frontiers | Frontiers in Oncology

TYPE Review
PUBLISHED 15 November 2022
DOI 10.3389/fonc.2022.988956

OPEN ACCESS

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SPECIALTY SECTION
This article was submitted to Pharmacology of Anti-Cancer Drugs, a section of the journal Frontiers in Oncology

RECEIVED 02 August 2022
ACCEPTED 01 November 2022
PUBLISHED 15 November 2022

CITATION
Zhang J, Han H, Wang L, Wang W, Yang M and Qin Y (2022) Overcoming the therapeutic resistance of hepatomas by targeting the tumor microenvironment.
Front. Oncol. 12:988956.
doi: 10.3389/fonc.2022.988956

Overcoming the therapeutic resistance of hepatomas by targeting the tumor microenvironment

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Hepatocellular carcinoma (HCC) accounts for the majority of primary liver cancers and is the third leading cause of cancer-related mortality worldwide. Multifactorial drug resistance is regarded as the major cause of treatment failure in HCC. Accumulating evidence shows that the constituents of the tumor microenvironment (TME), including cancer-associated fibroblasts, tumor vasculature, immune cells, physical factors, cytokines, and exosomes may explain the therapeutic resistance mechanisms in HCC. In recent years, anti-angiogenic drugs and immune checkpoint inhibitors have shown satisfactory results in HCC patients. However, due to enhanced communication between the tumor and TME, the effect of heterogeneity of the microenvironment on therapeutic resistance is particularly complicated, which suggests a more challenging research direction. In addition, it has been reported that the three-dimensional (3D) organoid model derived from patient biopsies is more intuitive to fully understand the role of the TME in acquired resistance. Therefore, in this review, we have focused not only on the mechanisms and targets of therapeutic resistance related to the contents of the TME in HCC but also provide a comprehensive description of 3D models and how they contribute to the exploration of HCC therapies.

KEYWORDS

hepatocellular carcinoma, tumor microenvironment, therapeutic resistance, therapeutic targets, novel drugs, patient-derived organoids

Introduction

Primary liver cancer is one of the most aggressive and lethal cancers worldwide, with an increasing number of patients suffering from chronic liver fibrosis and inflammation (1). According to the Global Cancer Statistics 2020, hepatocellular carcinoma (HCC) ranks sixth in terms of cancer incidence and has a high mortality rate worldwide (2). A substantial proportion of primary liver cancers comprise HCC, intrahepatic cholangiocarcinoma, and other mixed tumors (3). HCC is the most common primary malignancy, is associated with a poor prognosis and recurrence within 5 years, and is often diagnosed at the end stage of the disease (4). Currently, liver resection and transplantation are the most promising curative options for patients with HCC. However, most patients diagnosed with HCC miss the optimal time window for surgery. Some palliative measures include locoregional therapies such as radiotherapy, trans-arterial chemoembolization (TACE), trans-arterial radioembolization (TARE), and ablation (microwave, cryoablation, and ethanol). In the past few years, there have been significant advances in chemotherapy, immunotherapy, and targeted therapies. However, drug resistance largely limits efficacy and has a negative impact on patient prognosis (5, 6). It is widely known that cancer therapeutic resistance mechanisms are complicated and are composed of two groups: intrinsic drug resistance (resistance factors that existed before drug treatment) and acquired drug resistance (caused by enhanced efflux of drugs, growth factors, increased metabolism of xenobiotics, enhanced DNA repair ability, and epigenetic factors among other factors during the treatment process) (7). Several studies have demonstrated that therapeutic resistance has a strong relationship with the tumor microenvironment (TME), mainly referred to as acquired resistance (8–10).

A prototype of the TME was initially proposed in 1889 as "seed and soil," which vividly illustrated the relationship between tumor cells and the TME (11). This complex and dynamic TME mainly contains cellular components (cancer-associated fibroblasts [CAFs], immune cells, regulatory T cells [Tregs], myeloid-derived suppressor cells [MDSCs], tumor-associated macrophages [TAMs], and natural killer [NK] cells) and non-cellular components (tumor vasculature system, exosomes or extracellular vesicles (EVs), cytokines, and growth factors) (12, 13). Liver cancer is a rapidly growing solid tumor. The hypoxic microenvironment of liver cancer tissue is widespread, which not only stimulates the proliferation of liver cancer cells, causes angiogenesis, and accelerates invasion, but also has an important impact on drug tolerance (14). Moreover, dynamic changes in the TME mean that cells and extracellular secretions are constantly remodeled, which makes the microenvironment more conducive to the development of tumor drug resistance (15). Therefore, regulating the HCC microenvironment is an important treatment approach.

Immune checkpoint inhibitors (ICIs) and anti-angiogenesis therapies are the two main TME-targeted therapies; in particular, the IMbrave150 study demonstrated that the combination of the PD-L1 inhibitor atezolizumab with the anti-angiogenic agent bevacizumab had a survival benefit superior to the standard treatment of sorafenib in the order of many years (16). This regimen has also been approved by the FDA for the first-line treatment of HCC in many countries and regions. However, during treatment, new immune checkpoints, new angiogenesis patterns, and other factors in the microenvironment trigger acquired drug resistance and limit clinical efficacy. Therefore, targeting the TME as a battlefield to overcome many existing therapeutic limitations is a promising research direction. Simultaneously, it is necessary to accurately mimic the full appearance of the TME to explore tumor recalcitrant mechanisms and discover efficient therapies for HCC patients. For example, cultivation of three-dimensional (3D) organoid models *in vitro* provides a broader platform for preclinical studies. In this review, we mainly focus on the mechanisms and markers of drug resistance related to the HCC microenvironment (Figure 1) and elaborate on potential drugs for TME-targeted therapies. Furthermore, we discuss what the 3D organoid model is and how it contributes to HCC therapy exploration.

Mechanisms of therapeutic resistance in the HCC microenvironment

Vascular system

The emergence of vessel co-option and vasculogenic mimicry

Available data suggest that anti-angiogenesis can inhibit tumor growth, but compensatory angiogenesis is involved in anti-angiogenic therapeutic resistance. Important vascular stimulators have been studied in HCC, including vascular endothelial growth factor (VEGF)/vascular endothelial growth factor receptor (VEGFR), fibroblast growth factor (FGF)/fibroblast growth factor receptor (FGFR), platelet-derived growth factor (PDGF)/platelet-derived growth factor receptor (PDGFR), endoglin (CD105), and angioportin/tie (17). Among these factors, VEGF is the most important cytokine that facilitates angiogenesis and is composed of five different isoforms: VEGF-A, -B, -C, -D, and -E. The VEGF receptors VEGFR-1, -2, and -3 bind VEGF with different affinities (18). The combination of VEGF and VEGFR can not only trigger the proliferation of vascular endothelial cells but also activate lymphatic metastasis by forming new lymphatics. In 1971, Folkman suggested potential strategies against tumor progression by blocking tumor angiogenesis (19). Angiogenesis

