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Recent Developments in the Molecular Pathogenesis and Targeted Treatment of Hepatocellular Carcinoma

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Abstract

For the treatment of hepatocellular carcinoma (HCC), molecularly targeted drugs provide an important tool, and although such therapies can prolong patient survival, drug resistance and adverse effects limit the feasibility of their long-term application. The focus of current research has shifted to the discovery of new therapeutic targets, refinement of efficacy prediction criteria, and optimization of patient treatment plans to enhance survival quality and benefit. However, although studies of relevant biomarkers offer potential personalized strategies for targeted therapies, most of these studies are in their infancy and urgently need to be validated by clinical studies with large sample sizes and prospective designs. With the development of bioinformatics, it is expected that new therapeutic targets and criteria for evaluating therapeutic efficacy will be identified, leading to more scientific and effective therapeutic regimens customized for HCC patients. The aim of this review is to explore the molecular mechanisms, drugs, and biomarkers of targeted therapies for HCC in order to provide direction and evidence base for future studies.

Keywords: Hepatocellular Carcinoma; Molecular; Pathogenesis; Targeted Therapy; Research Progress

Introduction

Primary liver cancer, especially Hepatocellular Carcinoma (HCC), is globally recognized as one of the major health challenges, and its clinical importance and epidemiological data reveal the high incidence and lethality of the disease(1). According to the global statistics in 2020, the number of new primary liver cancer patients is 905,677 and the number of deaths is as high as 830,180, of which the number of new patients in China is about 410,038, accounting for nearly 45% of the world(2). This data not only highlights the importance of HCC in the global cancer burden, ranking sixth in the number of new patients and fourth in the mortality rate, but also highlights China's significant share of the global HCC disease burden. The high incidence and lethality of HCC is attributed to a variety of risk factors, including hepatitis B and C virus infection(3), consumption of aflatoxin-contaminated food(4), excessive alcohol consumption(5), overweight(6), type 2 diabetes(7), and Smoking(8). These factors contribute to the complexity and variability of HCC pathogenesis, thus making the choice of treatment and its efficacy very challenging. Since most patients with HCC are in the middle to advanced stages at the time of diagnosis, the opportunities for localized therapies such as surgical resection(9), radiofrequency ablation(1), or radiotherapy are limited(11). Moreover, chemotherapy is inefficient and natural survival without effective treatment usually does not exceed 3 to 4 months(12). Therefore, it is especially necessary to study the molecular pathogenesis and targeted therapy of HCC. Targeted therapy has become a hotspot in HCC therapeutic research due to its advantages of strong targeting, high specificity, low drug resistance and few side effects. This therapeutic strategy targets the key oncogenic sites of HCC for specific binding, thus demonstrating its anti-cancer effect. With in-depth research on the pathogenesis of HCC, the identification of new high-risk factors, and continued advances in molecular markers, diagnostic techniques, therapeutic approaches, and prognostic assessment techniques, a variety of molecularly targeted drugs for HCC have been put into use one after another, bringing new hope for the treatment of patients with advanced disease(13). Thus, the high morbidity and lethality of HCC, as well as the challenging nature of its treatment, emphasize the importance of studying the molecular pathogenesis of HCC and developing targeted therapeutic strategies. The future research needs to continue to deepen the understanding of the biology of HCC, explore new therapeutic targets, and validate the safety and efficacy of new targeted agents through clinical trials in order to improve the prognosis and quality of life of HCC patients.

Pathogenesis and molecular subtypes of hepatocellular carcinoma

Pathogenesis

The pathogenesis of HCC is a multistep, highly complex process characterized by genetic imbalances(14), aberrant activation of signaling pathways(15), dysregulation of cellular differentiation(16), and abnormal angiogenesis(55). The development of HCC arises in most cases from the process of dedifferentiation of mature hepatocytes, while a small proportion evolves directly from the progenitor cells of the liver(18). During the development of hepatocellular carcinoma (HCC), 50 to 70 mutations typically appear in the hepatocyte genome, and although the majority of these mutations are non-tumorigenic passive or incidental mutations, approximately 2 to 6 mutations are critical driver mutations that cause malignant transformation of the cell by affecting core signaling pathways within the cell(19). The major genes for these driver mutations include the telomerase reverse transcriptase promoter,

encoding β-linker protein, and somatotropic axis repressor 1.

