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Review

Developing Potent Therapeutics for Liver Cancer Chemoresistance via an RNA Nanotech and Series-Circuit-Christmas-Bulb Mechanism Targeting ABC Transporters

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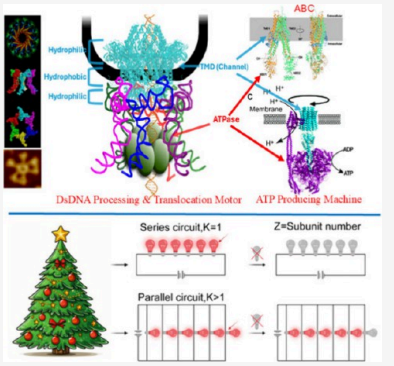
ABSTRACT: Liver cancer, particularly hepatocellular carcinoma (HCC), poses significant treatment challenges due to chemoresistance and cancer recurrence. Similar to customs at the border, the liver detoxifies incoming chemicals via efflux pumps and overexpresses ATP-binding cassette (ABC) drug exporters, leading to chemoresistance. ABC contains a multihomosubunit structure and a revolving transport mechanism, actively effluxing drugs from cancer cells, thereby reducing intracellular drug accumulation and therapeutic efficacy. Based on the understanding of the dsDNA translocating mechanism and complete inhibition observed in the multihomosubunit-revolving ATPase of the Phi29 DNA packaging motor, we report here an unprecedented approach to develop a potent HCC drug mimicking the series-circuits of "Christmas light bulbs", as described by the calculation formula: $x = (p+q)^z = \sum_{M=0}^{Z}\left(\frac{Z}{M}\right)p^{Z-M}q^{M} = \sum_{M=0}^{Z}\left(\frac{Z!}{M!\,(Z-M)!}\right)p^{Z-M}q^{M}$. RNA nanotechnology offers a novel "Christmas light bulb series circuit" strategy, inspired by the Phi29 hexameric RNA-driven DNA packaging motor, in which targeting a single subunit completely inhibits the entire cassette. The concept has been validated by delivering Paclitaxel and miRNA via RNA nanostructures to inhibit the homomeric multisubunit ABC drug efflux pump P-gp in HCC in a mouse model. The programmable and multivalent nature of RNA nanotechnology enables the codelivery of multiple high-payload therapeutics, combined with liver-targeting ligands such as GalNAc, thereby achieving synergistic anticancer effects. This review highlights the mechanistic insights into potent HCC drug design, the advantages of RNA nanotechnology, and the structure–function relationship of ABC and other ATPase transporters, emphasizing a targeted strategy to overcome chemoresistance in liver cancer.



KEYWORDS: *Hepatocellular Carcinoma (HCC), Drug Delivery, RNA Nanotechnology, Nanoparticle Design, ABC Drug Efflux Pump, Synergetic Therapy, Exosome-Based Delivery, Series-Circuit Mechanism, GalNAc*

1. INTRODUCTION

The liver is a multifaceted organ responsible for many critical physiological functions. Hepatocytes, its primary cells, act as hubs for complex differential processes including exogenous signal gradients, cellular localization cues, and a hierarchy of transcription factors directed toward detoxification, energy regulation, metabolism, and numerous other functions.[1] It is a marvel that such a myriad of molecular mechanisms can coalesce in a single highly active organ, albeit not without the potential for dysregulation, which can result in disease, cancer, or chemoresistance. This diverse and often interconnected nature presents significant challenges in identifying and targeting the molecular mechanisms in physiological conditions. This complexity is reflected globally, where liver cancer ranks as the seventh most commonly diagnosed malignancy and the third leading cause of cancer-related mortality worldwide. In 2020, there were approximately 906,000 new cases; despite the alarming number of cases, therapeutic options remain limited and treatment failure rates are high (Figure 1).[2,3]

The underlying risk factors of HCC are strongly associated with chronic liver disease, inflammation, alcoholic liver disease, hepatitis C infection, hemochromatosis, and aflatoxin B1-

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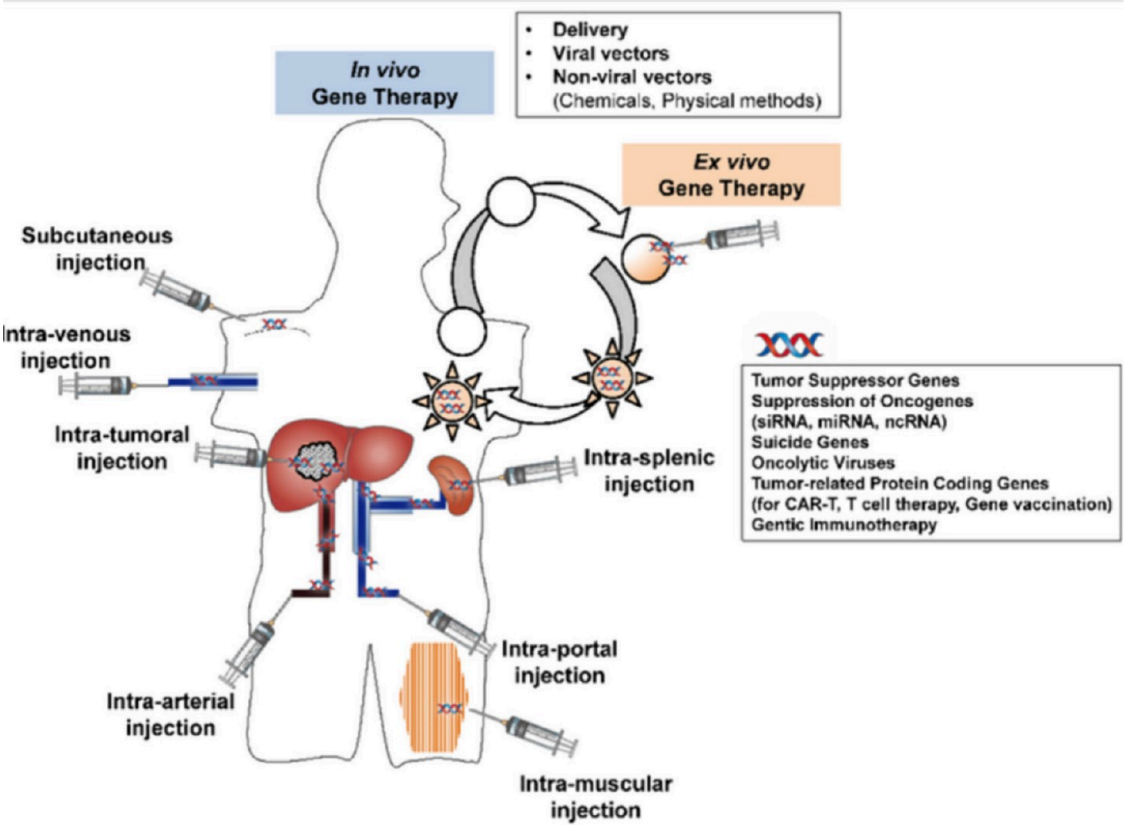

Figure 1. The liver is a key organ for metabolism, protein synthesis, detoxification, and endocrine function, and among liver diseases, including hepatitis, cirrhosis, malignant tumors, and congenital disease, liver cancer is one of the leading causes of cancer-related deaths worldwide. Adapted with permission from ref 4. Copyright 2019, the authors, under CC BY-4.0 https://creativecommons.org/licenses/by/4.0/.

induced toxicity.[5−7] Diabetes has also been associated with HCC due to the carcinogenic properties of antidiabetic agents such as sulfonylureas and insulin.[8,9] Additionally, other conditions related to obesity, such as biliary cirrhosis and autoimmune hepatitis, have shown a strong correlation with HCC.[7]

On a histological level, liver cancer/hepatic tumors are commonly subclassified as either focal nodular hyperplasia (FNH), cholangiocarcinoma (CC), hepatocellular adenoma (HCA), hepatocellular carcinoma (HCC), or combined HCC−CC.[10] Among these, HCC is the most common form of liver cancer and a major focus of clinical research, with its incidence being four times higher in males than in females.[5] Therapeutic options for HCC depend on multiple factors, including tumor stage, patient condition, liver functional reserve, and the extent of liver damage.[11,12] Several treatment options exist for early-stage liver cancer (Figure 2) with surgical resection and liver transplantation as primary approaches. Additional therapies include absolute ethanol injection, trans-arterial chemoembolization (TACE), trans-arterial radioembolization, and local ablation therapies like radiofrequency ablation (RFA) and microwave ablation (MWA).[7,9,11] Early stage HCC diagnosis only accounts for 20% of cases, with over 70% of patients who undergo surgical resection facing recurrence within 5 years.[13,14] Despite advancements in outcomes following liver resection and liver transplantation, the effectiveness of these treatment approaches remains largely confined to early stage hepatocellular carcinoma.[15]

For patients with intermediate or advanced-stage HCC (Figure 2), systemic therapies (such as immunotherapy, gene therapy, molecularly targeted therapies, etc.), TACE, and trans-arterial radioembolization (TARE) remain the only available options given the limited regenerative abilities of the liver.[11,16,17] TACE and TARE are mainly used for intermediate-stage HCC. However, due to suboptimal chemotherapeutics, TACE has shown limited efficacy in improving patient survival. The liver's natural drug resistance properties and dysfunctional drug delivery capability when cancerous result in chemically insensitive tumor environments.

Systemic therapies have considerably increased over the past decade and challenge conventional strategies for treating advanced-stage HCC (Figure 3).[17] This treatment model is less invasive than TACE and can be accomplished through intravenous or oral methods. Systemic therapies mainly consist of molecular targeting drugs such as Tyrosine Kinase Inhibitors (TKIs), immunotherapeutic drugs (Immune Checkpoint Inhibitors (ICIs)), monoclonal antibodies (mAbs), and gene therapy via RNAi (siRNA and miRNA).[18,19] To date, gene therapy has the potential for versatility in combinatory treatments. However, a central barrier to effective therapy is multidrug resistance due to the role of the ATP-binding cassette (ABC) transporter, which mediates multidrug resistance in liver cancer, involving an ATPase mechanism akin to a biological motor.[38] Inspired by this, we introduce a series-circuit "Christmas light bulb" strategy, in which targeting a single subunit can halt the entire transporter complex, providing a novel approach to overcoming chemoresistance.

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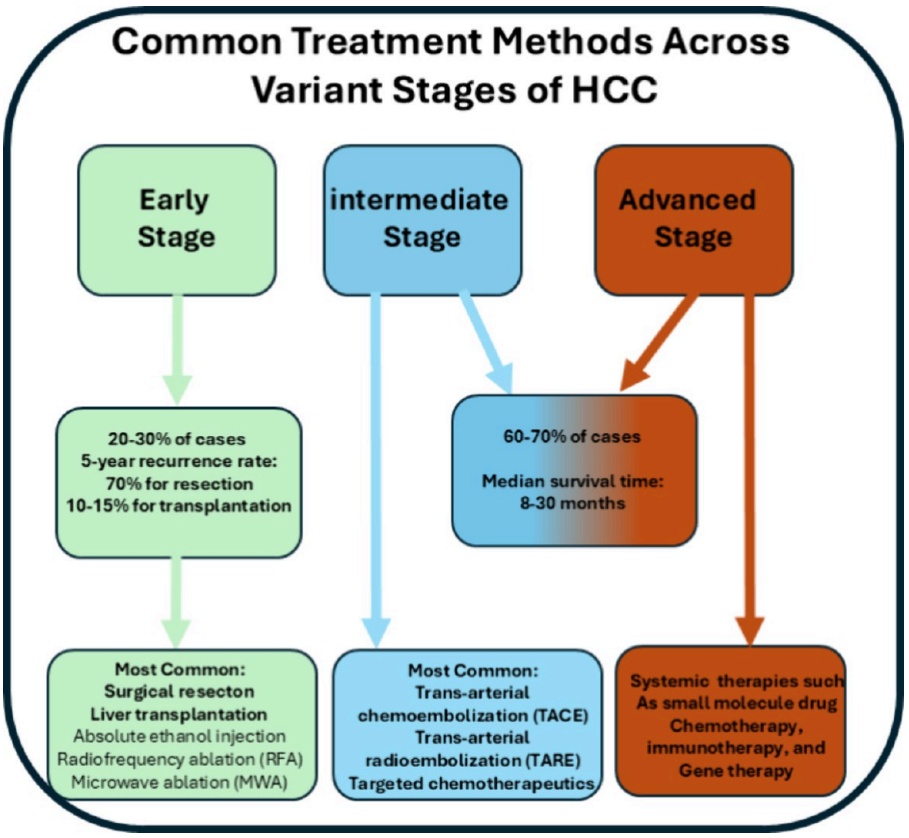

Figure 2. General schematic of current treatment strategies for HCC, based on the stage.

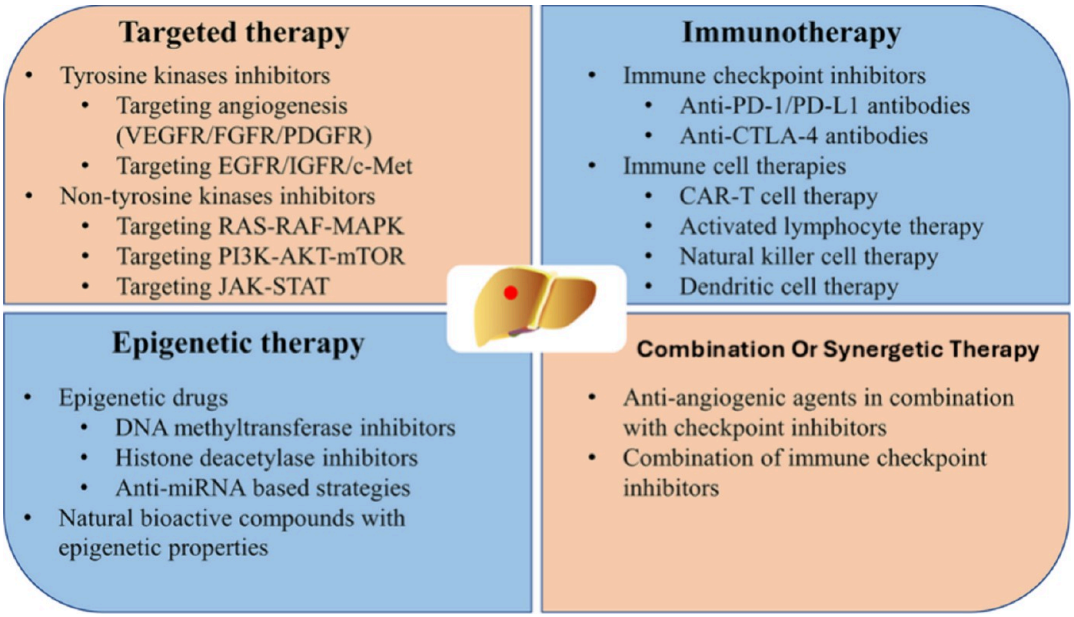

Figure 3. Recent advances in systemic therapy for hepatocellular carcinoma (HCC). Adapted with permission from ref 20. Copyright 2021, Springer Nature.