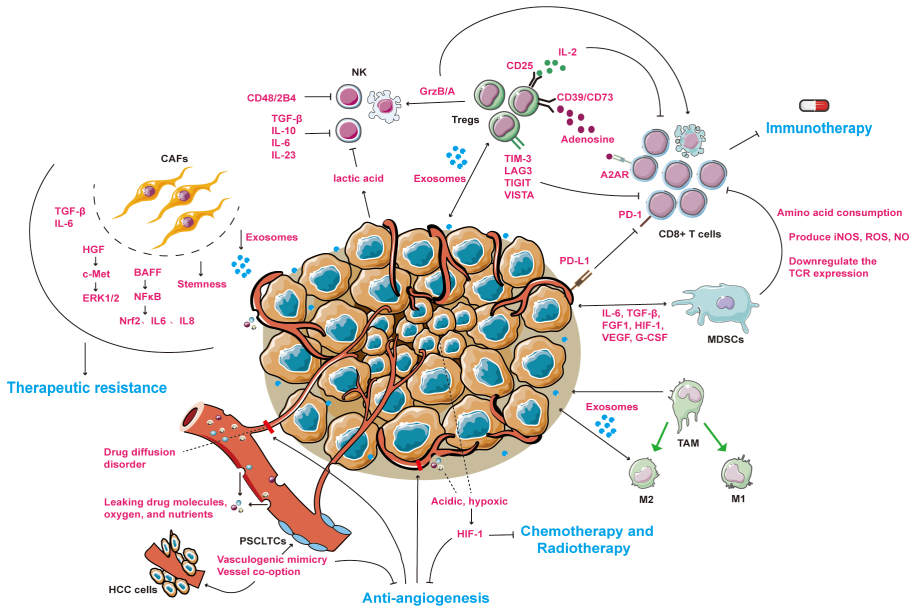

FIGURE 1
An overview of drug resistance mechanisms between tumor microenvironment and HCC cells. The microenvironment of HCC mainly includes cellular components (CAFs, NKs, Tregs, MDSCs, TAMs) and non-cellular components (tumor vasculature system, exosomes and cytokines). Under the combined effect of tumor and microenvironmental components, patients with liver cancer are resistant to immunotherapy, anti-angiogenesis or radiotherapy. → represents the pointing and promoting effect. ⊥ represents the inhibiting effect. The ↔ representative serves to enhance each other. The blue fonts represent the treatment for HCC patients. The red fonts represent microenvironmental molecules and their mechanisms of action. Black fonts represent cells name. CAFs, cancer associated fibroblasts; NKs, natural killer cells; Tregs, regulatory T cells; MDSCs, myeloid-derived suppressor cells; TAMs, tumor-associated macrophages; M1, tumor suppressor macrophages; M2, tumor-promoting macrophages. PSCLTCs, pluripotent stem cell-like tumor cells. HCC cells, hepatocellular carcinoma cells.

inhibitors are administered to patients with HCC, including bevacizumab, cabozantinib, lenvatinib, ramucirumab, regorafenib, and sorafenib, based on the National Comprehensive Cancer Network guidelines (20). Placental growth factor (PLGF) is a pro-angiogenic factor belonging to the VEGF family, which is usually secreted under pathological conditions. The overexpression of PLGF has been noted in several tumors resistant to anti-angiogenesis therapy, suggesting that PLGF is a prospective target in HCC treatment (21–24).

Although current anti-angiogenic drugs, together with immunotherapy, have prolonged the survival of patients to a certain extent, acquired resistance limits the therapeutic efficacy of HCC. To some extent, antiangiogenic drugs prevent the transport of chemotherapy drugs and limit the therapeutic efficacy. Furthermore, vasculogenic mimicry (VM) and vessel co-option (VC) are two emerging theories that explain resistance to anti-angiogenic drugs.

VM is a novel tumor microcirculation model independent of endothelial cells and was first developed by Maniotis et al. in 1999 (25). This model indicates that pluripotent stem cell-like tumor cells (PSCLTCs) acquire endothelium-like properties and form stable tubular structures without endothelial cells by secreting heparin sulfate, proteoglycans, collagen IV and VI, and laminin to transport nutrients for tumor growth (26). The VM process is unsurprisingly complex and is usually induced by a hypoxic TME and favored by many molecular mechanisms, mainly involving epithelial–mesenchymal transition (EMT), cancer stemness, response to hypoxia, and extracellular matrix remodeling (27, 28). VEGF/VEGFR inhibitors disrupt the formation of tumor blood vessels, leading to an acidic environment that compensates for the production of VM structures. Therefore, VM is an important cause of drug resistance after the application of anti-VEGF drugs to solid tumors. Sorafenib is a small-molecule tyrosine kinase inhibitor with anti-angiogenic activity. Clinical trials in breast cancer have been limited and have stopped at phase III. Mao et al. found that compared to the less invasive MCF7 cell line, only breast cancer stem-like cells (BCSLCs) and ALDH1+ MDA-MB-231 cells showed angiogenic ability and were directly involved in the development of VM. Sorafenib only inhibits endothelial angiogenesis and has no effect on VM. Mechanistically, they found that HIF-1α contributes to the proportion of BCSLCs (29). Furthermore, VM usually occurs in tumors with higher grades, shorter survival, and more aggressive disease (30, 31). Sorafenib resistance has been studied most frequently in HCC.

Shi et al. found that the high expression of ITGA5 and ITGB1 in sorafenib-resistant liver cancer tissues promoted the degree of hypoxia and the generation of VM structures (32). The most important molecular mechanisms favoring VM in HCC include the following: (a) HIF1-α promotes VM production by regulating LOXL2 and is positively correlated with poor prognosis of HCC patients (33); (b) m6A methyltransferase METTL3 promotes VM generation and HCC metastasis by enhancing the translation efficiency of YAP1 mRNA and is related to Hippo pathway (34); (c) exosomes derived from hepatocytes, circRNA-100338, can affect the angiogenesis of liver cancer, including VM, thereby promoting the metastasis of liver cancer (35); (d) high expression of CD276 protein can activate the PI3K/AKT/MMPs pathway to promote VM formation, which is related with poor prognosis in HCC (36); (e) BMP4, migration-inducing gene 7 (MIG7), long noncoding RNA n339260, RhoC/ROCK2, Slug, and osteopontin were also found to be factors inducing VM formation in HCC (37–42); (f) EMT pathway can be induced by Hsp90B, Notch 1, ZEB2 and Twist1 to promote VM formation (43–46). These molecules are thought to be potential therapeutic targets for tumors resistant to anti-angiogenic therapy in patients with HCC.

Vessel co-option is a non-angiogenic mechanism in which tumor cells adhere to the existing normal vessels of host organs and grow infiltratively by migrating along them without angiogenesis (26). Kuczynski et al. performed an experiment to illustrate the co-option process in sorafenib-resistant HCC using Hep3B-hCG orthotopic HCC xenografts. Under sorafenib treatment, angiogenic vessels of the tumor are depleted but co-opted pre-existing vessels are preserved. Meanwhile, EMT-like molecules are increased, leading to both tumor invasion and incorporation into the liver parenchyma, co-opting the normal liver vascular system. These changes can be reversed by discontinuing sorafenib treatment (47, 48). Interestingly, following the onset of sorafenib resistance, tumor cells begin to surround the hepatic sinuses and major blood vessels and invade rapidly. The tumor invasion signal weakens and becomes angiogenesis-dependent only when sorafenib treatment is discontinued (49). In cases of colorectal cancer with liver metastases, vascular co-option is associated with resistance to anti-angiogenic therapy. Cancer cells replace hepatocytes by inducing apoptosis proteins, motility, and EMT, and enter sinusoidal vessels to establish vascular co-option (50). However, these findings lack specific markers of co-opted vessels and need to be verified in patients with HCC in future studies. If possible, anti-angiogenic drugs together with anti-co-opted vessels will be an optimal choice for patients with metastatic HCC.

Potential drugs and mechanisms to overcome antiangiogenic resistance in HCC

Recently, some VM-targeted drugs have been developed to inhibit VM in HCC and have shown satisfactory results in reversing drug resistance. The promising drugs and functional mechanisms that inhibit VM formation and overcome anti-angiogenic therapy resistance are summarized in Table 1.

