence and pathological polymorphism of primary liver cancer was due to the blocking of liver stem cell differentiation. It is characterized by poorly differentiated liver cancer cells when they are blocked in their early stage of development, in between a HCC and a cholangio cell. However, when these cells are locked in later stages of differentiation, they are characterized as HCC and cholangio cells. More recently, the Henry Lilian Stratton basic research single theme meeting at the American association for the study of liver diseases reported on the research progress of stem cells in liver diseases and cancer. They focused on the identification of hepatic stem cells and liver cancer stem cells, research progress in our understanding of their functions and clinical transformation of these cells in patients. Liver stem cells begin as liver progenitor cells (LPCs), and then de-differentiate into pluripotent stem cells, and then transdifferentiate to become disease-specific liver stem cells. However, the occurrence of tumor-initiating stem-like cells and the abnormalities of some signaling proteins, such as transforming growth factor β, β-catenin and LPCs markers, become potential signs of chronic liver damage and liver cancer[34]. Among them, the original stem cell-like cells of the tumor play an important role in cell transcription and reverse transcription in the formation of liver cancer, and the detection and treatment for this kind of cell is considered to be the new focus liver cancer research and treatment[35].

TARGETED THERAPIES IN LIVER CANCER

The theory has been put forward that a potentially revolutionary change in tumor medical treatment will occur with the development of molecule-targeted therapy. With the continuous progress of tumor basic research, more and more new tumor targeted drugs are used in the clinic, effectively improving the survival time of patients with tumors. In our opinion, the recent decade has seen the fastest growth in tumor targeted drugs, creating a new approach for tumor treatment.

Molecule-targeting drugs for liver cancer

Sorafenib was approved by the Food and Drug Administration (FDA) as a molecule-targeting drug for the treatment of primary liver cancer, mainly for advanced hepatocellular carcinoma[36,37]. It was originally designed for the treatment of kidney cancer and non-small cell lung cancer, and was the first multiple targeted drugs against Raf kinase. Its main mechanism of action is to block signal transduction mediated by the Raf/MEK/ERK pathway, and inhibit various tyrosine kinases, including vascular endothelial growth factor-2 (VEGF-2), VEGF-3, PDGFR-β and c-Kit protein. All of these tyrosine kinases are associated with tumor growth, and inhibition of tumor growth can be demonstrated when these drugs are applied[38,39]. Currently, phase Ⅱ clinical trials have shown that sorafenib and doxorubicin is a safe and effective treatment for the degradation of microspheres for embolization, and it is worth noting that the efficacy and safety of sorafenib in patients with hepatic insufficiency is not yet clear. Studies have found that the iron chelator (deferoxamine) can inhibit the cell cycle and induce apoptosis to protect the liver and inhibit cancer formation. In recent reports, deferoxamine has shown satisfactory efficacy and safety in 10 patients with advanced liver cancer, which has suggested that deferoxamine is appropriate for patients with poor liver function and advanced liver cancer, and might be considered a useful supplement for sorafenib[40]. However, in 2013, Rimassa *et al*[41] reported a phase Ⅱ clinical trial that concluded that for those patients with advanced liver cancer after radiation treatment failure, when the sorafenib dose increased from 400 mg, 2 times/d, to 600 mg, 2 times/d, survival time and their quality of life failed to improve. This observation suggests that an increased dose of sorafenib does not necessarily translate into a clinical benefit for patients with advanced liver cancer. Brivanib is another promising targeted drug for the treatment of liver cancer. It is a small molecule tyrosine kinase inhibitor of VEGF and the fibroblast growth factor receptor family, whose main mechanism of action is to inhibit vascular endothelial growth factor and fibroblast growth factor receptors[42,43]. In 2011, phase Ⅱ clinical trials with brivanib as a first-line treatment for advanced liver cancer suggested that the drug is safe. In the study, a brivanib dose of 800 mg, 1/d was used and liver cancer patients on the drug showed a progression-free survival ratio of 18.2%. In this same patient population, the median disease-free survival was 2.7 mo, there was a complete remission in one case, a partial response (PR) in three cases, stable disease (SD) in 22 cases, and the median survival period was 10 mo[44]. Recently, Finn

Table 1 Molecular targets and potential therapeutic agents for hepatocellular carcinoma