The review explores the current landscape of chemotherapy and chemoresistance in liver cancer with a focus on overcoming drug resistance. Herein, the ABC (ATP-binding cassette) transporter plays a key role in liver cancer resistance and has a similar structure and function to ATPase biological motors.[38] This Review discusses systemic therapy options, the molecular basis of multidrug resistance, reporter applications of siRNA and miRNA, and the potential of RNA nanotechnology to overcome current therapeutic limitations, enabling precise, multivalent delivery of anticancer agents. Specifically, RNA nanotechnology allows the design of programmable nanoparticles that codeliver chemotherapeutics, regulatory RNAs, and targeting ligands such as GalNAc to achieve synergistic

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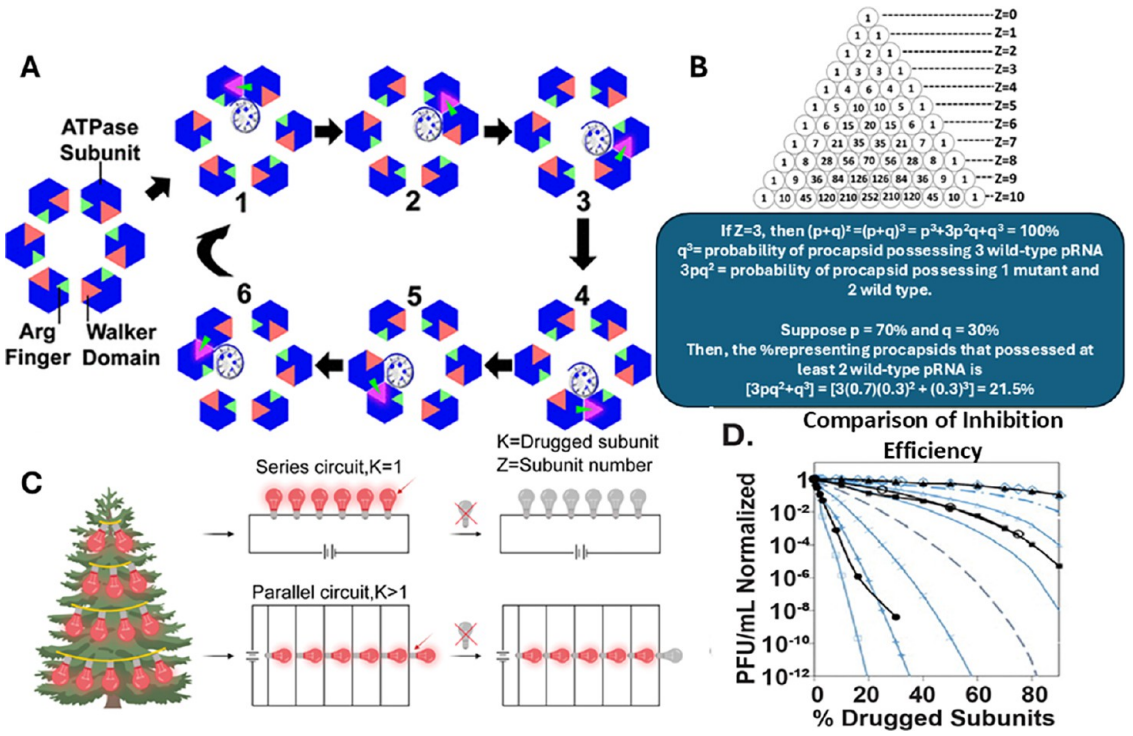

Figure 4. Sequential action and inhibition kinetics in multimeric viral DNA packaging motors and analogy with a series circuit breaking mechanism. A) Illustrating the sequential activation of ATPase homosubunits, facilitated by Arg finger and Walker domain interactions, drives progressive steps during viral DNA encapsulation. Steps 1 through 6 depict the orderly progression of motor action. Adapted with permission from ref 34. Copyright 2019, American Chemical Society. B) Pascal or Yanghui's triangle-based statistical model demonstrating inhibition scenarios, highlighting the distribution and probability calculations for mutant subunits. Example calculation provided for the scenario $Z = 3$, with given probabilities of wild-type and mutant subunit and inhibition rate calculation. Adapted with permission from ref 35. Copyright 1997 American Society for Microbiology. C) Analogy to series and parallel circuit configurations illustrated using strands of Christmas tree lights. Series circuits ($K = 1$) reflect sequential dependency wherein failure of a single bulb disables the entire strand, paralleling the sequential mechanism of the multimeric motor. Conversely, parallel circuits ($K > 1$) demonstrate robustness, with individual bulb failures minimally impacting overall function, contrasting the sequential motor model. D) Blue curves represent theoretical curves, and black curves represent experimental data, with the top curve representing a lower Z number and the lower curves representing a higher Z number. Adapted with permission from ref 35. Copyright 1997 American Society for Microbiology.

inhibition of ABC transporters and enhance drug efficacy in overcoming the shortcomings of current treatment strategies.

2. CHEMOTHERAPY OF LIVER CANCER

2.1. The Chemotherapeutics for Liver Cancer. The most common chemotherapy drugs for treating liver cancer include Gemcitabine (Gemzar), Oxaliplatin (Eloxatin), Cisplatin, Doxorubicin (PEGylated liposomal DOX), 5-fluorouracil (5-FU), Capecitabine (Xeloda), and Mitoxantrone (Novantrone). Sorafenib and Lenvatinib are the first-line drugs for systemic therapy of HCC, with Sorafenib targeting multiple kinases (e.g., Raf-MEK-ERK pathway, VEGFR, PDGFR, Ret, FLT3, and c-Kit).[11,21−23]

The limited effectiveness of chemodrugs in HCC is caused not only by the tumor's high chemoresistance but also by the restricted applicability of most chemotherapy regimens, which are limited by the presence of underlying liver disease. For a long time, no systemic standard of care has been available for patients with advanced HCC. Most HCCs have prevalent mutations affecting the TERT promoter, TP53, and the Wnt/β-catenin signaling pathway, which are still unsuitable for routine therapeutic targeting. Meanwhile, the established therapeutic targets in other tumors could only be identified in several of HCC cases.[10]

2.2. Side Effects and Mechanism of Chemoresistance in Liver Cancer. Chemotherapy can be administered either systemically or regionally. Systemic chemotherapy, administered intravenously or orally, circulates throughout the body and is effective for metastatic cancers but toxic to normal tissues. It is typically given in 2- to 3-week cycles, followed by rest periods for recovery.[5] On the other hand, regional chemotherapy, such as hepatic artery infusion (HAI), delivers the drug into an artery and continuously delivers it through a pump attached to a catheter in the hepatic artery, enabling higher local drug doses to the liver tumor with reduced toxicity.[9]

While chemotherapy drugs can inhibit liver cancer proliferation, they also affect normal cells, including those in the bone marrow, hair follicles, and the lining of the mouth and intestines, leading to side effects. Common side effects include hair loss, mouth sores, loss of appetite, nausea, vomiting, diarrhea, increased infection risk, easy bruising or bleeding, and fatigue. The severity of these side effects depends on the drug type and dosage. In some cases, drug doses may need to be reduced, delayed, or discontinued to prevent worsening side effects.[6,24]

Beyond these side effects, drug resistance in HCC is often driven by multiple cellular and molecular mechanisms. Overexpression of ATP-binding cassette (ABC) transporters,

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including P-glycoprotein (P-gp/ABCB1), breast cancer resistance protein (BCRP/ABCG2), and multidrug resistance-associated proteins (MRPs), actively expels chemotherapeutic agents from tumor cells, decreasing intracellular drug accumulation and effectiveness.[25] [26]The tumor microenvironment (TME), characterized by hypoxia, fibrosis, and immunosuppression, further promotes chemoresistance by activating hypoxia-inducible factors (HIFs), encouraging angiogenesis, impairing drug penetration, and triggering pro-survival signaling pathways like PI3K/AKT/mTOR.[26−29] Chemoresistance is also influenced by the regulation of genes involved in apoptosis, drug transport, and cell cycle control.[26,30] A thorough understanding of these mechanisms offers opportunities to improve treatment strategies, including combining chemotherapy that inhibits ABC transporters and RNA-based therapies, to boost treatment effectiveness while reducing systemic toxicity.[25,26]

3. MIMICKING THE SERIES-CIRCUIT-CHRISTMAS LIGHT BULB MECHANISM TO DEVELOP POTENT DRUGS TARGETING HOMOMULTIMERIC REVOLVING MACHINES

3.1. Study on the Asymmetrical Homohexameric ATPase Motors with a Revolving Mechanism Leads to a New Concept in Potent Drug Development. Drug discovery is a multidisciplinary science that includes the fields of medicine, biotechnology, and pharmacology.[31] While effort has been focused on screening new drug compounds, uncovering new drug targets, and functional pathways, less attention has been given to novel design strategies. We propose that drug inhibition efficiency depends on the stoichiometry of the biocomplex or biomachine that serves as the drug target. This concept defines stochiometry as the number of identical subunits comprising the complex rather than the traditional drug-to-target ratio. Herein, Phi29 was used as a viral component with defined variable stoichiometries as model drug targets to test the hypothesis. *In vitro* and *in vivo* assembly assays were conducted to compare inhibition efficiencies across targets with different subunit stoichiometries. Inhibition efficiency was analyzed using Yang Hui's (Pascal's) Triangle, as shown below.[32,33]

$$x = (p + q)^z = \sum_{M=0}^{z} \binom{Z}{M} p^{Z-M} q^M = \sum_{M=0}^{z} \left(\frac{Z!}{M!(Z-M)!} \right) p^{Z-M} q^M$$

Z represents the number of the homosubunits in the biomotor (e.g., ABC) complex, with $Z > 1$; K is the number of drug inactivated subunit in the complex, and $K = 1$; p is the total number of drugged subunits, q is the total number of undrugged subunits, and $p + q = 1$ (100%; M and N represent the motor number involved, and $M + N = 1$.

Drug inhibition depends on the ratio of the drug to the nondrugged complex (Figure 4). For $K = 1$ and $Z > 1$, inhibition efficacy follows a power function of Z, leading to increased potency, since inhibition of any subunit results in complete inhibition. For a single-subunit target, inhibition efficiency is proportional to the substrate targeting efficiency (p), making it first-order.[36−38,25] If the complex has $Z > 1$ identical subunit, then one inactivated subunit ($K = 1$) is sufficient to inhibit the whole system. The fraction of active (uninhibited) complexes is q^Z, where q is the undrugged subunit. Thus, inhibition efficiency is $1-q^Z$, with $1-q$ is the drugged fraction.

3.2. Mimicking the Series-Circuit-Christmas-Bulb Mechanism for Potent Drug Effects. *3.2.1. Cooperativity in Multiunit Biocomplexes Leads to High Inhibition Efficacy.* Drug inhibition efficiency is enhanced by the synergy of multisubunit complexes, where inactivating a single subunit can disable the entire complex.[39−41] This mechanism is analogous to the difference between parallel and series light bulbs. In a parallel circuit, the failure of one bulb does not affect the rest, whereas in a series circuit, the failure of one bulb disables the entire system. This concept implies that drug binding to one subunit is sufficient to inhibit the function of the entire complex, for example, in the case where the probability for $Z = 6$ and $K = 1$. A total of six subunits ($Z = 6$) are required for complex formation. Still, the presence of even a single drugged subunit ($K = 1$) is sufficient to inhibit its function, rendering all complexes containing 1 to 5 drugged subunits nonfunctional; only those composed entirely of undrugged subunits remain active. The probability of this is q;[6] therefore, the inhibition efficiency is $1-q$.[6,39,40,42] Fewer uninhibited targets result in greater efficacy. This contrasts with conventional approaches that focus solely on increasing drug binding affinity to each target.[53]

3.2.2. Inhibition Efficacy as a Power Function of Target Stoichiometry by the Phi29 Viral Assembly System. The hypothesis that drug inhibition efficiency follows a power function of the target stoichiometry has been proved using the Phi29 viral assembly system.[54] This well-defined *in vitro* assembly system comprises four components, comprised of different subunits that can act as the nanomachine target. Inhibition of viral assembly is achieved using mutant components that represent drugged target components.[32,43−46]

The entire Phi29 genome consists of 19,400 base pairs, with 6 copies of packaging RNA and 6 of ATPase protein gp16 (Figure 4). It is estimated that more than 10,000 ATP molecules are required to package a Phi29 genome. The hexameric structure of Phi29 pRNA has been extensively demonstrated through single-molecule techniques, AFM imaging, pRNA crystal structure determination, and statistical analysis. Similarly, the hexameric nature of Phi29 gp16 has been confirmed by native gel binding, capillary electrophoresis assays, Hill constant measurement, and titration of mutant subunits using binomial distribution.[27,35,36] The availability of a motor system with multiple well-defined and characterized components makes Phi29 an ideal model for studying the drug inhibition efficiency. The highly sensitive in vitro Phi29 assembly system was used to assess the effectiveness of drugs targeting multisubunit complexes and to validate a new method for developing potent drugs.[39,47−53]