Immune system

The immunosuppressive environment contributes to therapeutic resistance

The imbalance between the recruitment of immunosuppressive cells (MDSCs, TAMs, and Tregs) and the reduction of anti-tumor effector cells (cytotoxic T lymphocytes [CTLs], NKs, and dendritic cells [DCs]) results in an immunosuppressive microenvironment for HCC, leading to immunotherapy resistance (61). A high-level overview of these mechanisms is shown in Figure 1. The mechanisms and potential targets of drug resistance in these cells are described.

Tregs are a subset of CD4+CD25+ immunosuppressive T cells, characterized by Forkhead box protein P3 (Foxp3) expression, which determines the development and differentiation of Tregs. In HCC tissues, a high concentration of Tregs combined with a low concentration of CD8+T cells is an independent factor that affects the survival and recurrence of patients (62). However, the mechanisms by which Tregs induce resistance to ICIs and sorafenib in HCC have not been fully elucidated. However, a number of molecules and signaling pathways have been implicated in Treg-induced immune tolerance: (a) the emergence of other immune checkpoints from Tregs, such as TIM-3, TIGIT, LAG3, and VISTA, blocks effector T cell activation, and their high expression is often associated with poor prognosis in HCC patients after anti-PD-1/PD-L1 treatment (63, 64); (b) TGF-B secreted by Tregs induces EMT and promotes the invasion and migration of HEPA1-6 cells. The combination of TGF-B inhibitors significantly enhances the sensitivity of HCC cells to regorafenib and sorafenib (65, 66); (c) CD25 expressed by Tregs competitively consumes IL-2, reduces effector T cell activation, and induces metabolic disorders (67); (d) CD73, CD39, and cyclic AMP-regulated adenosine A2 receptor (A2AR) can reduce T-cell toxicity and shift the microenvironment into a tolerant state (68); (e) Tregs secrete granzyme B/perforin that directly lyses NK and CD8+ T cells (69). Moreover, CCR4+ Tregs have been found to be an important type of Tregs in HBV^+ HCC, correlated with sorafenib resistance and increased IL-10 and IL-35 levels, and treatment with a $CCR4^+$ antagonist has been shown to successfully reverse sorafenib resistance and sensitize liver cancers to PD-L1 checkpoint blockade (70).

MDSCs are heterogeneous and immature cells derived from the bone marrow, which can be induced in almost all types of tumors and in pathological conditions such as infection, autoimmune disease, and trauma. The mechanism of drug resistance in MDSCs is mainly through damaging T cell

TABLE 1 The novel targets and promising drugs to reverse resistance by inhibiting VM formation.

Molecule	Target	Target's expression	Molecule function	Cell lines	Ref
Myricetin	PAR1	UP	Myricetin reversed PAR1-mediated EMT and inhibits HCC cell invasion, metastasis, VM formation and angiogenesis	PLC-PRF-5	(51)
Daurisoline	RhoA/ROCK2/AKT, ERK-p38 MAPK	UP	Daurisoline dramatically sensitized HCC cell lines to sorafenib	MHCC-97H	(52)
IU1 (S7134)	USP14	UP	IU1 treatment decreased cell proliferation, invasion, migration, and VM formation under hypoxia conditions	HCCLM3 Huh-7	(53)
Regorafenib	ID1, Snail VE-cadherin,	UP	Regorafenib distinctly inhibited EMT in HCC cells *via* targeting ID1, leading to the suppression of cell migration, invasion and VM formation.	Huh7, PLC/PRF/5	(54)
Androgen receptor (AR)	Notch4, VE-cadherin,	UP	AR suppressed the VM formation by down-regulating circRNA7/miRNA7-5p/VE-Cadherin/ Notch4 signal	SKhep1, HA22T	(55)
Y27632	ROCK	UP	Y27632 inhibited VM formation *via* TGF-B1/ROCK induced EMT pathway	HepG2, MHCC-97H	(56)
Melittin	HIF-1α, p-AKT, MMP2/9	UP	Melittin suppressed VM formation by inhibiting HIF-1α/AKT pathway	SMMC-7721 Huh7, HepG2	(57)
NVP-BEP800	Hsp90B Twist1	UP	NVP-BEP800 suppressed VM formation by releasing Hsp90B and Twist1 interaction.	SMMC-7721	(43)
Biejiajian Pills	VE-cadherin, PI3K, RhoA ROCK	UP	Biejiajian Pills can inhibit the formation of VM in HCC cells *in vitro* possibly by inhibiting the RhoA/ROCK pathways and the expressions of VE-cadherin and PI3K.	HepG2	(58)
Polyphyllin I (PPI)	Twist1 VE-cadherin	UP	PPI impaired VM formation by decreasing Twist1 and VE-cadherin, and blocking PI3k/Akt pathway	SMMC-7721 PLC/PRF/5	(59)
Arsenic trioxide (As2O3)	VE-cadherin, MMP2, MMP9	UP	As2O3 inhibited VM formation through downregulating the expression of VE- cadherin, MMP2, and MMP9	HepG2	(60)

function: (a) MDSCs overconsume essential amino acids such as arginine-1 and cysteine in the body, leading to T cell proliferation and activation dysfunction, as well as tryptophan by overexpression of indoleamine-pyrrole 2,3-dioxygenase (IDO) (71–73); (b) Cystine deprivation downregulates the T-cell receptor (TCR) ζ chain of hepatic CD8(+) T cells and helps tumor cells to escape; and (c) MDSCs also express cytotoxic reactive oxygen species (ROS), inducible nitric oxide synthase (iNOS), and nitric oxide (NO) to influence T cell viability (74). Furthermore, many tumor-derived cytokines such as FGF1, HIF-1, VEGF, IL-6, and G-CSF have been shown to promote the accumulation of MDSCs and are related to therapy resistance (75–80). For example, Xu et al. argued that chemotherapy-resistant HCC cell-released IL-6 boosts the silencing and activity of MDSCs and, when anti-IL6 neutralizing antibody is combined with 5-FU chemotherapy, tumor growth is significantly decreased (80). TGF-B secreted by MDSCs participates in a variety of drug resistance mechanisms, such as EMT induction, CSC promotion, and immunosuppression (81). Downregulation of TGF-B expression can overcome sorafenib resistance in liver cancer (82). Furthermore, hepatoma-intrinsic CCRK inhibition reduces MDSCs immunosuppression and enhances the blocking effect of the immune checkpoint (PD-L1) (83). SB265610, a CXCR2 antagonist, may reverse immunosuppression by inhibiting the chemotaxis of MDSC to the tumor, thereby promoting the anti-tumor immunity of CD8+ T cells and inhibiting tumor immune escape (84). MDSC-targeted therapy has achieved great progress in solid cancers, such as melanoma, breast cancer, NSCLC, and glioblastoma, and has even entered the stage of clinical trials, but research on liver cancer is still lacking.