These driver mutant genes play a role in hepatocarcinogenesis by activating multiple signaling pathways. Which include signaling pathways that promote growth factor activation, telomere maintenance(20), apoptotic pathways in response to oxidative stress(21), and the Wnt/β-linker protein signaling pathway(22). In addition, aberrant epigenetic regulation, such as altered expression of DNA methylation(23), histone and chromatin remodelling(24), and long-chain non-coding RNAs(25), also play a key role in the formation and progression of HCC(26). Abnormalities of such epigenetic modifications are present in a significant proportion of HCC patients and provide new targets for the diagnosis and treatment of HCC.

Understanding the heterogeneity within and outside the tumour, which results from a combination of genetic mutations and epigenetic alterations, is essential for the identification of representative biomarkers that can help to screen patient populations that are more sensitive to specific targeted therapeutic approaches, thereby enhancing the efficiency and effectiveness of treatment. Addition, dysregulation of cellular differentiation and abnormal angiogenesis are also key factors in the development of HCC, which not only promotes tumour growth and spread, but also provides nutrients and oxygen to the tumour, exacerbating disease progression(27). Therefore, an in-depth understanding of the complex pathogenesis of HCC, especially in the regulation of gene imbalance, aberrant activation of signalling pathways, cellular differentiation and angiogenesis, is important for the development of new therapeutic strategies and the improvement of patient survival.

Molecular subtypes and heterogeneity

Molecular biological studies of HCC have revealed significant heterogeneity in the genetic and expression profiles of this malignancy, which is reflected in the different molecular subtypes of HCC. Based on gene expression levels, HCC can be broadly classified into two major groups: proliferative and non-proliferative, each of which accounts for approximately half of the total number of HCCs(28). Proliferative HCC is usually associated with hepatitis B virus (HBV) infection(29) and is characterised by an abnormal proliferation of hepatocytes, which occurs mainly through the activation of several key signalling pathways including the protein kinase B/mammalian target of rapamycin (AKT/mTOR) pathway(30), mesenchymal epithelial transforming factor (MET)(31), transforming growth factor-β (TGF-β)(32), insulin-like growth factor(33) and the Ras/promoter activated protein kinase pathway(34). Such HCC cells exhibit a lower degree of differentiation, higher chromosomal instability and aggressiveness. Non-proliferative HCC, on the other hand, is more common in cases of hepatitis C virus (HCV) infection or alcohol-related cases, and is characterised by the frequent occurrence of mutations in CTNNB1, the gene encoding β-conjugated proteins.

Targeted therapy

Sorafenib

Sorafenib is a potent orally active (Rapdily Accelerated Fibrosarcoma,Raf) inhibitor and a multikinase inhibitor, which induces cellular autophagy and apoptosis and possesses antitumour activity(35), and is used as targeted agent for the treatment of HCC. It exerts dual effects of anti-tumour cell proliferation and anti-tumour angiogenesis by blocking a variety of tyrosine kinase receptors, such as vascular endothelial growth factor receptor (VEGFR), platelet-derived growth factor receptor (PDGFR), and c-Kit, c-Kit, thereby inhibiting

the Raf/MEK/ERK signalling pathway(36). Both the multi-centre randomised controlled SHARP study and the Asia-Pacific patient-based ORIENTAL study have confirmed that sorafenib can effectively delay tumour progression and prolong patient survival in advanced HCC patients. In addition, sorafenib and its combination with a PD-1 inhibitor in the treatment of non-surgically resectable HCC has shown significant efficacy and manageable adverse effects in clinical practice(37), suggesting that this combination strategy is a safe and effective therapeutic option(39).

The role of regorafenib in the treatment of hepatocellular carcinoma (HCC) is not limited to the blockade of a single signaling pathway. Instead, it acts through multiple mechanisms that collectively affect the survival, proliferation, and angiogenesis of tumor cells. Firstly, sorafenib acts by directly inhibiting the activation of VEGFR and PDGFR, thereby blocking the tumor cells' response to these growth factors(39). This inhibitory effect directly intervenes in the angiogenesis process within the tumor microenvironment, weakening the tumor's ability to acquire nutrients and oxygen through newly formed blood vessels, thereby inhibiting further growth and spread of the tumor. Secondly, sorafenib's inhibition of c-Kit enhances its direct antiproliferative effects on tumor cells(40). c-Kit is a cell surface receptor tyrosine kinase closely associated with cell growth and survival. By inhibiting the signal transduction of c-Kit, sorafenib further blocks the internal survival signals of tumor cells, accelerating their apoptosis. Most crucially, sorafenib acts by blocking the Raf/MEK/ERK signaling pathway, directly affecting the proliferation and survival mechanisms of tumor cells(41). The Raf/MEK/ERK pathway is an important intracellular signal transduction route involved in cell proliferation, differentiation, and survival. The action of sorafenib leads to reduced activity of this pathway, thereby inhibiting the growth and division of tumor cells.