3.3. Targeting Biocomplexes for Developing Potent Drugs. In the history of drug development, stoichiometry is a critical property of most channel protein receptors. Many channel proteins, including most G-protein-coupled receptors (GPCR) proteins are expressed as dimers or oligomers on cell membranes.[54] Targeting of GPCR hetero- and homooligomers is generally considered for drug development, prompting the new development of models for multisubunit protein binding. The Hill equation has characterized binding affinities between ligand and multisubunit targets.[39,54]

Another target, the ATP-sensitive homotrimeric P2X7 receptor (P2X7R), acts as a ligand-gated ion channel. It

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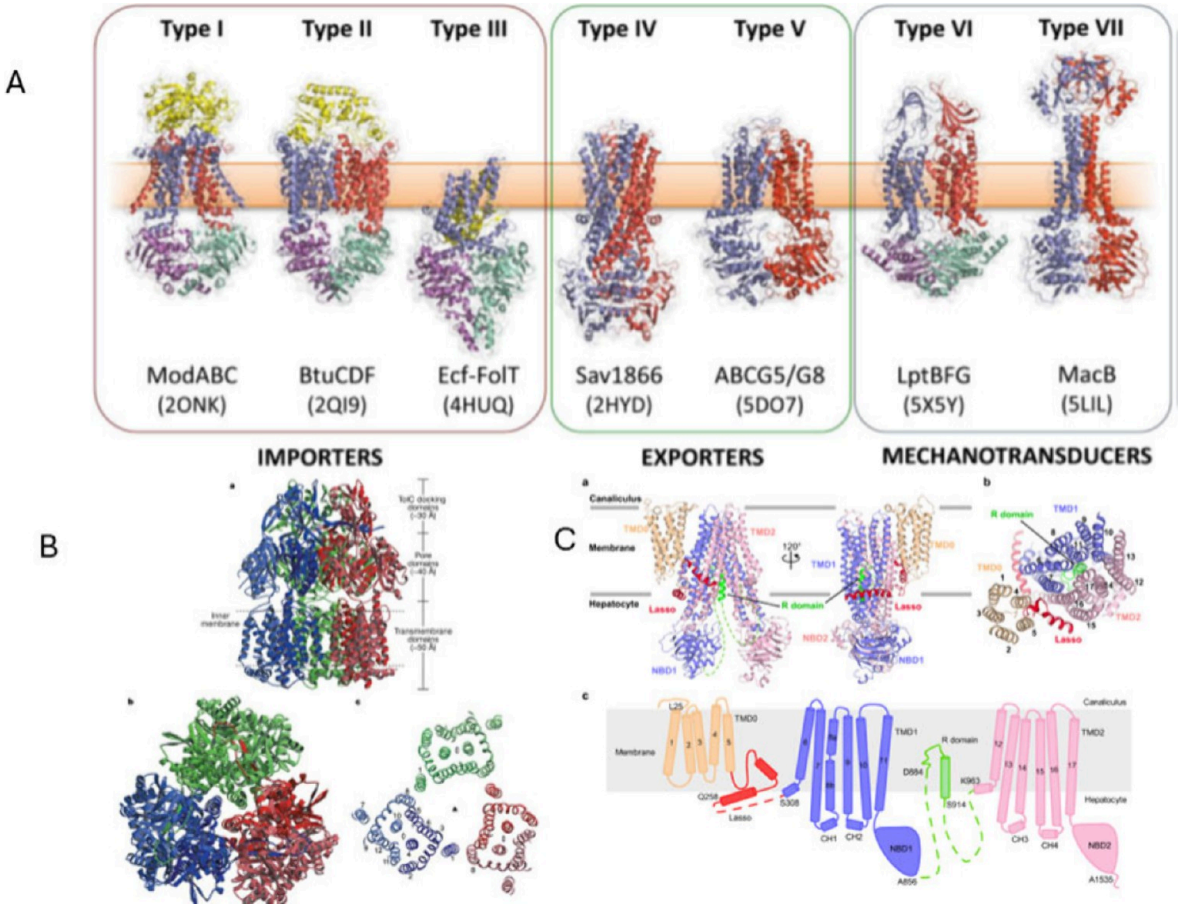

Figure 5. A) The cryo-EM structure of the *E. coli* MlaFEDB complex was modeled in a membrane environment using the PPM 2.0 server. The EcMlaD hexamer is tilted at an angle of ∼16° relative to the outer leaflet of the membrane. Adapted with permission from ref 62. Copyright 2018, Frontiers, under CC BY 4.0 https://creativecommons.org/licenses/by/4.0/. (B) Electron density (2Fo−Fc) maps highlighting Leu107 residues in EcMlaD_P41212 and EcMlaD_complex, showing the geometry of the central channel. Ball-and-stick models represent Leu107 residues (D1−D6 in EcMlaD_P41212; D1′−D6′ in EcMlaD_complex), with dotted lines indicating Cα−Cα distances between adjacent protomers. Adapted with permission from ref 57. Copyright 2002, Nature Publisher. (C) Schematic representation of EcMlaD conformational changes during phospholipid transport, proceeding through four steps: (1) resting state, (2) protomer movement, (3) ligand capture, and (4) return to resting state. Adapted with permission from ref 63. Copyright 2024, Nature Publisher.

forms a chalice-like structure with three ATP-binding sites at the interface of the three subunits. Activation requires occupancy of at least two of the three sites, which is necessary for activation of the receptors, which results in the opening of the channel pore and the passage of small cations (Na^+, Ca^{2+}, and K^+). P2X7R has received particular attention as a potential drug target for its widespread involvement in inflammatory diseases and pivotal roles in central nervous system (CNS) pathology.[55]

3.4. Targeting Homomeric Drug Transporters for Drug Development. The mechanism of the drug transporter is similar to the revolution motor involves entropy-induced transitions by ATP. Targeting multidrug efflux transporters with high stoichiometry improves the chances of overcoming multidrug resistance (Figure 5B).[56,57] For example, pyridopyrimidine derivatives have been reported to be promising drugs to treat multidrug-resistant pathogens by specific inhibition of the homotrimeric AcrB and MexB transporters.[58,59] The structural architecture of ABC transporters consists of two TMDs and two NBDs. These four individual polypeptide chains combine to form a full transporter, such as in the *E. coli* BtuCD.[60,61]

4. ROLE OF THE ABC TRANSPORTER IN LIVER CANCER

4.1. The Chemoresistance Induced by the Influx/Efflux Transporter. Drug resistance is a primary cause of cancer therapy failure and can lead to low chemotherapy benefits. Multiple pathways contribute to the development of multidrug resistance (MDR), primarily through increased drug efflux or decreased cell permeability mediated by energy-dependent transport proteins.[64] MDR has been linked to drug efflux transporters, including P-glycoprotein (P-gp, ABCB1), multidrug-resistance protein (MRP, ABCC), and breast cancer resistance protein (BCRP, ABCG2). In addition, epigenetic regulations, hypoxia-inducible pathway, PI3K/AKT pathway, tumor angiogenesis, and epithelial-mesenchymal transition (EMT) also contribute to drug resistance.[27]

The ABC transporters represent the most prominent family of transmembrane proteins that bind ATP and use the energy to drive the transport of various molecules across cell membranes. MIAD is an ABC analog that shares a similar hexameric structure with the ABC transporter that is related to MDR in HCC (Figure 6).[65,66] Based on the arrangement of molecular structural components, human proteins are classified into seven distinct families from ABCA to ABCG (Figure

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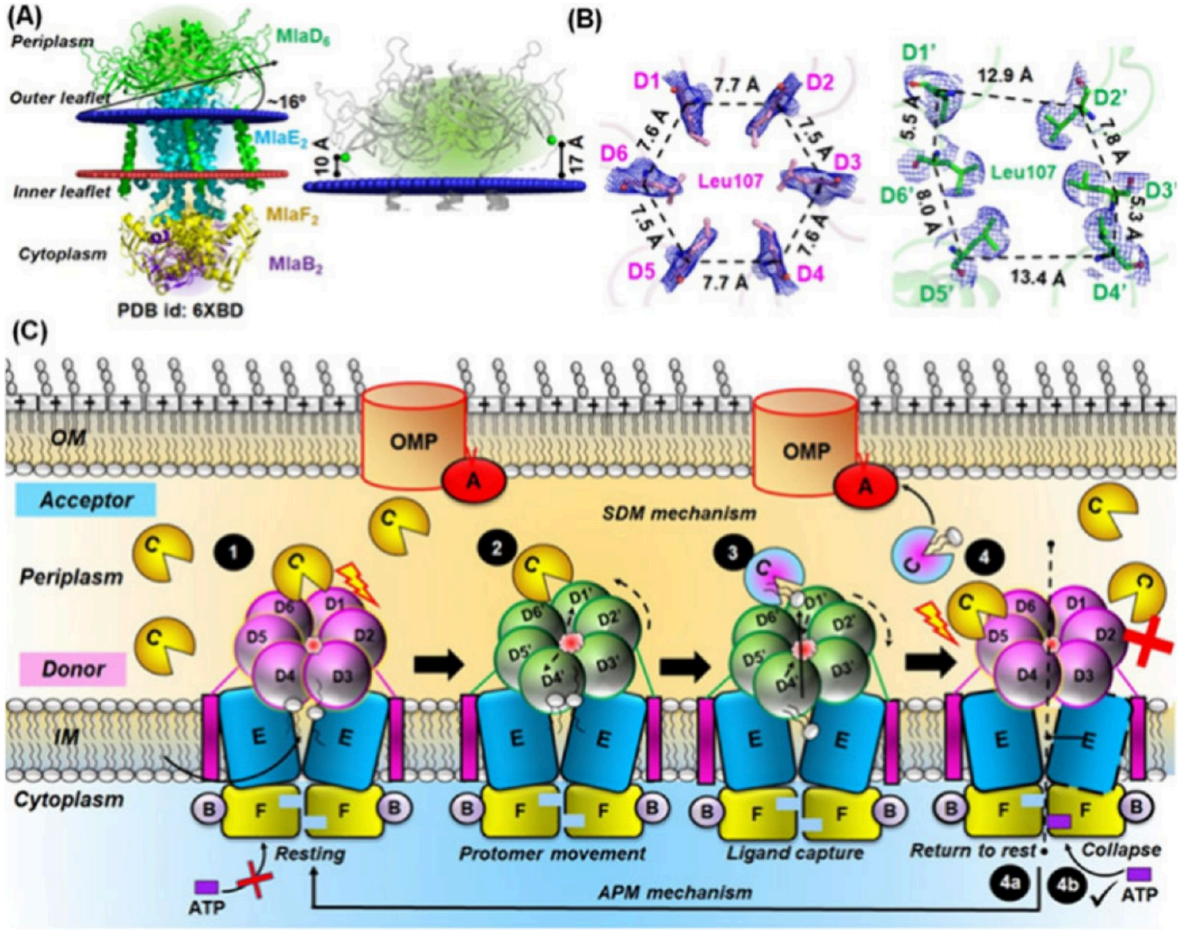

Figure 6. A) The cryo-EM structure of the *E. coli* MlaFEDB complex modeled in a membrane environment using the PPM 2.0 server. The EcMlaD hexamer is tilted at an angle of ∼16° relative to the outer leaflet of the membrane. (B) Electron density (2Fo−Fc) maps highlighting Leu107 residues in EcMlaD_P41212 and EcMlaD_complex, showing the geometry of the central channel. Ball-and-stick models represent Leu107 residues (D1−D6 in EcMlaD_P41212; D1′−D6′ in EcMlaD_complex), with dotted lines indicating Cα−Cα distances between adjacent protomers. (C) Schematic representation of EcMlaD conformational changes during phospholipid transport, proceeding through four steps: (1) resting state, (2) protomer movement, (3) ligand capture, and (4) return to resting state. Adapted with permission from ref 65. Copyright 2024, Springer.

5A).[62] Among them, ABCB1 (P-glycoprotein), ABCC1 (multidrug resistance protein 1, MRP1), ABCC2 (MRP2), and ABCG2 (breast cancer resistance protein, BCRP) are efflux transporters that are confirmed to be highly related to multidrug resistance to modulate the absorption, distribution, metabolism, excretion, and toxicity of xenobiotics.[67,68]

4.1.1. ABCB1 (P-Glycoprotein)-Mediated Chemoresistance. P-glycoprotein (P-gp), also named ABCB1 (Figure 5C) encoded by the MDR gene in rodents and humans, is an ATP-binding cassette transporter linked to drug resistance. It is expressed on the luminal surface of epithelial cells in the intestine, blood−brain barrier, adrenal gland, bile canalicular membrane in the liver, and kidney.[69,70] P-gp, with a molecular weight of ∼170 kDa, is a single-polypeptide chain and comprises two transmembrane domains (TMDs) and two nucleotide-binding domains in the plasma membrane.[71] In addition, P-gp can interact with various types and sizes of hydrophobic compounds, including natural products, linear and cyclic peptides, steroids, fluorescent dyes, and chemotherapeutic drugs.[72−74]

There are two kinds of MDR genes in humans, namely, MDR1 and MDR2. The MDR1 gene is highly responsible for multidrug resistance; the function of the closely related MDR2 gene is still unknown.[75] The intrinsic expression of the MDR1 gene is found in cancers from the kidney, liver, colon, pancreas, and adrenal. There are two well-studied examples of multiresistance in cultured cells, one is alterations in glutathione metabolism, especially glutathione S-transferase (GSTs),[76] and another is alterations in topoisomerase II,[77] which is the target for many natural products and anticancer drugs.