TAMs are another type of marrow-derived cells that are divided into M1 (tumor suppressor macrophages) and M2 (tumor-promoting macrophages) subtypes, with type M2 predominating in liver cancer (85). In multiple cancers, the infiltration of M2 TAMs is highly related to poor prognosis and treatment resistance (86, 87). The main mechanism by which TAMs exert immune tolerance in HCC is through the secretion of molecules. For example, hepatocyte growth factor (HGF), derived from polarized M2 TAMs, confers HCC resistance to sorafenib in a feed-forward manner. Accumulated HGF can activate the HGF/c-Met, ERK1/2/MAPK, and PI3K/AKT pathways in tumor cells, recruit more M2 TAMs, and promote tumor growth. Therefore, the combination of the HGF inhibitor

cabozantinib and sorafenib is reasonable for improving the efficacy of first-line systemic therapies (88). TAMs can also induce oxaliplatin resistance through autophagy in HCC cells (89). Therefore, blocking the recruitment of TAMs or reprogramming the polarization of TAMs is a promising measure to enhance the sensitivity of HCC treatment. Antagonists targeting the chemokine C-C motif ligand 2 receptor (CCR2) and stromal cell-derived factor 1 α (SDF-1α/CXCL12) have shown good efficacy in blocking TAM recruitment (90–92). Targeted colony stimulating factor (CSF-1), autophagy, NF-KB, Mir-214, IL-6, and toll-like receptors (TLRs) can transform TAMs from a pro-tumor phenotype into an antitumor phenotype (M1) (93–99).

NK cells are abundant in liver tissue, exerting their 'killing' function by regulating the activation receptors (NKG2C, NKG2D, CD244, CD266, and NCR) and inhibitory receptors (CD94/NKG2A and KIR) expressed on the surface of NK cells (100). The balance between the signals elicited by these receptors determines whether NK cells are activated and perform their effector functions. NK cell abnormalities induced by the TME are the main reason why tumor cells escape the immune response. For example: (a) immunosuppressive factors such as TGF-B, IL-10, IL-6, and IL-23 secreted by Tregs, MDSCs, or TAMs suppress NK cell function and induce tumor evasion and progression (101); (b) NK cells are excessively activated by mononuclear macrophages through CD48/2B4 interactions, thus inducing NK cell exhaustion and death (102); and (c) increased lactic acid content in tumor cells leads to metabolic reprogramming, resulting in NK cell dysfunction (103). In addition, the influence of HCC cells and their microenvironment can limit the sensitivity to NK cell cytotoxicity. NK cells targeting CSCs are known to inhibit tumor progression; however, the resulting overexpression of CEACAM1 renders $EpCAM^+$ HCC cells resistant to NK toxicity. Anti-CEACAM1 antibody has been shown to restore the cytotoxicity of NK cells against $EpCAM^+$ Huh-7 cells (104). Similarly, the inhibition of CNOT7 and the enhancement of zeste homolog 2 (EZH2), miR-889, granulin-epithelin precursor (GEP), and MICA/B can successfully reverse NK cell resistance, suggesting that these molecules are promising targets for immunotherapy in HCC patients (105–109).

Targeting immune environment to overcome drug resistance in HCC

As mentioned above, the immune system greatly influences tumor response to various treatments in the microenvironment. Therefore, based on the above immune tolerance mechanisms, significant efforts have been made to attempt to reverse tumor drug resistance, including: (a) combining immune checkpoint inhibitors; (b) inhibiting the recruitment of Tregs, MDSCs, and M2 TAMs in tumor tissue; (c) reprogramming the polarization of TAMs; and (d) reinforcing the anti-tumor capabilities of the immune system.

Multiple studies have shown that PD-1/PD-L1 inhibitors in combination with multi-tyrosine kinase inhibitors, VEGF inhibitors, or CTLA-4 inhibitors are superior to monotherapy (110). Two CTLA-4 inhibitors, ipilimumab and tremelimumab, have recently gained increasing attention, with FDA approval in 2011 for patients diagnosed with advanced or unresectable melanoma and in 2015 for patients with malignant mesothelioma (111, 112). In contrast to the immunonormalizing effect of PD-1/PD-L1 inhibitors, CTLA-4 suppression tends to largely enhance the toxic function of T cells, thus increasing the sensitivity to other treatments. Currently, the combination of ipilimumab and nivolumab/pembrolizumab has been associated with encouraging survival outcomes in patients with advanced HCC with primary resistance to prior immune checkpoint inhibitors (113). Moreover, this combination as a first-line therapy for patients with advanced HCC is currently undergoing evaluation (NCT04039607). Tremelimumab, another CTLA-4 inhibitor, in combination with durvalumab for patients with unresectable hepatocellular carcinoma, has been associated with a reasonable outcome (median overall survival of 18.7 months) and a satisfactory benefit-risk profile (114). With the emergence of more immune checkpoints and anti-PD-1/L1 resistance, the combination of immune checkpoint inhibitors to overcome drug resistance has become a popular trend. For example, the inhibition of TIGIT together with anti-PD-1 has been shown to significantly decrease tumor growth and increase the proportion of cytotoxic T cells in tumors (64). Fibrinogen-like protein 1 (FGL1) was recently identified as a major immune inhibitory ligand of LAG-3 and its overexpression was positively associated with poor prognosis, anti-PD-1 therapy resistance, and sorafenib resistance (115, 116). Although the exact mechanism by which FGL1/LAG3 regulates the immune environment remains unclear, synergistic inhibitory effects of anti-FGL1 and anti-PD-1 have been identified in animal studies. Similarly, a combined inhibitory effect of anti-LAG-3 and anti-PD-1 has been identified in several cancers such as melanoma, NSCLC, and breast cancer (117–119). Therefore, FGL1 and LAG3 can be used as next-generation immunotherapy targets independent of PD-1/PD-L1.

Anti-tumor efficacy can be enhanced by inhibiting Tregs, MDSCs, and TAMs, or by targeting important molecules to enhance NK cell activity. Related targets and potential reversal of drug resistance are described in **Section 2.2.1**. Adoptive cell transfer is an emerging therapeutic strategy with wide potential value in the treatment of HCC, and is deemed to be a highly individualized cancer therapy because of the adhibition of the patients' own effect factors (120). What's more, antiviral treatment for HBV-HCC was proved effective in increasing the postoperative survival (121). In the clinical study of PD-1 antibody, when ICIs repair immune function, HBV infection can be significantly reduced. In mouse models, it can also be seen that the virus infection rate of mice treated with PD-1 antibody is significantly reduced. Thus, the immune escape mechanisms of viruses and tumors actually work in the same pathway (122).

Therefore, it is worth exploring whether there is a relationship between viral load and treatment resistance and whether the combination of antiviral drugs and immunotherapy or anti-angiogenesis is more effective. At present, adoptive cell therapy has been developed into a phase III clinical treatment, including chimeric antigen receptor (CAR) T cell therapy, tumor-infiltrating lymphocyte (TIL) therapy, engineered TCR therapy, cytokine-induced killer (CIK) cell therapy, and NK cell therapy. However, more effort is needed to achieve the desired clinical effect. Thus, due to the high heterogeneity of HCC tissues, targeting the immune system requires a combination of other molecular targeting inhibitors or multiple types of therapies.

Cancer-associated fibroblasts and therapeutic resistance

It has been well documented that CAFs are the paramount population of stromal cells responsible for modulating neighboring cancer cells by way of autocrine, paracrine, and exosome functions (123). CAFs and their secreted soluble factors including cytokines (TGF-B, IL-4, IL-6), chemokines (CCLX and CXCL family members), pro-angiogenesis factors (VEGF, PDGF, and HIF), enzymes (MMPs), and ECM proteins (ectodysplasin-A and collagen type-I) contribute to tumor progression (124, 125). CAFs are highly heterogeneous and lack clear markers to distinguish functional subsets, which usually induces worse outcomes in anticancer therapy. With the participation of TME, CAFs are found contributory to increase the chemoresistance of HCC from multiple mechanisms (126–128).