Lenvatinib

Lenvatinib is a multitarget tyrosine kinase inhibitor, and its application in the treatment of primary hepatocellular carcinoma (HCC) has revealed its profound potential for intervention in molecular mechanisms. By targeting a range of key receptor tyrosine kinases, including VEGFR1 to VEGFR3, fibroblast growth factor receptors (FGFR) 1 to 4, PDGFRα, RET, and KIT, lenvatinib comprehensively blocks multiple signaling pathways related to tumor proliferation and angiogenesis(42). The inhibition of VEGFR primarily affects the angiogenesis of the tumor, weakening the tumor's ability to obtain nutrients and oxygen through neovascularization, thereby inhibiting tumor growth and metastasis(43). The inhibition of FGFR targets another crucial pathway for tumor cell proliferation and survival, further enhancing the antitumor effect of lenvatinib. The inhibition of PDGFRα affects the fibrotic process within the tumor microenvironment, which helps alleviate liver fibrosis and cirrhosis, particularly important for patients with hepatocellular carcinoma (HCC). Moreover, by blocking RET and KIT, lenvatinib can also impact other growth and metastasis pathways of tumor cells(44). Additionally, by blocking RET and KIT(45), lenvatinib can also affect other pathways of tumor cell growth and metastasis.

In clinical studies, lenvatinib has demonstrated its efficacy and safety in the treatment of advanced hepatocellular carcinoma (HCC). Phase II and III clinical trials have shown that lenvatinib has a comparable overall survival rate to sorafenib, as well as significant advantages in objective response rate and partial progression-free survival (PFS) times. Particularly in the subgroup analysis of Chinese patients, the OS time for the lenvatinib group was significantly extended, indicating

its effectiveness in specific populations. These results reflect the potential of lenvatinib in blocking multiple signaling pathways closely related to the development of HCC(46).

Donafenib

Donafenib is a novel deuterium-modified derivative of sorafenib, with an optimized molecular structure to enhance its pharmacological and pharmacokinetic properties, showing potential in the treatment of HCC(47). Clinical studies have found that compared to sorafenib, donafenib has a longer overall survival period (mOS) in patients with advanced HCC, and also has better safety and tolerability(48). Specifically, the comparison between donafenib and sorafenib shows that the plasma exposure of donafenib is significantly higher than that of sorafenib when it reaches a steady state, which may be due to its stable deuterated methyl group, making donafenib less likely to be metabolized by liver enzymes in the body, thus having a longer half-life, suggesting that donafenib may exhibit superior pharmacokinetics (PK) and metabolic characteristics in the human body. In addition, preclinical studies of donafenib also show that compared to sorafenib, donafenib has much higher exposure in plasma and tumor tissue, suggesting that donafenib may exhibit superior pharmacokinetics (PK) and metabolic characteristics in the human body. In addition, preclinical studies of Donafenib have also shown that, compared to Sorafenib, Donafenib has much higher exposure levels in plasma and tumor tissues, suggesting that Donafenib may exhibit superior pharmacokinetic (PK) and metabolic characteristics in the human body(49). In terms of molecular mechanisms, Donafenib acts as a multi-kinase inhibitor, exerting a dual anti-tumor effect by blocking multiple signaling pathways closely related to the development of HCC, such as VEGFR, PDGFR, and c-Kit(50), and inhibiting the Raf/MEK/ERK signaling pathway, thereby inhibiting tumor cell proliferation and anti-tumor angiogenesis. Through this mechanism, Donafenib can effectively intervene in the molecular mechanisms of HCC, blocking the growth and spread of tumor cells, thereby demonstrating a potential therapeutic advantage in the treatment of advanced HCC.

mTOR inhibitor

Everolimus, an inhibitor of the mammalian target of rapamycin (mTOR), plays a significant role in the treatment of HCC by regulating the mTOR signaling pathway(51), affecting cell growth, proliferation, and survival. The mTOR signaling pathway is activated in various cancers, including HCC, and its dysregulation promotes tumor growth and treatment resistance(52). Especially in HCC patients post-liver transplantation, Everolimus has demonstrated potential in enhancing transplant success rates, protecting renal function, and reducing the risk of HCC recurrence. Immunosuppressive therapy is essential for preventing acute rejection after transplantation. However, the adverse effects of conventional immunosuppressants, such as calcineurin inhibitors (CNIs), including nephrotoxicity and an increased risk of HCC recurrence, have limited their use(53). Everolimus offers an effective alternative, combining immunosuppressive effects with reduced renal toxicity and potentially better outcomes for HCC recurrence. Additionally, resistance to mTOR inhibitors like Everolimus may arise through feedback activation of compensatory pathways, such as the PI3K/AKT signaling pathway. Combining Everolimus with inhibitors targeting these alternative pathways can enhance the therapeutic effect on HCC. Studies have shown that the combined application of Everolimus with the AKT kinase inhibitor MK-2206 is more effective in synergistically inhibiting the proliferation of HCC cells than using either drug alone. This combination therapy effectively reduces AKT kinase activity and tumor cell growth, proposing a potential

strategy to overcome resistance to mTOR inhibition(54).