4.1.2. ABCC Subfamily Associated Chemoresistance. The discovery of a second distantly related ABC protein, multidrug-resistant protein 1 (MRP1, encoded by ABCC1; Figure 5) led to the identification of eight additional genes in the same ABC subfamily potentially involved in drug resistance, including MRP2 (ABCC2), MRP3 (ABCC3), MRP4 (ABCC4), and MRP5 (ABCC5).[36] MRP1 is widely expressed in tissues throughout the body, with high levels in the lung, testis, kidneys, skeletal muscle, and peripheral blood mononuclear cells, but lower expression in the liver.[78] In contrast, MRP2 shows high expression in the liver, blood−brain barrier, gut, placenta, and kidney.[79]

MRP1 and MRP2, both members of the ABC family, share 49% amino acid identity[78] and consist of five domains, including an additional NH_2-proximal MSD with five transmembrane segments and an extracytosolic NH_2-terminus, followed by the more typical four-domain core ABC transporter structure. P-gp is the primary transporter of

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drugs and confers resistance, while MRP1 and MRP2 efflux xenobiotics such as vincristine and daunorubicin through a cotransport mechanism with reduced glutathione (GSH). MRP1 and MRP2 transport metabolites of alkylating anticancer agents, including cyclophosphamide and chlorambucil.[80] MRP1 can efflux the antiandrogen flutamide, which is important for the hormonal treatment of prostate cancer.[81]

4.1.3. ABCG2-Promoted Chemoresistance. The ABCG subfamily consists of 1 half transporters that form homo or heterodimers to become active.[82] The ABCG2 is a breast cancer-resistant protein (BCRP), MXR, and ABCP, which contain one nucleotide-binding domain and one transmembrane region. Mediated unidirectional flux from the cytoplasm.[82] Like ABCB1, ABCG2 is expressed on the apical membranes of multiple healthy organs including the liver, kidney, intestine, and brain. It plays an important role in removing toxic substrates from cells; ABCG2 governs the absorption, distribution, and excretion of various clinically essential drugs.[82]

ABCG2 confers resistance to a narrower set of anticancer agents such as anthracyclines, mitoxantrone, and topoisomerase-I inhibitors (e.g., camptothecin),[83] but not to the Vinca alkaloids, epipodophyllotoxins, paclitaxel, or cisplatin.[83] Its resistance is consistent across overexpression cell lines, likely due to the three variants of BCRP/ABCG2. Similar to P-gp, and unlike MRPs, it operates independently of glutathione (GSH)[84]

4.1.4. ABC Mechanism within Liver Cancer and Therapeutic Homosubunits. Transporter-mediated uptake was first demonstrated in bile salts, revealing carrier-mediated, sodium-dependent uptake governed by Michaelis−Menten kinetics, leading to the identification of the sodium-taurocholate cotransporting polypeptide (NTCP). Transmembrane drug transporters from the ATP-binding cassette (ABC) (Figure 5) and solute carrier (SLC) families significantly influence pharmacokinetics and pharmacodynamics.[63]

In HCC, ABCs such as ABCB1 (P-glycoprotein), ABCC1 (MRP1), and ABCG2 (BCRP) are associated with MDR by actively effluxing chemotherapeutics via ATP hydrolysis.[85] The overexpression of ABC transporters is closely associated with poor treatment outcomes and disease recurrence, enabling tumor proliferation and drug resistance.[26] Additionally, ABC family members like ABCA1 and ABCG1 regulate lipid efflux and metabolism, contributing to a tumor-supportive microenvironment[26] while others (e.g., ABCB5) facilitate epithelial-mesenchymal transition (EMT), promoting invasion and metastasis..

Multidrug resistance-associated proteins MRP3 (ABCC3) and MRP4 (ABCC4) are basolateral ABC effluxers expressed in the liver and other barrier tissues, responsible for transporting various endogenous and xenobiotic organic anions, including vinca alkaloids, methotrexate, and nucleoside analogs. MRP3 primarily transports glucuronidated aiding acetaminophen detoxification, while MRP4 effluxes bile acids, uric acid, steroid hormones, and cyclic nucleotides, with its upregulation in cholestasis potentially serving as a protective detoxification response. MRP2, a canalicular ABC transporter, mediates the biliary excretion of methotrexate, etoposide, ezetimibe, and bilirubin-glucuronide, reducing the bile salt toxicity. MDR3, a phosphatidylcholine transporter, is essential for bile composition, and its inhibition by azole antifungals (e.g., itraconazole) may increase bile toxicity and cause cholestatic liver injury.

Homosubunit-targeted therapies in liver cancer inhibit ABC transporters by interfering with nucleotide-binding domain (NBD) dimerization, a critical step for ATP hydrolysis, and block the conformational changes required for substrate efflux. These therapies counter MDR in HCC and restore chemotherapeutic efficacy by disrupting this process.[26] Therapeutic strategies targeting ABC transporters in HCC aim to overcome drug resistance. Inhibitors like ceefourin-1 and MK-571 block ABCC3 (MRP3)-mediated efflux of drugs such as methotrexate, improving intracellular retention and efficacy.[86] Third-generation inhibitors such as tariquidar selectively target the homodimeric ABCB1, blocking drug efflux and enhancing intracellular retention of agents such as doxorubicin and Sorafenib, which enhances intracellular drug retention and tumor cell apoptosis.[87] Preclinical studies using murine HCC models demonstrate that subunit-specific inhibitors, such as Ko143 for ABCG2, synergize with chemotherapy to suppress tumor growth and metastasis.[88] Structural insights from cryo-EM studies further validate the therapeutic potential of disrupting NBD interfaces in ABC transporters, offering new alternatives for rational drug design.

4.2. Example of Potent Drugs That Target the Asymmetrical Homohexameric ATPase Motors.

4.2.1. Hexameric pRNA as a Target. In 1987, an RNA component was discovered in the packaging motor of bacteriophage phi29.[89] The phi29 DNA-packaging motor employs a third mechanism: a revolution without rotation. It consists of three coaxial rings of hexameric RNA, a hexameric ATPase, and a dodecameric channel.[89] Over the past decade, antiviral agents that inhibit asymmetrical homohexameric ATPase motors associated with hexameric packaging RNA (pRNA) have been shown to halt viral replication by sequentially blocking these multisubunit DNA-packaging systems.[90]

Another study in structural studies of hexameric pRNA structures in the phi29 DNA-packaging motor, has demonstrated its potential for application in nanotechnology and therapy.[91,328−331] Another discovery followed this in the high-resolution structure of the hexameric herpesvirus DNA-packaging further revealed a revolving mechanism that serves as a promising target for antiviral agents. and cancer therapy.[92,93]

Recent studies have revealed that fluoxetine inhibits enterovirus 2C AAA + ATPase at an allosteric site, stabilizing its hexameric complex and blocking replication.[94,330] The 2C protein functions as an RNA-activated ATPase with a two-stroke mechanism for targeting antiviral targets. This reveals the function of an arginine finger that drives the stepwise action of the asymmetrical hexameric ATPase motor and antiviral targeting.[95−97]

4.2.2. F1F0 ATPase. F1F0-ATPases or ATP synthases are membrane-bound enzymes that produce cellular energy by generating ATP via a proton gradient across mitochondrial, bacterial, and chloroplast membranes.[98] These enzymes are found in the mitochondria of bacteria and the thylakoid membranes of chloroplasts. They are central to the process of oxidative phosphorylation as well as photosynthesis.[99] ATP synthases are important to cellular metabolism, since they generate the majority of ATP for cellular functions. The proton gradient across membranes is involved in the ATP synthesis process, and its disruption can lead to severe metabolic effects.[99] Over the years, various drugs that target ATP

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Table 1. Overview of Representative ABC Transporter Inhibitors and Potency toward HCC

Inhibitor	Target Transporter	Inhibitor Potency (IC_{50})	Effect on HCC	Clinical Development	ref.
Tariquidar	ABCB1, ABCG2	10−80 nM	Improve doxorubicin delivery (toxicity problem)	Phase 1	117
Elacridar	ABCB1, ABCG2	100−500 nM	Combination with Lenvatinib suppresses HCC	Phase I	123
Ko143	ABCG2	25−50 nM	Enhanced doxorubicin accumulation, particularly in liver cancer[124]	Preclinical	119
Zosuquidar	ABCB1	30−70 nM	Enhancing drug uptake, apoptosis, and reducing hepatotoxicity in HCC	Phase I−III	125
MK-571	ABCC1 (MRP)	70−90 μM	Near-maximal MRP1 inhibition	Preclinical	126
Valspodar (PSC833)	ABCB1	100−200 nM	Enables to reduction of paclitaxel dose without compromising systemic drug toxicity.	Phase I	127−129
Reversan	ABCB1 and ABCC1	1−5 μM	Dual-targeting ability and low toxicity make it a promising candidate for future translation.	Preclinical	130
Fumitremorgin C (FTC)	ABCG2	1−10 μM	Dual targeting ability and low toxicity, suitable for future translation	No clinical trial (toxicity problem)	118,131
Biricodar (VX-710)	ABCB1	0.1−0.3 μM	Effectively restores drug sensitivity to vinblastine, paclitaxel, daunorubicin, and other P-gp substrates	Phase I/II	120
Sitravatinib	ABCB1	nM range	Overcomes Sorafenib resistance	Phase II	132
Imanitib	ABCB1 and ABCG2	μM range	Downregulates ABCB1 and increases doxorubicin uptake	Clinical trial	133
Nilotinib	ABCG2 and ABCB1	Not disclosed	Enhance the accumulation of paclitaxel and doxorubicin in drug-resistant tumor xenografts.	Phase I/II (CML)	134
Gefitinib	ABCG2	μM range	Enhances mitoxantrone efflux	Preclinical	135
Verapamil	ABCB1	μM range	P-gp blocker; used in HCC	Phase I/II	136
Probenecid	ABCC2	40−150 μM	Restores oxaliplatin and SN-38 uptake in HCC models	Preclinical	118

synthase have been synthesized in research and therapy to investigate cellular energy regulation and treat diseases.

Oligomycin, which suppresses mitochondrial ATP synthase by closing the proton channel in F0, prevents the passage of protons from flowing back into the mitochondrial matrix. This inhibition delays ATP production and is a powerful tool for studying mitochondrial dysfunction. The study suggested that oligomycin can disrupt the metabolic processes of rapidly dividing tumor cells, impeding their energy supply.[100] Another inhibitor, dicyclohexylcarbodiimide (DCCD) binds to rotary catalysis of the F1F0, which indicates that DCCD binds randomly and reacts to one of the 10C subunits that block proton movement and ATP synthesis.[101]

Additionally, Bedaquiline, a potent inhibitor of *Mycobacterium tuberculosis* ATP synthase, targets the bacterial F0 subunit and inhibits proton pumping and subsequent ATP synthesis, making bacteria unable to survive. Studies by Vestergaard et al. demonstrate that Bedaquiline has shown remarkable efficacy in treating drug-resistant tuberculosis.[102] Moreover, FCCP (Carbonyl Cyanide-p-Trifluoromethoxyphenylhydrazone) is a traditional uncoupler that destabilizes the proton gradient in mitochondrial membranes, resulting in uncoupling of oxidative phosphorylation from ATP synthesis.[103,104]

Further studies of ATP synthase inhibitors resulted in the discovery of atractyloside, a glycoside from a plant that is able to inhibit the ATP/ADP translocase. This enzyme complex is part of the mitochondrial F1 complex.[105,106] In addition, Gramicidin, a peptide antibiotic, has been investigated regarding its effect on the proton gradient across bacterial membranes and affecting the function of bacterial ATP synthase.[107−109]

4.2.3. ABC Drug Efflux Pump P-pg. Asymmetrical homohexameric ATPase motors, specifically those associated with ATP-binding cassette (ABC) drug efflux pumps, play a pivotal role in the active transport of various substrates, including drugs, across cellular membranes.[110] One of the transporters, P-glycoprotein (P-gp, ABCB1), is central to multidrug resistance (MDR), thus lowering intracellular drug concentrations and reducing therapeutic efficacy.[110]

Several drugs have been developed to prevent drug efflux and improve the efficacy of the treatment. Drug such as Verapamil are calcium channel blocker that inhibits P-gp and blocks the efflux of chemotherapy drugs, rendering resistant cancer cells sensitive to treatment.[111,112] Another compound called Tariquidar is another P-gp inhibitor capable of inhibiting the ATPase activity of the transporter and restoring the effectiveness of chemotherapeutic drugs such as paclitaxel and doxorubicin.[113,114] An immunosuppressant agent, Cyclosporine A, is a potent inhibitor of P-gp and other ABC transporters, enhancing the intracellular level of chemotherapeutic drugs and efficacy.[115] Elacridar, a dual inhibitor of both P-gp and BCRP, has demonstrated potential to overcome drug resistance in chemotherapeutic drugs like docetaxel and vincristine.[116]

4.2.4. Analysis of Key ABC Inhibitors. To date, multidrug resistance is a prominent obstacle that limits the efficacy of chemotherapeutic agents for HCC treatment due to the overexpress of ABC, such as ABCB1 (P-gp), ABCC (MRPs), and ABCG2 (BCRP).[25] ABC transporters actively efflux various chemotherapeutic drugs, which leads to a low efficacy of the drugs. The inhibition of ABC transporters is a promising strategy to reverse multidrug resistance (MDR) and increase its therapeutic effect.[117−120] According to the 2022 Nature Scientific Data publication comprehensive data set, at least 1,167 ABC transporter inhibitors and 604 substrate compounds have been analyzed against multiple transporters (ABCB1, ABCC1−4, ABCG2).[121] These inhibitors originated from various molecules-repurposed drugs (e.g., verapamil, flavonoids), synthetics, kinase inhibitors with off-target ABC activity, and natural products.[122] Despite their abundance, only a small minority have progressed to clinical trials, primarily due

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to issues like toxicity, poor selectivity, and unfavorable pharmacokinetics.[122]

Table 1 discusses the comparison between ABC transporter inhibitors relevant to their application in HCC.

5. RNA AS THE THIRD MILESTONE IN THERAPEUTIC PHARMACEUTICAL DRUG DEVELOPMENT

RNA nanotechnology for therapeutics has been extensively studied in recent years.[137−139] Notably, the advancement of RNA multiway junctions, including 3WJ and 4WJ, has enabled a highly stable RNA nanoparticle to overcome drug resistance.[140−142] Incorporation of the Phi29 pRNA motif into RNA nanoparticles enables precise delivery to cancer cells. When designed to target ABC transporters, these nanoparticles can impair the drug efflux mechanism, ultimately restoring chemosensitivity in resistant tumor cells.