First, paracrine signaling plays an important role in crosstalk between CAFs and tumor cells. For example, CAF-derived HGF increases the resistance of $CD73^+$ cancer cells to sorafenib or cisplatin through the HGF-c-Met-$ERK_{1/2}$ pathway in HCC (129). CAF-derived TGF-B, as the most crucial characteristic in the HCC inflammatory process, is becoming an inducer of resistance in various tumors, including HCC (126, 130–132). Recently, Liu suggested that valproic acid (VPA) could reverse TGF-B-induced sorafenib resistance in HCC cells by reducing the Jagged2-mediated Notch1 signaling pathway and altering the EMT phenotype (133). CAF-derived IL-6 can impair the activity of tumor-infiltrating T cells and neutralization of IL-6 reverses anti-PD-L1 resistance in an HCC mouse model (134).

On the contrary, cancer cells affect the activation and expression of CAFs to protect themselves from toxic drug attacks. Gao et al. noted that when co-cultured with sorafenib-resistant Huh7 cells, the BAFF/NF-KB axis could be activated in CAFs and simultaneously induce the upregulation of Nrf2, IL6, and IL8, which contributed to the development of drug resistance in non-resistant Huh7 cells (135). The positive feedback of the CAF-HCC cell loop makes the tumor more resistant.

Furthermore, in view of the critical role of CSCs in therapy resistance, CAFs can promote stem cell action to increase cancer cell resistance. The C-MET/ERK/FRA1/HEY1 axis is mediated by CAF-derived HGF to promote the stemness of tumor-initiating cells (136). In line with HGF, IL-6 can regulate STAT3/NOTCH1/NICD/HES1 signaling to enhance the stem cell-like properties of HCC, either (137). The neutralizing antibody HGF or IL-6 has been used to successfully reverse the stem cell viability of CD24+ HCC cells *in vitro* and *in vivo* through the HGF/C-MET/STAT3 or IL6/IL6R/STAT3 signaling pathways (138). COMP, derived from CAFs, can induce EMT and stemness in HCC cells. RvD1 has been shown to damage COMP by targeting FPR2/ROS/FOXM1 signaling to eliminate the recruitment of COMP promoters by FOXM1 (139). The cellular crosstalk between CAFs and HCC cells does not stop. CAF-derived CLCF1 increases the self-renewal ability of HCC cells by binding to CNTFR expressed in an autocrine manner. Cancer cells then secrete CXCL6 and TGF-B, which partially account for the CLCF1-regulated stemness of HCC cells (140). Moreover, cessation of autophagy is also an effective way to attenuate CAF-promoted stemness in HCC (141).

Finally, some small molecules carried by CAF-derived exosomes help markedly. CAF-derived exosomes containing circZFR enhance chemoresistance to cisplatin by inhibiting the STAT3/NF-KB pathway in HCC (142). CAF-secreted exosomal miR-1247-3p is associated with the production of IL-6 and IL-8 in CAFs through B1-integrin–NF-KB signaling, which increases the stemness and EMT of HCC (143). Evidence suggests that DNA methyltransferase 3 beta (DNMT3b) is upregulated in HCC tissues and is associated with poor progression. DNMT3b-targeted therapy with annamycin A has been shown to significantly reverse sorafenib resistance in cells (144). Moreover, CAF-derived exosomal miR-29b can inhibit HCC progression by targeting DNMT3b, serving as a potential drug (145).

In conclusion, the above literature findings illustrate the mechanisms of drug resistance between CAFs and HCC cells, suggesting that CAF-targeted therapy is a promising method for reversing drug resistance in HCC (Table 2). Apart from the inhibitors mentioned above, some traditional Chinese medicines that exert antifibrotic properties have been reviewed recently (146). Several anti-HGF/c-MET antibodies, such as onartuzumab, tepotinib, capmatinib, and tivantinib are already in the clinical stage of HCC treatment evaluation (NCT01897038, NCT02115373, NCT01737827, NCT02029157).

Hypoxia

Hypoxia and acidic environment

Abnormal vascular morphology in HCC tumors results in a preliminary anoxic and acidic microenvironment after the treatment of anti-angiogenic therapy (147). The lack of oxygen

TABLE 2 CAFs-derived molecules promote chemotherapy resistance in HCC.

CAFs-derived molecules in HCC	Effect on HCC	Mechanism	Referance
HGF	Resistance to sorafenib or cisplatin	HGF enhances the resistance of $CD73^+$ cancer cells to sorafenib or cisplatin through HGF-c-Met-$ERK_{1/2}$ pathway	(129)
	Induce stemness	C-MET/ERK/FRA1/HEY1 axis is mediated by HGF to promote the stemness of tumor-initiating cells	(136)
TGF-B	Resistance to sorafenib	TGF-B induces stemness, EMT and metabolic reprogramming in CAFs	(126)
IL-6	Resistance to anti-PD-L1 therapy	Activated IL-6/STAT3 signaling can resist anti-PD-L1 therapy in HCC	(99)
COMP	Induce EMT and stemness	COMP is derived from CAFs in a paracrine manner and initiate EMT and resistance in HCC.	(139)
CLCF1	Induce stemness	–	(140)
CircZFR	Resistance to cisplatin	Exosomal circZFR enhanced chemoresistance to cisplatin by inhibiting the STAT3/NF-KB pathway of HCC cells	(142)
MiR-1247-3p	Induce EMT and stemness	Exosomal miR-1247-3p was associated with the production of IL-6 and IL-8 in CAFs through B1-integrin–NF-KB signaling	(143)

and nutrients often causes cancer cells to undergo glycolysis rather than metabolism, producing large amounts of lactic acid. The degree of hypoxia in the HCC microenvironment is heterogeneous. As the degree of hypoxia increases along with depth from the blood vessels into the tumor, HCC cells are exposed to a smaller concentration of oxygen and the pH decreases (148, 149). Tyrosine kinase inhibitors, anti-angiogenic drugs, or other treatments that restrict the blood supply within the tumor can exacerbate the development of an anoxic and acidic environment, leading to tumor progression and drug resistance (150). Hypoxia-regulated transcription factor HIF-1 is an extensively authoritative marker to modify cancer sensitivity to therapeutic agents, and comprises a heterodimeric DNA-binding complex composed of α and B subunits (151–154). Hypoxia-induced HIF-1α elevation and multiple mechanisms contributing to chemotherapy resistance are related to: (a) alterations in metabolic pathways. Glucose uptake and glycolysis are strongly activated in hypoxic environments to meet the demands of tumor cell growth. HIF-1α induces many glycolytic genes including glucose transporter type 1 (GLUT1) and hexokinase 2 (HK2) (155, 156). Genistein, as a natural isoflavone, can directly downregulate HIF-1α, thus inactivating GLUT1 and HK2 to inhibit aerobic glycolysis, and therefore induce apoptosis of aerobic glycolysis HCC cells. At the same time, Genistein enhanced the sensitivity of sorafenib resistant HCC cells *in vivo* and *in vitro* (157); (b) crosstalk between autophagy and mitophagy. In the context of increased metabolic demands, mitophagy and autophagy may play key roles in inducing chemoresistance by regulating the adaptation of tumor cells to hypoxia, increasing oxidative stress and DNA damage, and providing nutrition and energy to tumor cells (158). B-cell lymphoma-2/adenovirus E1B 19 kDa-interacting protein 3 (BNIP3) and BNIP3-like protein X (NIX) are hypoxia-induced HIF-1α target genes that can bind LC3 and trigger the mitophagy response (159). Upregulation of BNIP3 by HIF-1α can promote autophagy and oxidative resistance in HCC cells (160). However, treatment with sorafenib did not inhibit its cytoprotective action (161); (c) drug efflux. ATP-binding cassette (ABC) transporters, such as MDR1, can pump chemotherapeutic drugs from the intracellular to extracellular regions of HCC cells to produce drug resistance. The reporter gene assay and electrophoretic mobility shift assay proved that HIF-1α is a critical factor for MDR1 gene overexpression (162); and (d) apoptosis inhibition. HIF-1α is the promoter activator of CBR1, and CBR1 overexpression induces apoptosis resistance by reducing oxidative stress associated with hypoxia, cisplatin, and doxorubicin treatment (163). Furthermore, several important signaling pathways and other gene responsible for chemotherapy resistance under hypoxia are summarized in Table 3; however, whether they are regulated by HIFs has not been verified.