Bevacizumab

Bevacizumab is an antibody drug targeting Vascular Endothelial Growth Factor A (VEGF-A), which can effectively block tumor angiogenesis, thereby inhibiting tumor growth and metastasis. By targeting VEGF-A, Bevacizumab reduces the formation of new blood vessels around the tumor, limiting the tumor's ability to acquire nutrients and oxygen, thereby inhibiting tumor growth. In the treatment of HCC, the application of Bevacizumab, especially when used in combination with other treatments such as targeted drugs or chemotherapy, has been included in treatment guidelines as a first-line or second-line treatment option in multiple countries. A study explored the effects of Sintilimab combined with Bevacizumab in the treatment of patients with unresectable hepatocellular carcinoma, as well as changes in serum VEGF and MMP-9 levels. The results indicated that this regimen can effectively control disease progression, significantly reduce the levels of tumor-related factors in the serum, improve patient survival rates, and has better safety compared to conventional treatments(55). Furthermore, by intervening in the VEGF pathway, Bevacizumab helps to overcome treatment resistance caused by increased VEGF expression in liver cancer patients, thus providing a new treatment strategy for advanced or surgically unresectable HCC patients. Overall, Bevacizumab offers a mechanistic innovation in the treatment of HCC by targeting angiogenesis. Its combined use can effectively improve patient prognosis and, to some extent, enhance the quality of life for patients.

Biomakers

Alpha-Fetoprotein (AFP)

Alpha-fetoprotein (AFP) indeed has significant value in the diagnosis of hepatocellular carcinoma (HCC), but its sensitivity and specificity for early-stage liver cancer screening are controversial. AFP is a protein synthesized by the fetal yolk sac and liver during pregnancy, and normal adult liver cells do not typically produce AFP, resulting in low serum AFP levels. When liver cells become cancerous, the AFP gene is reactivated, leading to increased serum AFP levels. However, not all liver cancer patients will experience an increase in AFP levels, especially in the early stages of the disease. Some studies suggest that combining AFP with other liver cancer biomarkers, such as AFP-L3%, DCP, or the GALAD model, may enhance the sensitivity and specificity of screening. Therefore, the use of AFP as the sole indicator for liver cancer screening has limitations(56).

Circulating tumor DNA (ctDNA)

In recent years, with the advancement of molecular biology techniques, novel biomarkers such as circulating nucleic acids (e.g., circulating tumor DNA or ctDNA) have been proposed for the early screening and diagnosis of liver cancer. These circulating nucleic acid biomarkers may offer higher sensitivity and specificity, allowing for the detection of early changes in liver cancer even in the absence of noticeable symptoms. For instance, ctDNA, which are fragments of DNA released into the bloodstream by tumor cells, can reflect the genetic information of the tumor. Studies have shown that ctDNA testing holds promise in the early screening of liver cancer, potentially detecting tumors earlier than AFP, especially in cases where the tumor volume is small or AFP levels have not increased, thus enabling earlier prediction of patient relapse risk. (57)

VEGF and VEGFR

Vascular endothelial growth factor (VEGF) is a key factor that regulates angiogenesis and plays a crucial role in the development of hepatocellular carcinoma (HCC). VEGF supports tumor growth and spread by promoting the formation of new blood vessels and providing tumors with essential nutrients and oxygen(58). The expression level of VEGF and its receptor, especially VEGFR-2, is closely related to tumor growth rate, aggressiveness, and patient prognosis(59). Studies have shown that the VEGF/VEGFR-2 axis plays a central role in the angiogenesis of HCC and that VEGFR-2 expression is associated with poor prognosis in HCC patients. Therefore, the VEGF/VEGFR axis has become an important target in HCC therapy.

At present, a variety of anti-angiogenic drugs have been developed to target the inhibition of VEGF or VEGFR-2, thereby blocking the vascular supply of tumors and limiting their growth and metastasis. These drugs include antibody drugs, small molecule tyrosine kinase inhibitors, and other types of VEGFR-2 inhibitors. Clinical studies have shown that these anti-angiogenic agents can improve overall survival and disease-free survival in HCC patients, especially when combined with other treatments.