Molecular targets	Therapeutic agents	Phase study	Mechanism of action
VEGF/VEGFR	Bebacizumab	II	Anti-angiogenic
	Vatalanib (PTK787)	I - II	
	Cediranib (AZD2171)		
	Brivanib		
	Sunitinib	II - III	
	Linifanib (ABT869)		
	Ramucirumab		
EGFR/ErbB1/Her1	Cetuximab		EGFR inhibitor
EGFR/ErbB1/Her1	Erlotinib	III	
EGFR/ErbB1/Her1	Gefitinib	I - II	
EGFR/ErbB1/Her1 and ErbB2/Her2/Neu	Lapatinib		
IGF/IGFR	OSI-906		
	IMC-A12		
	AVE1642		
	BIIB922		
Ras/Raf/MEK/ERK	Sorafenib	III	Multi-kinase inhibitor
PI3K/Akt/mTOR	AZD8055		mTOR inhibitor
	Everolimus	III	
	Sirolimus	I - II	
	Temsirolimus		
Wnt-β-catenin	PFK118-310		
	PFK115-584		
	CGP049090		
MET	Tivanitib	II	HGF/c-MET inhibitor
HSP-90	STA-9090	I - II	HSP-90 inhibitor

VEGFR: Vascular endothelial growth factor receptor; EGFR: Endothelial growth factor receptor; IGF: Insulin-like growth factor; mTOR: Mammalian target of rapamycin; MET: MNNG HOS transforming gene; HGF: Hepatocyte growth factor; HSP: Heat shock protein; STA-9090: Ganetespib, Hsp90 inhibitor.

et al[45] reported on clinical trials of brivanib as second-line therapy in advanced liver cancer in patients that were receiving the same dose of brivanib (800 mg, 1/d). In the 46 patients studied, there was PR in two cases (4.3%), SD in 19 cases (41.3%), and progressive diseases in 19 cases (41.3%). The tumor response rate was 4.3%, the disease control rate was 45.7%, and the median survival time as 9.79 mo. Ultimately, they came to the conclusion that brivanib and sorafenib are safe and effective treatments in patients with advanced liver cancer. Table 1 lists the results of the most recent clinical trials, and the various therapies currently used to treat HCC.

Bottleneck of molecular targeted therapy of liver cancer
Molecule-targeted therapy is the most active area of in the tumor treatment research, and we have made great progress in improving the survival time of cancer patients using these types of therapies. However, there is not one drug completely designed for liver cancer, and further development in the research of liver targeted drugs is now almost stagnant. The main reasons for this are as follows: (1) The mechanism of liver cancer is complex, so it is difficult for the development of specific targeting drugs. Liver cancer is the result of the combined action of multiple factors, all of which we know very little about, and the liver cell has its own characteristics and proliferates rapidly. Once the cancer occurs, the hyperplasia or resistance mechanism of the liver cancer cell varies, so it is not easy to find specific targets; (2) The most targeted therapeutic drugs are less effective, and the curative effect is not ideal; (3) The selectivity of targeted drugs for the treatment of liver cancer targets is not high, there are adverse reactions, there is a high resistance such as the "off-target effect", and the research cost is high, so it is difficult to put into widespread use; and (4) Different responses to targeted drugs may occur in liver cancer patients, based upon extrinsic or intrinsic factors such as ethnic and gender differences. We are still unable to accurately detect and monitor liver cancer cell change sat the molecular level, so the potential for disease monitoring is not sufficient.

OPPORTUNITIES AND CHALLENGES

The treatment of liver cancer fundamentally depends upon the systemic understanding of the pathogenesis of liver cancer. Surgery, interventional embolization, chemotherapy and radiation are still the main treatments for liver cancer. A better recognition and re-development of existing treatments may likely bring about new hope for the treatment of liver cancer. With the development of new biological technologies and an increase in our knowledge of the molecular mechanisms of liver cancer, the treatment of liver cancer is facing new opportunities and challenges. Molecule-targeted therapy will gradually become a new favorite for the treatment of liver cancer, and also represent the future developmental direction of the treatment of liver cancer. Furthermore, basic

research breakthroughs will create more effective methods of liver cancer targeted therapy, and in conjunction with normalized and individualized clinical treatments, they will eventually result in new successes in the treatment of liver cancer.

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