Hypoxia is a typical factor that leads to radiotherapy resistance in solid tumors. Radiation can directly damage the DNA strands or damage the DNA strands through the production of radicals, resulting in the death of tumor cells (182). In an aerobic environment, oxygen reacts with free radicals on broken DNA strands, forming a more stable pattern of DNA damage. It's also a stable hydrogen peroxide structure, which can promote more DNA breaks. In anoxic environments, tumor cells repair DNA damage by removing hydrogen from free sulfhydryl groups, thereby developing resistance to radiotherapy (183). Similarly, HIF-1α overexpression during hypoxia is an important factor in radioresistance. Bai Bing et al. found that inhibition of the EZH2/Mir-138-5p/HIF-1α pathway could enhance the sensitivity of radiotherapy (184). In addition, the activation of PI3K/AKT signaling is known to induce radioresistance in various tumors by increasing HIF-1α translation efficiency, and elevated PDK1 is a driver of PI3K/AKT/mTOR signaling in HCC, suggesting that this pathway is a potential therapeutic target to reverse radiotherapy resistance (185, 186).

TABLE 3 hypoxia related gene targets and mechanisms induced chemotherapy resistance in HCC.

Mechanism Type	Targets	HIF-1αrelated	Mechanism	Reference
Mitophagy	ATAD3A	Unknown	MiR-210-5P/ATAD3A/PINK1/PARKIN axis regulates hyperactivated mitophagy to induce sorafenib resistance in HCC under hypoxia.	(164)
	NIX and BNIP3	Yes	NIX and BNIP3 are HIF-1α mitotic targets related to mitophagy activity to induce sorafenib resistance.	(161)
Autophagy	FOXO3	Unknown	RNA m6A methylation regulates sorafenib resistance in liver cancer through FOXO3-mediated autophagy in hypoxic TME.	(165)
	FOXO3a	Unknown	FOXO3a-dependent transcriptive activation of beclin-1 is responsible for hypoxia-induced autophagy in sorafenib-resistant HCC	(166)
	BAG5	Unknown	Deletion of PRMT6 induces autophagy and promotes drug resistance of HCC by regulating the stability of BAG5-related HSC70 through post-translational methylation of BAG5	(167)
	14-3-3ζ/ beclin1	Unknown	14-3-3ζ binds to and stabilizes phospho-beclin-1(S295) and induces autophagy in HCC cells to resist cis-diammined dichloridoplatium in hypoxic TME.	(168)
	ADRB2	Yes	ADRB2 signaling negatively regulated autophagy, leading to hypoxia-inducible factor-1α stabilization, reprogramming of hepatocellular carcinoma cells glucose metabolism, and the acquisition of resistance to sorafenib.	(169)
	Egr-1	Unknown	Hypoxia-induced Egr-1 expression enhanced drug resistance of HCC cells likely through autophagy.	(170)
	BNIP3	Yes	Upregulation of BNIP3 contributes to autophagy and anoikis resistance of HCC cells	(160)
Apoptosis inhibition	carbonyl reductase1 (CBR1)	Yes	Hif-1α can activate the promoter of CBR1, and CBR1 overexpression can inhibit apoptosis by reducing oxidative stress under hypoxia, cisplatin and doxorubicin treatment.	(163)
	COX-2	Yes	COX 2 induces apoptosis resistance *via* HIF 1α/PKM2 pathway in HCC cells.	(171)
	YAP and TAZ	Unknown	YAP and its paralog TAZ induce apoptosis resistance of HCC cells under hypoxia.	(172)
Drug efflux	ABCB1	Unknown	NRF2/ABCB1-mediated efflux and PARP1-mediated DNA repair contribute to doxorubicin resistance in chronic hypoxic HepG2 cells.	(173)
	ABCG2	Yes	ABCG2-mediated drug efflux induces cancer stemness of HCC cells	(174)
Metabolic pathways	GLUT1/3	Yes	GLUT1 and GLUT3 are upregulated to enhance the resistance to 5-caffeylquinic acid	(175)
	USP29	Yes	USP29 promotes sorafenib resistance by upregulating glycolysis	(176)
	MCT-4	Yes	Glycolysis conversion of HIF-1 and McT-4 reduces hepatocellular carcinoma cell apoptosis	(177)
	HK2	Yes	Hypoxia prevents hepatocellular carcinoma cell apoptosis through HIF-1α-dependent induction of hexokinase II expression	(155)
Important signaling pathways	NF-KB axis	Yes	NF-KB activation induce HMGB1 mediated cisplatin resistance or sorafenib resistance.	(178, 179)
	ERK/MAPK axis	Yes	ERK/MAPK pathway promotes the formation of MDR through P-gp, MRP1, LRP genes	(180)
	NPM1/ PTPN14/ YAP axis	Unknown	NPM1/PTPN14/YAP axis mediates the hypoxia-induced chemoresistance to sorafenib	(181)
	mTORC1/ p70S6K/RP-S6 axis	Yes	mTORC1/p70S6K/RP-S6 axis is a target to reverse the resistance to sorafenib by preventing HIF-1α synthesis to block the cytoprotective mitophagy induced by the hypoxic microenvironment.	(161)
Other	FBI-1	Yes	FBI-1 regulates the miR-30c/HIF-1α to promote the Warburg effect and enhance the resistance to molecular targeted agents	(154)

Furthermore, HIF-1α can also be upregulated by mTORC1 to confer radiotherapy resistance to HCC through the anabolic integration of glucose and cardiolipin (187).

Targeting anoxic and acidic environment to reverse drug resistance

Hypoxia is a characteristic of HCC and contributes to chemotherapy and radiotherapy resistance. Therefore, reprogramming the hypoxic environment using immunosuppressants, nanoparticles, natural products, and HIF-1α inhibitors is an attractive direction for future research. Rapamycin, an mTOR inhibitor, reverses resistance to adriamycin during hypoxia by decreasing hypoxia-induced HIF-1α accumulation (188). Moreover, rapamycin can increase the sensitivity of HCC cells to cabozantinib (a c-Met inhibitor), which has a synergistic inhibitory effect on HCC cells (189). Nanoparticles are an emerging tool to improve the hypoxic environment of HCC. Nanoparticle delivery of oxygen-generating MnO_2 and anti-angiogenic drugs can normalize the TME by inhibiting hypoxia-induced invasion,

EMT, and metastasis, and increasing the expression of M1-type macrophage genes (Nos2, IL-6, CCL5, CXCL9, and CXCL11) (190). Bruceine D and silymarin are two newly discovered natural products that reverse drug resistance by decreasing HIF-1α expression and can directly block the inhibitor of B-catenin and T-cell factor/B-catenin interaction to regulate HCC cell metabolism, and silymarin reduces the expression of MDR1 and P-glycoprotein (191, 192). Furthermore, sorafenib inhibits HIF-1α synthesis and shifts the hypoxia response from the HIF-1α- to HIF-2α-dependent pathway. HIF-2α overexpression activates the TGF-α/EGFR pathway, thereby inducing sorafenib drug resistance. This means that to inhibit cancer growth, it may be necessary to target both HIF-1α and HIF-2α. The HIF-1α mRNA antagonist RO7070179 has shown primary success in a phase Ib study (NCT02564614).