5.1. siRNA Therapeutics to Inhibit the Chemoresistance Gene. Small interfering RNA (siRNA) with 19−23 nucleotides plays a key role in developing new therapeutics for cancer, viral disease, inherited diseases, and metabolic disease. In HCC, siRNA linked to chemoresistance, such as efflux transporters or essential proteins, to enhance and improve the sensitivity to the chemical drugs.[143−145] Table 2 lists siRNA molecules implicated in MDR in liver cancer.

HCC also arises from cumulative epigenetic mutations that disrupt the regulation of proliferation, angiogenesis, invasion, and metastasis. In addition, RNAi therapy has been employed to modulate genes involved in the oncogenic processes of HCC.[18] These include adenomatous polyposis coli (APC),[146] phosphoinositide 2-kinase. (PI3K),[147] and fibroblast growth factor substrate 2 (FRS2).[148] All of those play an important role in signal transduction pathways in HCC pathogenesis, such as VEGF,[149] FGF, Wnt/β-catenin,[150] and PI3K/AKT/mTOR[28] Pathways.

Combining chemical drugs with an siRNA-resistant gene may improve the anticancer effect through synergistic effects. One study investigated the effects of vector-based siMDR1 on the reversal of multidrug resistance in Doxorubicin (DOX)-resistant HCC could efficiently knock down the expression of MDR1 mRNA and P-glycoprotein and reverse the multidrug resistance of HCC cells.[151] Similarly, calcium phosphate-based nanoparticles were used to coload nanoplatin and siRNAi-1 and achieved considerable anticancer efficacy and counter-regulated drug tolerance.[152]

Further advancements in siRNA-based combination therapies have shown promising results in overcoming drug resistance and enhancing anticancer efficacy in HCC. For instance, calcium phosphate nanoparticles were developed to codeliver FTY720, a new immunosuppressive agent, to enhance anticancer efficacy by targeting the Beclin gene that is related to autophagy.[153] Based on these, CPP-modified lipid nanoparticles were engineered to encapsulate narciclasine, an AMPK activator, with siULK1. This strategy not only inhibited autophagy but also suppressed tumor growth and promoted apoptosis.[154] In another approach, modified bone-marrow-derived mesenchymal stem cells (BM-MSC) to produce exosomes expressing siGRP78 to deliver Sorafenib.[155] This targeted delivery sensitized resistant cancer cells to Sorafenib, effectively reversing resistance and suppressing tumor growth, invasion, and metastasis.

Additionally, investigation of the role of Sineoculis Homeobox Homologue 1 (SIX1) in regulating PTX resistance in HCC cells by affecting reactive oxygen species (ROS) and the autophagy pathway.[156] PTX treatment upregulated the expression of SIX1 and caused drug resistance. Downregulating SIX-siSIX1 increased the sensitivity to PTX, upregulated the ROS levels, and increased autophagy, which promoted cell

Table 2. Lists of siRNA Molecules Implicated in Liver Cancer

SiRNA	Function of the target gene	Pathway	ref
	Multidrug resistance		
siMDR	Drug resistance	MDR gene	151
siP-gp	Drug resistance	P-gp gene	
siBeclin	Autophagy and drug resistance	Autophagy pathway	153
siULK1	Drug resistance	Inhibit the unc-51-like kinase and the autophagy pathway	154
siGRP78	Drug resistance		155
siSIX1	Drug resistance	ROS and the autophagy pathway	157
siUBC9	Drug resistance and apoptosis	ERK1/2 and p38 and Bcl-2 pathway	158
	Proliferation		
siEGFR	Proliferation and apoptosis	EGFR/HER3	158
siSurvivin	Proliferation, apoptosis, and invasion	Apoptosis protein	158
si-β-catenin	Proliferation	Wnt/β-catenin pathway	30
si-FOXA1	Proliferation and apoptosis	FOXA1	30
si-c-Met	Proliferation and metastasis	c-Met	
si-HES5	Proliferation and apoptosis	Repress the transcription of Hash1	54, 165
siTBLR1	Proliferation, apoptosis, and angiogenesis	Wnt/β-catenin pathway	167
siAEG-1	Proliferation	Retinoic acid induced gene expression	168
siCXCL1	Proliferation and apoptosis	STAT3, NF-κB, and HIF-1	169
siMucin-1	Proliferation and tumorigenesis	JNK/TGF-β pathway	170
siMIF	Proinflammatory and immunoregulator	Cyclin D1	171
siRRM2	DNA replication and proliferation	p21	172
siKSP	Mitosis	Kinesin spindle protein	173
siKNTC2	Proliferation	Phosphorylation of HH3 at serine 10	
siRTKN2	Antiapoptotic	GEPase effector	175
siE2F1	Cell cycle arrest		
siSphk2	Proliferation		177, 178
siGPC3	Apoptosis	Cyclin D1	179
	Angiogenesis		
siVEGF	Angiogenesis	Inhibit the microvessel formation	183
siHIF-1α	Improve drug sensitivity and angiogenesis	Hypoxia inducible and VEGF pathway	184
	Invasion, migration, and metastasis		
SiNotch-1	Metastasis	Notch-1/Snail1/E-cadherin pathway	186, 187
siGP73	Invasion, metastasis, and EMT	Golgi protein 73	188
siCXCR7	Migration and invasion	STAT3 pathway	144
siNET-1	Migration and invasion		189

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apoptosis and enhanced the PTX sensitivity. UBC9 is an E2-conjugating enzyme required for SUMOylation and overexpressed in tumors, including lung adenocarcinoma, ovarian carcinoma, and hepatocellular carcinoma. UBC9 is involved in the regulation of several critical cellular pathways. Knockdown of UBC9 reduced the expression of Bcl-2 and Bcl-xl. It increased the expression of cleaved-caspase 3.[157] Downregulation of UBC9 increased the chemosensitivity to DOX in HCC cells, which is possibly associated with ERK1/2 and p38 activation.

Beyond gene knockdown by siSX1 or siUBC9, siRNAs have become a powerful tool to inhibit tumor growth by silencing oncogenes and cell cycle regulators. Effective siRNA-based treatments need targeted delivery to tumor cells to prevent off-target effects and improve the uptake. Ligand−receptor systems can be used for this; for example, N-acetylgalactosamine (GalNAc) and the asialoglycoprotein receptor (ASGPR) can be engineered to bind specific cancer-associated surface proteins like EpCAM or HER2.[158] Conjugating siRNA with tumor-targeting ligands increases the precision of gene silencing, boosts chemosensitivity, and effectively suppresses tumor growth by simultaneously blocking survival and resistance pathways.

5.1.1. Inhibiting Tumor Proliferation. To inhibit tumor proliferation, EGFR has emerged as a key target for siRNA; EGFR is overexpressed in 40%−70% of preneoplastic HCC and is an important target for siRNA therapy of HCC.[159,143,144] Treatment with RNA interference and inhibition of phosphorylation of EGFR/HER3, the antitumor proliferation and pro-apoptotic ability of Sorafenib were significantly improved.[158]

Another study employed extracellular vesicles decorated with EpCAM aptamer-3WJ scaffolds were engineered to deliver cytosol of si-β-catenin to liver cancer stem cells (LCSC).[160] EpCAM, a stem cell biomarker, and β-catenin mutations that activate the Wnt-β-catenin pathway were targeted to suppress LCSC proliferation. A CD133 + cell-derived mouse model mimicking early and advanced liver cancer stages showed that FOXA1 siRNA showed significant therapeutic benefits in early stage HCC by enhancing apoptosis and suppressing stem cell proliferation, although it was less effective in advanced tumors.[30] Another promising target, C-Met, a hepatocyte growth factor (HGF), is highly overexpressed in HCC and is associated with metastasis phenotype and poor prognosis.[161−163] siRNA targeting c-Met has demonstrated strong therapeutic potential, especially when delivered using superparamagnetic iron oxide (SPIO) nanoparticles coated with (Gal) ligands and polyethyleneamine (PEI), which protect siRNA from degradation and effectively inhibit tumor growth.[164]

Hairy and enhancer of split 5 (HES5), a transcription factor represses cell differentiation, is also implicated in HCC development.[54,165] Selenium-based nanoparticles functionalized with hyaluronic acid (HA) and PEI were employed to deliver siHES5, leading to G0/G1 cell cycle arrest, apoptosis induction, and potent antitumor activity in both in vitro and in vivo models.[166] Additionally, a folate-targeting and MRI-detecting nanomedicine to deliver si-TBLR1 can inhibit HCC growth and combine MRI detection with MRI detection simultaneously. Transducing β-like protein 1-related protein (TBLR1) is a key HCC oncogene relating to HCC proliferation, antiapoptosis, and angiogenesis by regulating the Wnt/β-catenin pathway.[167]

Expanding on combinatorial strategies, Gal−PEG-PAMA polymer-based nanoparticles have been synthesized to deliver si-AEG-1 and all-trans retinoic acid (ATRA), which could efficiently inhibit tumor proliferation, induce apoptosis, and suppress HCC growth by silencing AEG-1, a critical driver of hepatocarcinogenesis that also impairs retinoic acid-induced gene expression and cell death.[168] Furthermore, Chemokine (C-X-C motif) ligand 1 (CXCL1) was upregulated, and CXCR1 was downregulated in tumor tissues compared to peritumor tissues by chemotaxis assay. A study found that downregulating the CXCL1 expression by siRNA could inhibit the proliferation and promote apoptosis of HCC cells by reducing the activity of STAT3, NF-κB, and HIF-1.[169]

Continuing the exploration of siRNA-based strategies for HCC therapy, Mucin 1 (MUC1), an oncogene overexpressed in HCC, promotes tumor progression via the JNK/TGF-β pathway. Targeting this, treatment with siMUC1 combined with the JNK inhibitor SP600125 effectively inhibited HCC cell proliferation.[170] Similarly, macrophage migration inhibitory factor (MIF), a proinflammatory chemokine involved in cancer progression, was targeted using siMIF1. This approach reduced MIF and cyclin DI expression, resulting in suppressed tumor growth and enhanced apoptosis in HCC cells.[171] Ribonucleotide reductase M2 (RRM2) is highly related to DNA replication and the proliferation of tumor cells, as demonstrated by the inhibition of HCC growth by codelivery of Doxorubicin and siRNA-RRM2 via liposomes.[172] Treatment with siRRM2 could downregulate the expression of RRM2, inhibit HCC cell proliferation, and achieve a synergetic anticancer effect with Doxorubicin by regulating p21 expression.

Kinesin spindle protein (KSP), essential for mitosis, was targeted with siKSP, which induced mitotic arrest, inhibited proliferation, promoted apoptosis, and increased Doxorubicin sensitivity.[173] Another promising target, Kinetochore-associated protein 2 (KNTC2), is specifically upregulated in HCC. Its silencing via siKNTC2 inhibited tumor growth by regulating histone H3 phosphorylation at serine 10.[174] Rhotekin 2 (RTKN2), an antiapoptotic Rho-GTPase effector overexpressed in most HCC cases, was also effectively silenced using siRNA, leading to reduced proliferation, increased apoptosis, and impaired invasion.[175]

E2F1, a key transcription factor in cell cycle regulation, was downregulated using galactosylated polyaspartamide copolymers to deliver siE2F1, resulting in G1/G0 cell cycle arrest and decreased proliferation.[176]

Additionally, Midkine was targeted by chitosan-based nanoparticles while Sphingosine kinase 2 (Sphk2) was delivered by lipid nanoparticles to decrease exosomal oncogenic miRNA content and inhibit tumor growth.[177,178]

Finally, a combination of Sorafenib and siGPC3 delivered via PEI-modified liposomes effectively inhibited HCC cell proliferation by jointly suppressing the antiapoptotic GPC3 and the proliferative cyclin D1 genes, demonstrating potent synergistic anticancer effects.[179]

5.1.2. Inhibiting Tumor Angiogenesis. VEGF is a major factor responsible for tumor angiogenesis and pathogenesis and is frequently overexpressed in highly vascularized HCC.[180] Several siRNA therapeutics target the VEGF pathway for HCC therapy. After interference with the VEGF signaling with siVEGF, knockdown of VEGF expression efficiently reduced endothelial cell proliferation and tube formation in vitro, and decreased tumor growth and microvessel density in orthotopic