Multitarget tyrosine kinase inhibitors (TKIs)

Multi-targeted tyrosine kinase inhibitors (mTKIs) play a crucial role in the treatment of hepatocellular carcinoma (HCC) by inhibiting multiple tyrosine kinases associated with tumor growth, angiogenesis, and cancer cell signaling. This inhibition effectively blocks the tumor's blood supply, curbing the proliferation and spread of cancer cells(60). Targeted therapies, including drugs like sorafenib, lenvatinib, and ramucirumab, rely to some extent on the activity of specific signaling pathways within tumor cells. For instance, HCC patients who respond well to sorafenib treatment may exhibit certain molecular characteristics in their tumors, such as a low neutrophil-to-lymphocyte ratio or specific gene expression patterns(61).

The prospects of targeted therapy application

With the advancement of molecular biology techniques, targeted therapy has become an integral part of the treatment strategy for hepatocellular carcinoma (HCC). In this process, biomarkers play a crucial role. By identifying and utilizing specific biomarkers, physicians can predict patients' responses to certain targeted treatments, thereby creating more personalized treatment plans that improve efficacy and reduce unnecessary side effects. Moreover, as our understanding of the molecular mechanisms of HCC deepens, new biomarkers and therapeutic targets are continually being discovered, opening the door to the development of innovative targeted drugs. These advancements not only enhance the precision of treatment but also bring new hope to patients.

Conclusion

A profound understanding of the molecular mechanisms of hepatocellular carcinoma (HCC) is essential for the discovery and development of effective targeted therapies. Advances in molecular biology have progressively unveiled key molecular events in HCC, such as overexpression of the Epidermal Growth Factor Receptor (EGFR), aberrations in the Wnt/β-catenin signaling pathway, mutations in the p53 gene, and activation of the Hepatocyte Growth Factor (HGF)/c-Met axis. The elucidation of these mechanisms not only deepens our comprehension of the pathogenesis of HCC but also guides the development of targeted therapeutic drugs. For instance, the application of the multi-kinase inhibitor sorafenib is based on an understanding of the role of multiple signaling

pathways in the progression of HCC. Thus, there is a symbiotic relationship between molecular biological research in liver cancer and targeted therapy; the former provides a theoretical foundation and experimental evidence for the latter, while the effective implementation of targeted therapy strategies further validates the relevance of molecular mechanisms, creating a mutually reinforcing cycle.

Table 1: Molecular Targeted Therapies for Hepatocellular Carcinoma and Their Characteristics

Drug Name	**Mechanism of Action**	**Research Findings/Conclusions**	**Reference Numbers**
Sorafenib	RAF/MEK/ERK pathway inhibitor, inhibits tumor cell proliferation and anti-tumor angiogenesis	SHARP and ORIENTAL studies demonstrated effective prolongation of survival in advanced HCC patients	(35-39)
Regorafenib	Multi-target inhibitor, affecting tumor cell survival, proliferation, and angiogenesis	Direct inhibition of VEGFR and PDGFR blocks tumor cells' response to growth factors	(39-41)
Lenvatinib	Multi-targeted tyrosine kinase inhibitor, blocking tumor proliferation and angiogenesis-related signal pathways	Clinical studies show similar overall survival rates compared to sorafenib, with better objective response rates and PFS times	(42-46)
Donafenib	Deuterated derivative of sorafenib, with optimized molecular structure	Longer overall survival and better safety and tolerability in advanced HCC patients compared to sorafenib	(47-49)
Everolimus	mTOR inhibitor, modulating the mTOR signaling pathway	Potential to enhance transplant success rates, protect renal function, and reduce the risk of HCC recurrence in HCC patients	(51-54)
Bevacizumab	Antibody drug targeting VEGF-A, effectively blocking tumor angiogenesis	Effective in improving patient prognosis when used in combination with other treatments such as targeted drugs or chemotherapy for HCC	(55)

Prospect

Looking ahead, the widespread adoption of high-throughput sequencing technologies and the application of cutting-edge techniques like single-cell sequencing will enable a more detailed characterization of the molecular heterogeneity of hepatocellular carcinoma (HCC). This will not only facilitate the crafting of personalized treatment strategies and optimize the use of existing targeted drugs but may also unveil novel therapeutic targets, offering hope to patients with intractable HCC.

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Author contributions

Concept and design of the study: W-PY. Drafting of the manuscript: W-PY . Critical revision of the manuscript for important intellectual content and study supervision: Sher Zaman Safi.All authors contributed to the article and approved the submitted version.

Competing Interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Not Applicable.

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