Exosomes

TME-derived exosomes and therapeutic resistance

Exosomes function as vectors to transport a large number of molecules between cells in the TME, including messenger RNA (mRNA), long noncoding RNA (lncRNAs), microRNAs (miRNAs), circular RNA (circRNAs), lipids, proteins, and nucleic acids. These molecules are secreted by cancer or stromal cells in the TME and are involved in TME remodeling, tumor progression, invasion, angiogenesis, metastasis, and drug resistance (193). On one hand, exosome-associated therapeutic resistance is achieved through transport from drug-resistant cancer cells to drug-sensitive cells. For example, Fu et al. found that exosome miR-32-5p from a multidrug-resistant cell line (Bel/5-FU) activated the PI3K/Akt signaling pathway by inhibiting PTEN and induced drug resistance in sensitive cells by facilitating EMT and angiogenesis (194). MiR-221/222 from chemotherapy-resistant MCF-7 cells can also render sensitive MCF-7 cells resistant, but this has not been verified in HCC (195). In contrast, exosomes may swallow drug molecules and pump them out with the help of the ATP-binding cassette (ABC) transporter family (such as ABCB1) (196, 197). Recently, several researchers have made progress in the study of exosome drug resistance in HCC. Li et al. found that miR-27a-3p derived from M2 macrophage exosomes is responsible for HCC stemness and directly negatively regulates TXNIP, which has been reported as a tumor repressor gene. Upregulation of TXNIP can reverse drug resistance induced by elevated miR-27a-3p levels (198). Zhang et al. found that overexpressed plasma exosome circUHRF1 could increase TIM-3 expression caused by miR-449c-5p decline to inhibit NK cell function and induce anti-PD-1 therapy resistance in patients with HCC (199).

Targeting TME-derived exosomes in HCC has advantages and disadvantages

Communication between cells is a double-edged sword that can not only transmit information to each other to promote the occurrence and development of tumors and drug resistance but also provide treatment targets. In fact, some exosome-mediated RNAs have shown satisfactory potential in relieving therapy-resistant stress. MiR-199a-3p, miR-744, and miR-122 are downregulated in HCC tissues, and chemotherapy resistance in HCC has been successfully reversed through exosome transport (200–202). Furthermore, exosomes as drug carriers have great advantages and a good precedent for the treatment of HCC patients. First, compared to artificial liposomes, exosomes have higher packaging efficiency and stability. Second, exosomes can reduce immune responses *in vivo*. Third, because of the specific molecules on the exosome surface, interaction with antibodies or coagulation factors is limited, thereby reducing the occurrence of immune responses *in vivo* (203). Notably, several major applications in exosome packaging include small-molecule chemical drugs, proteins, peptides, and nucleic acid drugs. Zhang et al. designed a neutrophil-derived exosome-like vesicle loaded with doxorubicin and decorated it with superparamagnetic iron oxide nanoparticles, which showed precise targeting in gastric cancer, hepatoma, and colon cancer cell lines (204). Another exosomal miR-155 inhibitor markedly improved cisplatin susceptibility in a cisplatin-resistant oral squamous cell carcinoma 3D model by decreasing MET and drug efflux transporter proteins (205). Although great progress has been made over the last few decades and multiple preclinical trials show promising results, there are still obstacles to be overcome (206). For example, there is a lack of standardized exosome isolation and purification techniques. Traditional separation techniques are often contaminated by other types of exosomes, which can significantly affect therapeutic efficiency. Second, thorough and accurate research on exosome characterization is required because exosomes from different cell sources may have opposite therapeutic effects. These technical barriers must be addressed so that exosomes can be used for the diagnosis or prognostic monitoring of cancer and innovative, personalized exosome-based therapies.

Organoid models for better understanding therapeutic resistance in HCC

An organoid is a type of three-dimensional micro-organ cultivated *in vitro*, which has a complex structure similar to real organs and can partially simulate the physiological function of the source tissue or organ. In early 2009, Clevers et al.

constructed the first mouse gut organoids with single sorted Lgr5 (+) stem cells, which maintain the ability of self-renewal and maintain the villous structure of the intestinal gland fossa (207). Over the past five years, research on human-derived organoids in preclinical platforms has developed rapidly. Compared with two-dimensional (2D) cell line cultures, organoids can mimic tumor heterogeneity, cell-cell interactions, and cell-extracellular matrix communication, although not completely (208). Compared to the disease mouse model, organoids have physiological and pathological characteristics that are unique to humans (209). Based on these advantages, organoids can provide more possibilities for personalized therapy, not only to verify the mechanism of the microenvironment in tumors *in vitro*, but also to facilitate the progress of new drug screening. In addition, the fact that many drugs show positive results *in vitro* or in mouse models followed by negative effects in clinical trials underscores the importance of exploring realistic hepatocellular organoid models to simulate efficacy.

Maturation of human hepatocyte organoids

Since the first attempt to construct a 3D cell culture system in the 1990s, the development of human hepatocellular organoids has gradually matured (210). Organoid material sources range from mice to humans, and the maintenance of organoid function increases from a few days to eight months. The research scope of organoids in patients with liver cancer has gradually expanded, including exploration of the mechanism of microenvironmental drug resistance, drug screening, and personalized treatment. Table 4 shows several typical examples of the maturation process of hepatocellular organoid development.

Contributions to therapeutic resistance in HCC microenvironment

The most common method for organoid culture of liver cancer is to transport liver cancer patient tissues back to the laboratory for 3D culture (218). Organoid structures contain a variety of specific cell types and their spatial structures are similar to those of their corresponding tumor tissues. With improvements in physiological modeling methods, organoid culture techniques can be combined with *in vitro* TME techniques to maintain diverse cell populations. The cultivation system of organoids simulating the tumor microenvironment, screening for sensitive clinical drugs, and achieving precise individualized medicine are three aspects of great significance in exploring clinical drug resistance in patients with HCC (Figure 2).

Co-culture of HCC organoids with matrix cells or extracellular cytokines helps explain the effects of the TME on HCC growth. In 2017, Wang et al. constructed HCC organoids and organoids with fibroblasts and endothelial cells. Immunofluorescence results showed that the addition of non-parenchymal cells greatly enhanced the expression of EMT-related molecules (MMP9, vimentin, and TGF-B), tumor-related

TABLE 4 The maturation process of hepatocellular organoid development.

Cellular source	Characteristics	Meaning	Reference
Single mouse Lgr5+ liver stem cells	Such clonal organoids can be induced to differentiate *in vitro* and to generate functional hepatocytes upon transplantation into Fah(-/-) mice.	Mark the first organoid from murine	(211)
Adult hepatic duct cells	Clonal organoids are genetically stable	Mark the first organoid from human	(212)
Commercially purchased HCC cells	Initiating the co-culture with non-parenchymal cells such as fibroblast and endothelial cells	Demonstrate the importance of microenvironment on the composition of HCC organoids	(213)
Healthy liver resections derived organoids	The organoid can identify different tumor tissue and subtypes and preserves the histological architecture, gene expression and genomic landscape of the original tumor	Identify the ERK inhibitor SCH772984 as a potential drug for HCC	(214)
Needle biopsies from HCC patients	HCC organoids maintain the genomic features of their originating tumors during long-term culturing for up to 32 weeks.	Illustrate the function of testing sensitivity to sorafenib and providing a tool for developing tailored therapies.	(215)
Reprogrammed human hepatocytes (hiHeps)	We employed hiHeps to establish an improved organoid model possessing liver architecture and function	Prove c-Myc-induced human HCC initiation was associated with the alteration of MAMs* and RAS-induced lineage conversion from hepatocytes to ICC* cells can be prevented by the combined inhibition of Notch and JAK–STAT	(216)
Distinct regions of liver tumor	A total of 27 liver cancer lines were established and 129 cancer drugs were tested	Demonstrate the usage of cancer organoid drug testing as part of a drug discovery pipeline	(217)