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Table 3. Downregulated miRNAs in HCC

miRNA name	Function of the target gene	Pathway	ref
	Chemoresistance		
miR-7203	Drug resistance and autophagy	Suppressing PI3K/AKT pathway	202
miR-216a/217	Drug resistance	Activating the TGF-β pathway	200
miR-27a	Hypoxia-induced chemoresistance	Upregulated by the HIF-1α pathway	29
miR-182	Drug resistance	P53	201
miR-107	Drug resistance	Maspin expression	212
miR-145	Drug resistance	Suppress the expression of P-gp and BCRP	17
miR-142-3p	Drug resistance, autophagy, and apoptosis	Autophagy-related protein	202
miR-31	Drug resistance	NDRG3	206
miR-33a-5p	Drug resistance		204
miR-375	Doxorubicin hydrochloride resistance		205
miR-539	Arsenic trioxide resistance		207
	Cell proliferation		
miR-122	Repress proliferation and induce apoptosis	Pyruvate kinase muscle 2 (PKM2), DLX4 and ADAM10	208, 209
miR-490-3p	Proliferation and migration	Downregulate the aurora kinase gene (AURKA)	210
miR-98	Proliferation	Zeste homologue 2 (EZH2)	215
miR-21	Promotes proliferation	PTEN and PDCD4	211
miR-195	Arrest the cell cycle and induce apoptosis	Wnt3	216
miR-1299	Cell cycle	Cyclin-dependent kinase 6 (CDK6)	218
miR-506	Cell cycle and apoptosis		217
miR-96-5p	Apoptosis	Caspase-9	219
miR-337/miR-370	Inhibit proliferation and promote apoptosis	HMGA2 and PIM1	221, 222
miR-25	Inhibition of miR-25 enhances cell apoptosis	TRAIL and PTPEN/PI3K/AKT/Bad pathway	223
miR-107	Increase proliferation and inhibit apoptosis	HMGA2	212
miR-155-5P	Elevate proliferation and inhibit apoptosis		226
miR-203a-3p	Improve proliferation	IL-24	227
miR-217	Inhibit apoptosis	MTDH	200
	Angiogenesis		
miR-146a	Metastasis and angiogenesis	Downregulate VEGF	233
miR-199a-3p	Angiogenesis	Decrease VEGF secretion and suppress VEGFR1 and VEGFR2	234
miR-451	Angiogenesis	Block VEGFR2 pathway	235
miR-638	Angiogenesis	Inhibit VEGF	236
miR-1301	Angiogenesis	Downregulate VEGFA, BCL9, and β-catenin	251
miR-338-3p	Promote angiogenesis	Upregulate VEGF	237
miR-497	Promote angiogenesis and metastasis	Inhibit VEGFA	238
	Invasion and metastasis		
miR-367-3p	Suppress the metastasis	MDM2/AR/FKBP5/PHLPP	242
miR-30e	Enhance metastasis and EMT	MTA1	243
miR-31-5p	Proliferation, migration, and invasion by SP1		
miR-345/miR-638	Increase the number of invasions and EMTs		236
miR-214-5p	Inhibit migration, invasion, and EMT		245
miR-212	Inhibit migration	Forkhead M1 (FOXM1) and Wnt/β-catenin pathway	246
miR-495/miR-613	Inhibit cell proliferation and invasion	IGF1R/YWHAZ	247
miR-122	Repress cell proliferation, invasion, and EMT	Snail1 and snail2	214
miR-187-3p	Inhibit EMT and metastasis		249
miR-199b-5p	Inhibit EMT and metastasis		234
miR-1301	Inhibit EMT and metastasis		251
miR-146a	Repress invasion, migration, and metastasis		233
miR-186	Repress invasion, migration, and metastasis		253
miR-199	Repress invasion, migration, and metastasis		203
miR-370	Repress invasion, migration, and metastasis		222
miR-520f	Repress invasion, migration, and metastasis		254
miR-634	Repress invasion, migration, and metastasis		255
miR-1207-5p	Repress invasion, migration, and metastasis		256
miR-25	Facilitate EMT	Inhibit Rho GDP dissociation inhibitor alpha	223
miR-135a	Promote migration and invasion	Forkhead box O1 (FOXO1)	258
miR-203a-3p	Improve migration and invasion	IL-24	227
miR-892a	Enhance migration and invasion		259
miR-1246	Enhance migration and invasion		260
miR-197↓	Inhibit migration and invasion	KAI1/CD82 pathway	261

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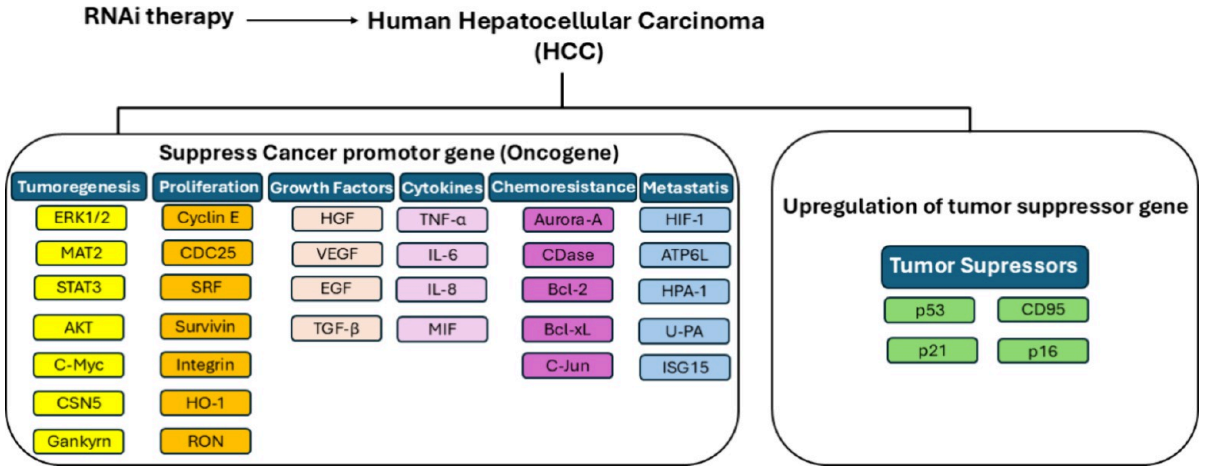

Figure 7. Approach for RNA interference-based therapy in hepatocellular carcinoma (HCC) by suppressing cancer promoter genes (oncogenes) or up-regulating tumor suppressor genes.

HCC tumor models.[181,182] A study demonstrated that an ASGPR targeting mesoporous silica nanocarrier to deliver siVEGF and Sorafenib achieved synergistic anticancer efficacy.[183] siHIF-1α was combined with TAE and improved the effectiveness of TAE by inhibiting the expression of HIF-1α and VEGF effectively.[184]

5.1.3. Suppressing Tumor Invasion and Metastasis. Expanding on siRNA strategies targeting key signaling pathways in HCC, Notch signaling contributes to tumorigenesis in many cancers, and aberrant high expression of Notch-1 has emerged as a critical contributor to tumorigenesis and metastasis.[185] Silencing Notch-1 via siNotch-1 shRNA effectively reduced HCC cell mobility and inhibited liver-to-lung metastasis through modulation of the Notch-1/Snail1/E-cadherin pathway, highlighting Notch signaling as a promising therapeutic target. Complementarily, knockdown of Notch4 using a lentiviral siRNA vector disrupted vasculogenic mimicry (VM) and impaired the migration and invasion of HCC cells.[186,187] In relation to the epithelial-mesenchymal transition (EMT), a key driver of invasion and metastasis, Golgi protein 73 (GP73) has been implicated in aggressive HCC phenotypes. Silencing GP73 using siRNA was shown to reduce EMT-associated behaviors, leading to decreased invasion and metastasis of HCC cells[188]

C-X-C chemokine receptor type 7 (CXCR7) is overexpressed in tumor endothelial cells (TECs) and contributes to the migration and invasion of cancer cells. Downregulation of CXCR7 via shRNA reduces the phosphorylation of STAT3 and its downstream targets, MMP2 and VEGF, thereby inhibiting TEC motility through the STAT3 signaling pathway.[144] To further enhance targeted delivery, siNET-1, combined with GPC3 antibodies was encapsulated in the nanobubbles. This system significantly improved transfection efficiency and effectively inhibited the migration and invasion of HCC cells, highlighting the potential of multifunctional nanocarriers in siRNA-based therapy.[189]

5.2. miRNAs Present Multivalent Expression Changes in and against Liver Cancer. MicroRNAs (miRNAs) are small noncoding RNAs with a length of 21−23 nucleotides, which act as important regulators heavily involved in the modulation of gene expression and regulation.[190] Most miRNAs are initially transcribed as nonfunctional precursors and become mature through two processing steps. They bind to the 3′-UTR of target mRNAs, leading to mRNA degradation and gene silencing.[191] As post-transcriptional gene suppressors, miRNAs can act as eitheroncogenic or anticarcinogenic agents. Its role in a wide range of miRNAs in both human and mouse cancer has also been investigated.[192,278] Several miRNA dysregulations are known to be directly involved in cancer, whether by suppressing apoptotic genes and preventing cell death or through their underproduction against oncogenic targets.[193] The miR-17−92 cluster, for instance, is shown to be overexpressed in lung cancer, and miR-223 is repressed in leukemia and hepatocellular carcinoma.[194,195] In a systematic analysis, miR-221/222, miR-195, and miR-199a were also shown to be differentially expressed in the HCC samples.

Accordingly, miRNAs can be utilized as antisense molecules to inhibit oncogenic miRNAs, thereby restoring the expression of tumor-suppressive genes and overcoming drug resistance conferred by multidrug resistance (MDR) gene networks. This approach is particularly relevant for targeting ABC transporters and other MDR-associated pathways, which often limit the efficacy of chemotherapeutic agents. By fine-tuning the expression of miRNAs involved in MDR regulation, we enhance treatment outcomes. Moreover, the combinatorial delivery of miRNAs with chemotherapeutics using nanocarriers may provide a synergistic strategy to tackle chemoresistant tumors, offering a promising avenue for translational cancer therapy.[191]

5.2.1. Circumventing Chemoresistant Gene-Mediated MDR. While numerous anticancer drugs are available, the development of drug resistance remains a key challenge and the main reason treatments often fail. Cancer cells develop drug resistance via a variety of mechanisms, including enhancing drug efflux, increasing DNA damage tolerance, reducing cell permeability, and more.[196] Central to these mechanisms is the acquisition of new genetic alterations. The miRNAs can silence gene expression and present potential in drug resistance. In addition, miRNAs have been involved in the pathways of chemoresistance in cancers, including HCC.[197,198] Table 3 presents the list of miRNA that are involved in HCCn.

MiRNAs' regulatory mechanisms are multifaceted. In some instances, the overexpression of certain miRNAs contributes to drug resistance. For example, Sorafenib, the most used drug in HCC treatment, faces the challenge of Sorafenib resistance

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(SR). Two separate studies have demonstrated the links between miR-7 and miR-216a/217 and SR. The first study revealed that miR-7 can act as a tumor suppressor in both in vitro and in vivo HCC models while simultaneously suppressing the expression of TYRO3, a member of the TYRO3-AXL-MER family of receptor tyrosine kinases, and thus a contributor to Sorafenib resistance.[199] The second study showed that sustained overexpression of miR-216a/217 in HCC cells downregulated Phosphatase and Tensin Homologue (PTEN) and Mothers Against Decapentaplegic Homologue 7 (SMAD7), both of which antagonize the Transforming Growth Factor Beta (TGF-β) type 1 receptor.[200]

MiR-27a, linked with various types of cancer, is overexpressed in hypoxic environments during hypoxia-induced chemoresistance.[29] On the other hand, miR-182 has also been reported to induce resistance toward cisplatin in HCC.[201] Hepatitis B Virus (HBV) induced HCC has been reported to be modulated by miRNAs downregulating the tumor suppressor Maspin, i.e., miR-7, miR-107, and miR-21.[202]

Furthermore, miRNAs have been implicated in multidrug resistance (MDR), another major challenge in HCC treatment. MiR-145 was identified as a key player in combating MDR by suppressing two key proteins involved in drug resistance, P-glycoprotein (P-gp) and Breast Cancer Resistance Protein (BCRP).[17]

The relationship between miRNAs and autophagy, a major obstacle in chemotherapy and targeted therapy, also offers interesting avenues for research. MiR-142-3p, for example, was found to control autophagy and, consequently, the sensitivity of HCC cells to Sorafenib.[203] Other miRNAs like miR-33a-5p, miR-4301, miR-455-3p, miR-483-3p, and miR-488-5p were found downregulated in cisplatin-resistant HCC cells, suggesting their potential role in this form of resistance.[204−207]

5.2.2. miRNA Therapeutics to Inhibit the Chemoresistance Gene. The regulatory mechanisms of miRNAs are varied. Sometimes, overexpression of some miRNAs leads to drug resistance (Figure 7). MiR-7[199] was related to overcoming Sorafenib resistance by suppressing TYRO3 by the PI3K/AKT pathway. Overexpression of miR-216a/217 will induce Sorafenib resistance by activating the TGF-β pathway.[200] The miR-27a[29] Expression will be up-regulated by the HIF-1α pathway in a hypoxic environment and involved in hypoxia-induced chemoresistance in various cancers, which has been identified as an oncogenic molecule in liver cancer, ovarian cancer, and prostate cancer. Upregulating miR-182 could increase cisplatin resistance in HCC by regulating tumor p53-induced nuclear protein 1.[201] Overexpression of miR-7/21/107 induced by HBV X protein could promote drug resistance of HCC cells by directly suppressing target maspin expression.[212]

Study found that TGF-β1 induced MDR in HCC through upregulating P-gp and BCRP via the SMAD4/HOTAIR/miR-145 axis.[17] It was found that TGF-β1 upregulated HOX transcript antisense RNA (HOTAIR) expression in HCC cells. When Drosophila mothers against decapentaplegic 4 (SMAD4) was silenced, HOTAIR expression was also reduced. Besides, miR-145 expression was increased when HOTAIR was silenced. As a result, miR-145 can suppress the expression of P-gp and BCRP by binding to the 3′-untranslated regions (3′-UTRs) of P-gp and BCRP.

MiR-142-3p[213] levels were reduced significantly by Sorafenib treatment, but upregulation of miR-142-3p could improve the sensitivity of HCC cells to Sorafenib by targeting autophagy-related 5 (ATG5) and autophagy-related 16-like-1 (ATG16L1) and enhance apoptosis induced by Sorafenib and inhibit cell growth. Inhibition of miR-33-5p could increase cisplatin resistance and reduce sensitivity in HCC.[204] MiR-31 could sensitize HCC cells to Adriamycin by targeting the NDRG3 gene.[206] Mir-375 could overcome doxorubicin hydrochloride resistance in HCC.[205] Overexpression of miR-539 could increase sensitivity to arsenic trioxide in HCC.[207]

5.2.3. miRNA Therapeutics to Inhibit Tumor Proliferation. Tumor cell proliferation and apoptosis are highly involved in the progression of tumor formation, which can be a potential therapeutic target to control HCC development and progression (Figure 7). As a liver-specific tumor suppressor miRNA frequently downregulated in HCC, miR-122 has drawn increasing attention in recent years.[214] Overexpression of miR-490-3p could suppress the proliferation and migration of HCC cells by downregulation of the aurora kinase A gene (AURKA).[210] Overexpression of miR-98 could arrest the cell cycle in G0/G1 phase and repress cell proliferation by targeting enhancer of zeste homologue 2 (EZH2).[215] Overexpression of miR-195 could arrest the cell cycle in the G1 phase and promote apoptosis by targeting Wnt3 in HCC.[216] MiR-506 could arrest the cell cycle in G1/S phase and induce apoptosis.[217] Overexpression of miR-1299 could inhibit the cell cycle from G0/G1 to S phase by targeting cyclin-dependent kinase 6 (CDK6).[218] MiR-96-5p could inhibit apoptosis by targeting caspase-9.[219] Overexpression of miR-122 could repress proliferation and induce apoptosis by targeting pyruvate kinase muscle 2 (PKM2) in HCC,[214] and inhibit proliferation by downregulating oncogenic distal-less 4 (DLX4).[220] Overexpression of miR-337[221] and miR-370[222] could inhibit cell proliferation and promote apoptosis in HCC by targeting HMGA2 and PIM1. Low levels of miR-337 could suppress apoptosis by inhibiting Bcl-xL expression[221]

Some miRNAs enhance proliferation when overexpressed and inhibit proliferation when downregulated. Inhibition of miR-25 could enhance cell apoptosis caused by TNF-related apoptosis-inducing ligand (TRAIL) by PTPEN/PI3K/AKT/Bad signaling pathway.[223] Overexpression of miR-107 could contribute to proliferation by targeting Axin2 in HCC,[224] while repression of miR-107 could increase proliferation by targeting high mobility group A2 (HMGA2).[225] MiR-155-5p could elevate proliferation and inhibit apoptosis in HCC.[226] High expression of miR-203a-3p could improve proliferation by targeting interleukin-24 (IL-24) in HCC.[227] Overexpression of miR-217 could inhibit apoptosis by targeting metadherin (MTDH) in HCC.[228]

5.2.4. miRNA Therapeutics to Inhibit Tumor Angiogenesis. Angiogenesis provides abundant nutrition for tumor cells, which is essential for tumor growth and metastasis in solid tumors.[229] HCC is a kind of common solid tumor that has abundant and deformed blood vessels.[230] As previously mentioned, VEGF is one of the most effective cytokines in the process of angiogenesis.[231] High expression of VEGF usually implies high invasion and metastasis of tumors.[232] Some miRNAs are reported to be involved in the regulation of angiogenesis by VEGF.

Overexpression of miR-146a could downregulate VEGF and repress HCC angiogenesis and tumor metastasis.[233] MiR-199a-3p could directly decrease VEGF secretion and suppress VEGFR1 and VEGFR2 expression to repress the angiogenesis of HCC.[234] MiR-451 could suppress VEGF expression, block the VEGFR2 pathway, and reduce angiogenesis.[235] MiR-638

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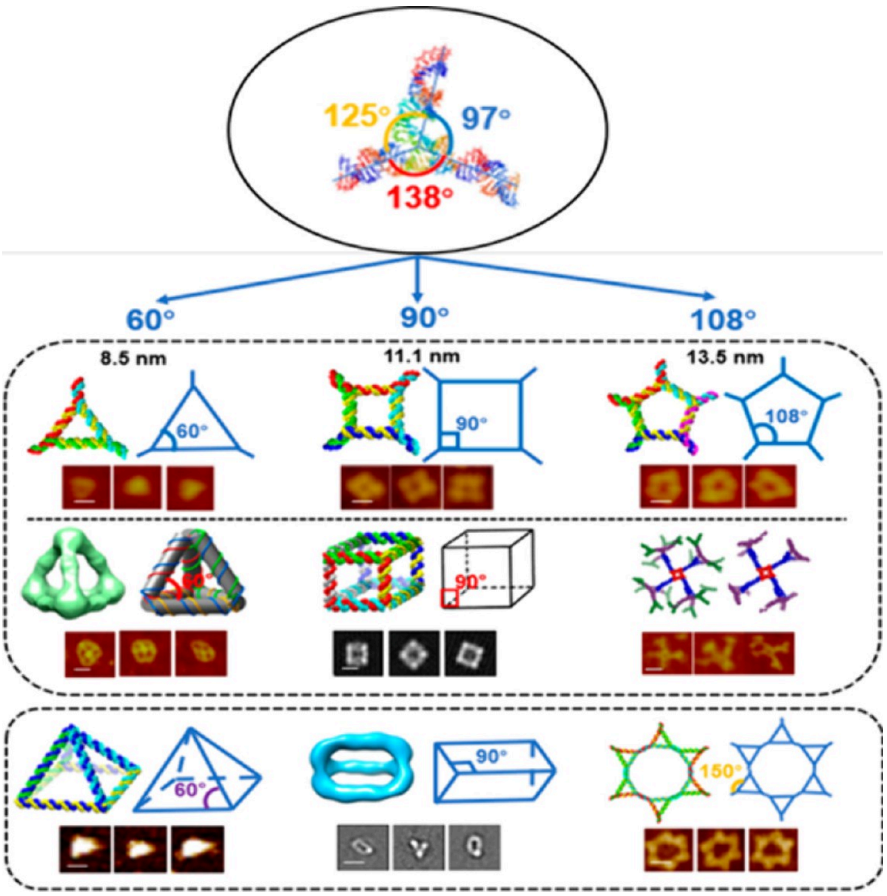

Figure 8. Construction and characterization of different RNA nanoparticles based on the three-way junction (3WJ). Angles of an original 3WJ were stretched to accommodate different shapes of 2D or 3D RNA nanoparticles. Adapted with permission from ref 267. Copyright 2024 American Chemical Society.

could inhibit VEGF and suppress the angiogenesis and tumor growth of HCC cells.[236] While some miRNAs could upregulate VEGF and enhance angiogenesis. Suppression of miR-338-3p could upregulate VEGF and promote angiogenesis in HCC.[237] Downregulation of miR-497 could promote angiogenesis and metastasis by inhibiting VEGF.[238]

5.2.5. miRNA Therapeutics to Inhibit Tumor Invasion and Metastasis. Invasion and metastasis are two significant factors for cancer prognosis, which are the dominant causes of cancer death, accounting for 90% of cancer deaths.[239,240] Tumor metastasis usually undergoes the following: implantation of metastatic organs in a distant place, substantial infiltration, adaptation to a new environment, and secondary tumor growth.[241] EMT is a very essential procedure for tumor cell invasion and metastasis. It is reported that some miRNAs are involved in regulating cancer cell invasion, EMT, and metastasis, which may provide a new platform for antimetastasis therapies.

MiR-367-3p increases Sorafenib efficacy by suppressing the metastasis by MDM2/AR/FKBP5/PHLPP/ (pAKT and pERK) signals.[242] Downregulation of miR-30e could enhance metastasis and EMT by enhancing MTA1 in HCC.[243] Besides, low levels of miR345[244] and miR638[236] Could heighten the invasion and EMT of HCC cells. Overexpression of miR-214-5p could inhibit migration, invasion, and EMT in HCC.[245] Overexpression of miR-212 could inhibit the migration by targeting forkhead M1 (FOXM1) and suppressing the Wnt/β-catenin pathway in HCC cells.[246] Overexpression of miR-495 and miR-613 could inhibit cell proliferation and invasion by targeting IGF1R and YWHAZ in HCC.[247] Upregulation of miR-122 could repress cell proliferation, invasion, and EMT by targeting Snail1 and Snail2 in HCC.[248] Overexpression of miR-187-3p,[249] miR-199b-5p,[250] and miR-1301[251] were reported to inhibit EMT and metastasis in HCC. Besides miR-146a,[252] miR-186,[253] miR-199,[203] miR-370,[222] miR-520f,[254] miR-634,[255] and miR-1207-5p[256] could repress invasion, migration, and metastasis in HCC.

Overexpression of miR-25 could facilitate EMT formation by inhibiting Rho GDP dissociation inhibitor alpha in HCC.[257] Overexpression of miR-135a could promote migration and invasion by targeting forkhead box O1 (FOXO1) in HCC cells.[258] Overexpression of miR-203a-3p could improve HCC migration and invasion by targeting IL-24.[227] Upregulation of miR-892a[259] and miR-1246[260] could enhance migration and invasion in HCC. Inhibition of hypoxia-inducible miR-210 combined with CXCR4 blockade cooperatively enhances therapeutic efficacy in CCA through reducing invasion, inducing cell apoptosis, and reversing drug resistance. Downregulation of miR-197 could inhibit migration and invasion by targeting KAI1/CD82 pathway in HCC.[261]

The miRNA-based therapeutic strategies present a promising and multifaceted approach to tackling the complex problem of drug resistance in HCC, providing an exciting area of research for more effective cancer treatments.

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6. THE ADVANTAGE OF RNA NANOTECHNOLOGY-BASED TREATMENT OPTIONS

RNA nanotechnology offers a novel pathway in targeted drug delivery for cancer therapy.[262,143,263−266] The properties of RNA allow researchers to deliver prodrugs directly to cancer cells and demonstrate the capabilities of RNA nanoparticles as a drug delivery vehicle (Figure 8).[267−269] Building on this, RNA nanotechnology-based treatment can be integrated with the series-circuit "Christmas light bulbs" strategy to overcome multidrug resistance by targeting multisubunit ABC transporters. In this approach, silencing or inhibiting a single essential subunit of the transporter—analogous to RNA nanotechnology, can disable the entire efflux pump, preventing the extrusion of chemotherapeutic drugs from cancer cells. RNA nanoparticles can functionalize to target ABC transporter subunits, while conjugation with targeting ligands such as GalNAc, aptamers, or other tumor-specific molecules ensures selective uptake by tumor cells.[141,270] The modular and multivalent nature of RNA nanostructures, including 4-way and 6-way junctions, further allows codelivery of transporter inhibitors with chemotherapeutic agents, enabling a synergistic effect that enhances intracellular drug accumulation, overcomes drug resistance, and improves therapeutic efficacy.[141,270]

Therapeutic siRNA and miRNA sequences can be integrated into the platform for exact control of gene expression in targeted cells, biocompatible and biodegradable.[191,270,271] RNA nanoparticles can harbor therapeutic RNAs, such as ribozymes,[272,273] riboswitch,[274,275] siRNAs,[276−278] miRNAs,[141,279] antisense RNAs,[280] and aptamers.[259]

6.1. RNA Nanotechnology Targeting ABC Transporters for Effective HCC Therapy. RNA nanotechnology involves constructing a nanometer-scale structure of highly stable RNA as a building block. RNAs have a unique characteristic in that it has deformative behavior as well as high thermodynamic stability.[280−282] Traditional RNA tends to internal fold and intramolecular interactions, specifically secondary and tertiary structures. RNA nanotechnology shifts toward intermolecular interactions that create quaternary structure. These modular structures are analogous to LEGO bricks, where rationally designed RNA motifs can be designed to produce robust self-assembly RNA nanoparticle structures.[283]

These RNA structures (RNA nanoparticles) form multivalent, branched frameworks capable of carrying various payloads such as small interfering RNA (siRNA), microRNA (miRNA), aptamers, antisense oligonucleotides, targeting ligands, prodrugs, and imaging agents such as fluorescence dyes. With this understanding, RNA nanoparticles are highly effective at delivering therapeutics specifically to target sites while reducing toxicity to healthy tissue. Several factors contribute to this: RNA's negative charge limits nonspecific cellular uptake; it naturally accumulates in tumor cells; unabsorbed RNA is quickly filtered out and excreted via urine; it can be multivalently functionalized; and its immunogenicity can be adjusted based on shape, size, and sequence composition.[282,283]

In the context of targeting ABC transporters for effective HCC therapy, RNA nanoparticles offer a versatile and powerful platform. Incorporation of ABC transporter inhibitors or siRNA can disturb ABC transporter genes such as ABCB1 or ABCG2. The RNA nanoparticles can inhibit the efflux pump that contributes to multidrug resistance (MDR).[26] Furthermore, the versatility of RNA nanoparticles allows cofunctionalization with aptamers, ensuring the specificity of RNA nanoparticles. Altogether, these properties make RNA nanoparticles particularly well-suited to overcome the limitations of conventional gene delivery systems and pave the way for more effective combination therapies in hepatocellular carcinoma.