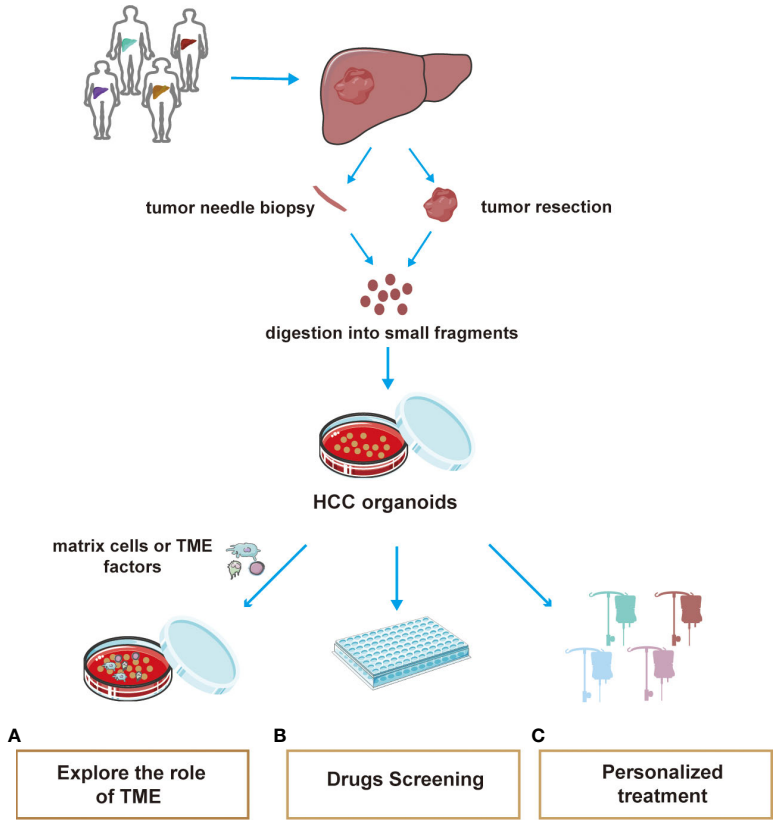

FIGURE 2
A summary of the main applications of HCC organoid. The tissue used in organoid culture of hepatocellular carcinoma is mostly obtained by pathological biopsy and surgical resection. Tissues were cultured for 3D using special media. At present, the application of organoids mainly includes three aspects: **(A)** Co-culture of HCC organoids with matrix cells or extracellular cytokines helps explore the effects of the TME on HCC progression or therapeutic resistance powerfully. **(B)** Efficient culture organoids are suitable for high-throughput drug screening **(C)** Organoid drug screening helps to develop individualized treatment regimen for different patients.

inflammatory factors (TNF-α, CXCL12, and CXCR4), and neo-angiogenesis-related markers (VEGF, VEGFR2, and HIF-α), which are important factors in the tumor microenvironment for tumor growth and drug resistance (213). This was a very successful integration of microenvironment and organoid models. Furthermore, to demonstrate the effect of FSTL1 secreted by fibroblasts in the HCC microenvironment on drug resistance in HCC, Loh's team constructed the patient-derived HCC organoid model and used FSTL1 overexpressing conditioned medium to culture the organoids. The results showed that FSTL1 enhanced the ability of hepatocytes to resist sorafenib through the AKT/mTOR/4EBP1/c-MYC signaling axis. In the mouse model, the administration of FSTL1 antibody enhanced the sensitivity of sorafenib, demonstrating the accuracy of *in vitro* organoid assays (219). Although research on the organoid-TME model in liver cancer has not been popularized, there is no doubt that this model provides novel ideas and important potential for the study of the mechanisms of drug resistance in the HCC microenvironment.

The organoid model has a short culture cycle and high success rate, which is conducive to high-throughput drug screening (220). Broutier et al. found that cultured HCC organoids were insensitive to BRAF and/or MEK inhibitors. However, in a patient-derived organoid, SCH772984 selectively inhibited ERK phosphorylation and significantly inhibited tumorigenesis (214). Numerous studies have demonstrated that the TME is greatly involved in the development of drug resistance in patients with HCC. Therefore, when drug resistance occurs in clinical patients, organoids can be cultured *in vitro* by biopsy puncture or surgical resection and then the drug-sensitive patients can be screened in batches.

Personalized treatment can be achieved through organoid drug screening. Recently, some patient-derived organoids from different individuals cultured with hydrogel capsules, which can simulate the TME of liver cancer, were tested for sensitivity to cabazitaxel, oxaliplatin, and sorafenib. As a result, sensitivity was found to differ among individuals. Magnetic resonance imaging and biochemical examinations were used at later follow-up to

test the cost-effectiveness of organoid screening drugs for patients (221). Therefore, it is a promising platform for realizing economical personalized treatment. Currently, several preclinical studies on HCC organoids are underway (NCT05384184, NCT02436564, and NCT02718235).

Conclusion and future directions

In this review, we have mainly discussed the relationship between the microenvironment and drug resistance associated with liver cancer treatment from various aspects and summarized the targets and directions of future treatment of liver cancer. Currently, the standard treatments for hepatocellular carcinoma remain surgical resection, radiofrequency ablation, TACE, and systemic therapy. Due to drug resistance and high recurrence rates, most patients cannot benefit from existing therapies. Especially in cases of HCC with high heterogeneity, it is even more difficult to precisely target biomarkers. Accumulating evidence has shown that the interaction between tumor cells and the tumor microenvironment is crucial for tumor cell survival, proliferation, acquisition of stem cell characteristics, invasion, metastasis, and drug resistance. Therapies targeting the TME represent a breakthrough in addressing therapeutic resistance. Accordingly, most researchers have suggested that combination therapy may be the mainstay treatment for HCC in the future, including ICI, lenvatinib, sorafenib, and targeted therapy.

However, due to the heterogeneous subpopulations that develop during the process of tumor progression, some significant markers such as TERT, TP53, ARID2, ARID1A, and the WNT signaling regulator CTNNB1 cannot be targeted efficiently, which reduces the utility of the predictor from a treatment perspective. Moreover, some drugs perform efficiently *in vivo* and *in vitro* but do not work in clinical settings. These facts highlight the importance of accurate preclinical model prediction. In recent years, human-derived organoids have made great progress in many cancers, which supplements the lack of microenvironmental influence and anthropogenic models compared to traditional 2D and mouse xenograft models. Currently, we have identified heterogeneous stem cell populations and tested hundreds of drugs developed using HCC organoids. Although more time and resources are required to culture organoids *in vitro* than cancer cell lines, the development potential of organoids is substantial. The use of more accurate 3D models to explore microenvironment-targeted therapy is a promising prospect for hepatocellular carcinoma.

Author contributions

YQ designed this study and carefully reviewed the manuscript. JZ and HH participated in the design and wrote the first manuscript. JZ and LW were mainly responsible for the design of the images. WW and MY were involved in the process of document collection and review. All authors contributed to the article and approved the submitted version.

Funding

The study was supported by National Natural Science Foundation of China (no. 81872264).

Acknowledgments

Thanks to Peiming Yan and Daidi Zhang for their support during this study.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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