6.2. Motility and Deformability Enable the Spontaneous Targeting of Cancer. RNA stands out as a unique biomaterial due to its motility and ability to deform. These dynamic qualities come from the RNA's exceptional structural and chemical characteristics. These include: canonical and noncanonical base pairing and stacking interactions that enable a wide formation of pseudoknots; base and strands transient driven by Brownian motion of nucleotides; induced-fit adaptations; nearest neighbor effect; and conformational capture. Together, this feature allows RNA to undergo dynamic configuration, allowing it to move and slide like an amoeba.[282] Results by optical tweezers have shown that RNA nanoparticles can repeatedly deform under applied force and return to their original form multiple times.[267]

RNA nanoparticles can be administered through an IV injection and pass through the leaky vasculature of the tumor. RNA nanoparticles have proven effective in inhibiting cancer biodistribution in a mouse model with high tumor accumulation and trace amount accumulation or no accumulation in healthy organs.[284] These results surpass those of more EPR results from more common gold and iron oxide nanoparticles. Furthermore, RNA nanoparticles penetrate deeper and stay in the tumor environment longer, with more than 5% delivered dose accumulated in the tumor within 0.5 to 1 h.[285]

6.3. Motility and Deformability Enable Rapid Renal Filtration, Lowering the Risk of Toxicity. The dynamic and motile properties of RNA enable it to pass the glomerular filtration barrier in the kidney. Glomerular filtration is responsible for removing small contaminants in blood that are smaller than 5 nm as they pass through. Particles larger than 5 nm typically remain in the circulation.[286] However, in the case of RNA nanoparticles, they still undergo renal excretion, even though the size exceeds 5 nm, which limits their retention in healthy organs and minimizes off-target interactions and toxicities. RNA nanoparticles have been detected in urine 0.5 to 1 h after IV injection and still retain their original structure, which indicates that RNA nanoparticles did not accumulate in the system.[267]

6.4. Negatively Charged RNA Minimizes Nonspecific Accumulation in Healthy Cells and Organs. RNA nanoparticles have a negative charge due to their phosphodiester backbone; this characteristic eliminates the need for additional surface modification required by other delivery systems. The negative charge of RNA causes electrostatic repulsion between RNA nanoparticles and the negatively charged lipid membrane of cells and organs. Consequently, RNA nanoparticles target only the cancer cells without targeting healthy cells. Typically, RNA nanoparticles are sized between 10 to 15 nm, which is well positioned to evade clearance by immune cells such as macrophages and Kupffer cells.[211,267] Comprehensive PK/PD study and biodistribution of RNA nanoparticles show low organ accumulation compared to other types of nanoparticles with the same size.[287]

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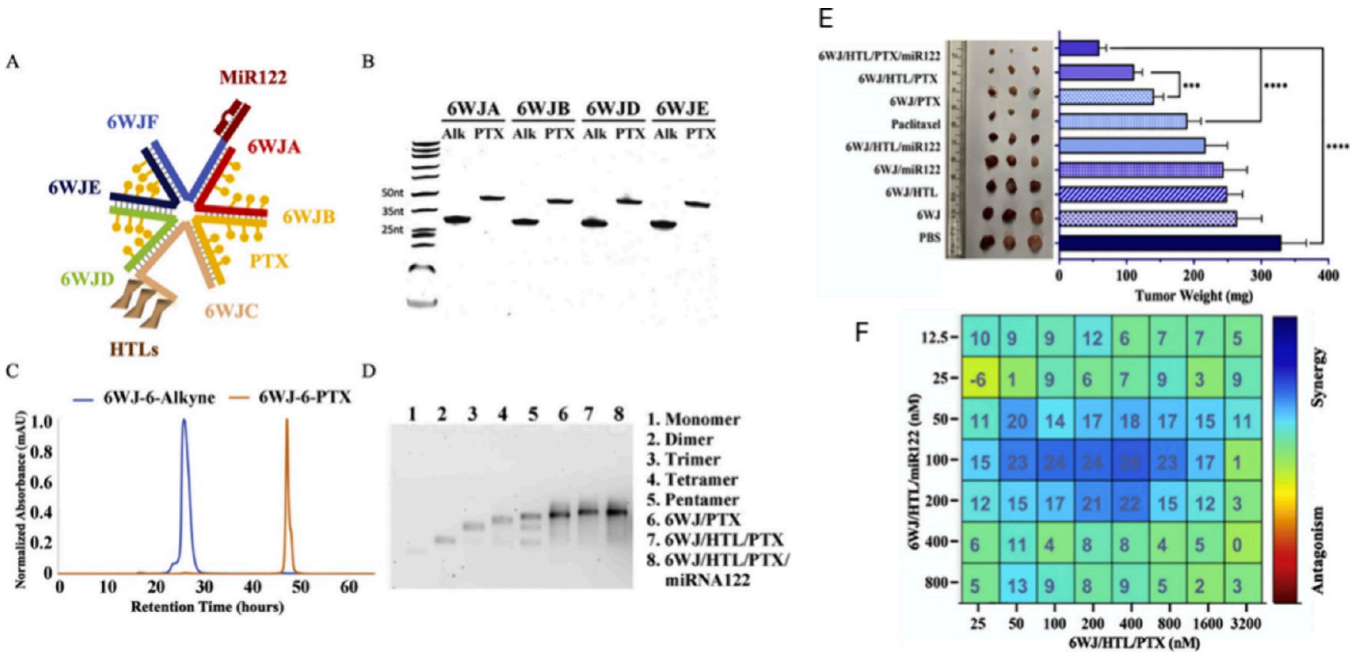

Figure 9. A) Design and construction of 6WJ/HTL/PTX/miR122 RNA nanoparticles. B) Gel-electrophoresis showing a change in gel migration for each strand after addition of PTX. C) HPLC chromatograph comparison between 6WJ-6-alkyne with 6WJ-6-PTX. D) Gel-electrophoresis showing bottom-up self-assembly of 6WJ/HTL/PTX/miRNA122 RNA nanoparticles. E) Images of liver cancer tumors harvested from mice after treatments. F) Synergetic cytotoxic effect between 6WJ/HTL/miR122 and 6WJ/HTL/PTX nanoparticles was assayed using the HSA synergy model. Adapted with permission from ref 141. Copyright 2021 Elsevier.

6.5. The Immunostimulatory Properties of RNA Are Dependent on Size, Shape, Sequence, and Stoichiometry. The immunogenicity of RNA nanoparticles is highly dependent on their size, shape, sequence, and stoichiometry. These RNA nanoparticles have a modular nature, which allows for the integration of multiple functional domains, such as targeting ligands, therapeutic payload, within a stable nanostructure.[288−291] Rationally designed Nucleic acid nanoparticles (NAN) present novel therapeutic approaches that overcome the limitations of conventional nucleic acid therapies by precisely controlling the composition and specific active components. NANs can be tailored to optimize stability, targeting, and pharmacological efficacy. For example, using peripheral blood mononuclear cells (PBMCs) transfected with NANPs provides a highly predictive model of cytokine responses.[288−291]

RNA Nps without single-strand extensions are commonly inert and do not activate the immune system.[292−294] However, the addition of a specific sequence, particularly a CpG motif, can significantly increase immune system activation by interaction with the TLR receptor.[292,293]

RNA nanoparticles whose size is between 10 to 20 nm tend to provoke a cytokine response. Moreover, larger and 3D nanoparticles are more likely to provoke cytokine responses than smaller or planar structures. These factors indicate that geometric configuration and molecular composition (strand composition) are critical to modulating immune response.[292,293,295] These tunable properties are beneficial to enhance their pharmacokinetics, targeting ability, and cellular uptake. Such control significantly influences the biodistribution and tumor penetration. The modularity and structural flexibility of RNA nanoparticles make them a highly promising multifunctional platform for targeted drug delivery and cancer therapy.

6.6. Biocompatibility and Biodegradation. RNA nanoparticles offer biocompatibility and biodegradability properties derived from native RNA. Since RNA nanoparticles come from native RNA, their interaction with biological systems is nontoxic, attributed to their nanoscale size and shape.[211,296,297,143] The instability of RNA is enhanced due to the formation of unique tertiary structures created through Watson−Crick and noncanonical base pairings. Further can be achieved via chemical modifications.[298]

Another important aspect for *in vivo* applications of RNA nanoparticles is their biodegradability. Unlike synthetic nanoparticles, which can accumulate in tissues and cause toxicity, RNA nanoparticles are designed to degrade into their constituent nucleotides by nucleases when they release the therapeutic payload.[279,299] These properties significantly reduce the risk of bioaccumulation and related toxicity in liver cancer.

6.7. Structural Versatility and Programmability. The properties of RNA offer remarkable structural versatility and programmability that can be translated into therapeutic potential.[275,300,143] The primary sequence of RNA determines its secondary and tertiary structures. The Watson−Crick base pairing enhances the design of RNA nanoparticle structures. It accommodates pseudoknots, triple helices, and G-quadruplexes, various forms of noncanonical interactions that add to the structural complexity and flexibility of RNA nanoparticles.[276,301,278] The flexibility of RNA's self-assembly into complex, diverse geometric shapes and sizes, such as triangles, squares, pentagons, and hexagons, allows further precision and tailoring of nanoparticle design.[302,303]

RNA can be programmed to enable multiple functionalities within a single nanoparticle. Multifunctional RNA nanoparticles can be engineered to load various therapeutic agents,

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targeting ligands, and imaging probes through RNA self-assembly and programmable properties.[279]

6.8. Targeted Delivery and Cellular Uptake. Targeted delivery is defined as increasing the specificity and efficacy of the drugs while reducing off-target effects and toxicity to improve patient outcomes.[304,141] Targeted delivery can be achieved by incorporating a target ligand or RNA aptamer within RNA nanoparticles.[305,18,306,307] These ligands or aptamers interact with specific receptors, driving receptor-mediated endocytosis and promoting cellular uptake.[259,308] For example, functionalized RNA nanoparticles with folate have been used for targeted delivery to cancer cells that overexpress folate receptors.[309] Another key factor of targeted delivery is avoidance of nonspecific uptake by RES, for prolonging the circulation time of RNA nanoparticles in the bloodstream and thus increasing their probability to reach the tumor site.[284,303] The physical and chemical properties of RNA nanoparticles, such as size, shape, and surface charge, can be tuned to reduce RES recognition and uptake.[303] RNA nanoparticles may also leverage the enhanced permeability and retention (EPR) effect. A characteristic sign of solid tumors, such as liver cancer, for passive targeting EPR effect describes leaky vasculature plus impaired lymphatic drainage within the tumor microenvironment, allowing accumulation of nanoparticles in tumor tissue.[310,311]

An innovative approach for the selective targeting of liver HCC cancer cells involves the incorporation of three copies of GalNAc as ligand into the multivalent rubber-like 6WJ RNA nanoparticle bearing 24 copies of therapeutic drugs Paclitaxel and one copy of miR122 to combat issues related to efflux drug transporters and chemoresistance.[141,270] The RNA 6WJ accumulated in HCC tumor sites and inhibited HCC growth. The combination of the multivalent effect of Paclitaxel and miR122 has led to the high efficiency of inhibition of HCC cancer growth in mice models (Figure 9).

6.9. Reduced Immunogenicity and Toxicity. In addition to their biocompatibility, structural versatility, programmability, and targeted delivery, RNA nanoparticles exhibit reduced immunogenicity and toxicity, making them a particularly suitable therapeutic option for liver cancer treatment. Apart from aiding in flexibility and design, RNA self-assembly circumvents the use of toxic chemistries associated with fabricating other nanoparticle types.[274]

In contrast to viral vectors and DNA therapies, RNA nanoparticles can be designed and engineered to be non-immunogenic.[312] Recent studies on RNA nanoparticles show minimal activation of Toll-like receptors (TLRs) and pro-inflammatory cytokine expression upon administration.[302]

The immunogenicity of RNA nanoparticles is reduced by incorporating pseudouridine or 2-thiouridine to reduce RNA recognition by TLRs and other innate immune sensors during in vitro transcription.[313]

7. STRATEGIES TO IMPROVE BIODISTRIBUTION AND PHARMACOKINETICS

7.1. Avoiding the Macrophage Phagocytic System (MPS). The MPS is one of the main biological barriers to nanoparticle delivery. This system consists primarily of macrophages and dendritic cells that recognize explicit foreign materials, like nanoparticles. MPS recognition is often mediated through an opsonization mechanism by which plasma proteins adsorb to nanoparticles, marking them for clearance. To prevent macrophage phagocytic system (MPS) clearance, nanoparticles can be encapsulated with hydrophilic polymers such as polyethylene glycol (PEG) and mixed-charged dendritic lipopeptide zwitterions.[314] These modifications create a steric hindrance, thereby reducing protein adsorption and recognition by the MPS.[315,316]

7.2. Navigating the Extracellular Matrix (ECM) to Enhance Cellular Uptake. The extracellular matrix (ECM), which is composed of fibrous proteins and polysaccharides in solid tumors, hinders the mobility of therapeutics.[317] Reducing the size of nanoparticles helps nanoparticles penetrate the tumor. Once at the tumor site, RNA nanoparticles with a size of around 10 nm favor the interaction with the EMC and entry into cancer cells.[318] To locate and enter the cancer cells, RNA-based therapies must first penetrate the ECM, be internalized via receptor-mediated endocytosis, and subsequently escape from endosomes to activate their therapeutic effect.[281]

7.3. Renal Clearance of Nanoparticles. Renal clearance poses a significant challenge in nanoparticle delivery. Composition and size play crucial roles in this process. Nanoparticles smaller than ∼5.5 nm tend to be quickly excreted. This rapid clearance limits its circulation time and prevents accumulation in tumors. Moreover, distal tubular secretion actively transfers nanoparticles from peritubular capillaries into the tubular lumen for urinary excretion, which further hampers their effectiveness in treating liver cancer.[319] Therefore, it is essential to carefully consider the size and characteristics of nanoparticles to avoid rapid renal clearance.

7.4. Ligand−Receptor Interactions. Targeting specific ligand−receptor interactions is an intelligent and precise way to deliver RNA nanoparticles to liver cancer cells. This method not only enhances the uptake of nanoparticles by cells but also minimizes unwanted side effects. Several receptors that are overexpressed in liver cancer cells have been identified.

The asialoglycoprotein receptor (ASGPR) is frequently overexpressed in hepatocellular carcinoma (HCC). GalNAc, a high-affinity ligand for ASGPR, can effectively target cells that express this receptor. Research has shown that RNA nanoparticles conjugated with GalNAc improved RNA nanoparticle uptake in HCC cells.[270] Glypican-3 (GPC3) is another overexpressed receptor in HCC that can be conjugated to RNA nanoparticles for effective delivery.[179] Additionally, the transferrin receptor (TfR) is a valuable target for liver cancer cells due to its overexpression and role in iron metabolism. TfR-targeted RNA nanoparticles have shown promising results in in vitro and in vivo studies.[320]

Ligand−receptor interactions for RNA nanoparticle targeting offer unique opportunities and challenges, while minimizing off-target toxicity

7.5. RNA Ligand-Displaying Exosomes for Targeted Delivery and Treatment. Liver cancer, especially hepatocellular carcinoma (HCC), remains a tough challenge in the field of oncology. Although many small-molecule drugs have been thoroughly studied, they often come with adverse side effects.[321] Exosomes have interesting similarities with RNA nanotechnology including biocompatibility, effective biodistribution, and internalization through endocytosis. Recent research suggests that the therapeutic potential of exosomes can be improved by attaching RNA molecules to their surface. The combination of hydrophobic small drug properties with hydrophilic RNA nanoparticle via conjugation has shown a lot of promise; for instance, an RNA 6-way-junction (6WJ) conjugated with PTX and miR122 has been explored for liver cancer treatment (Figure 10).[141]

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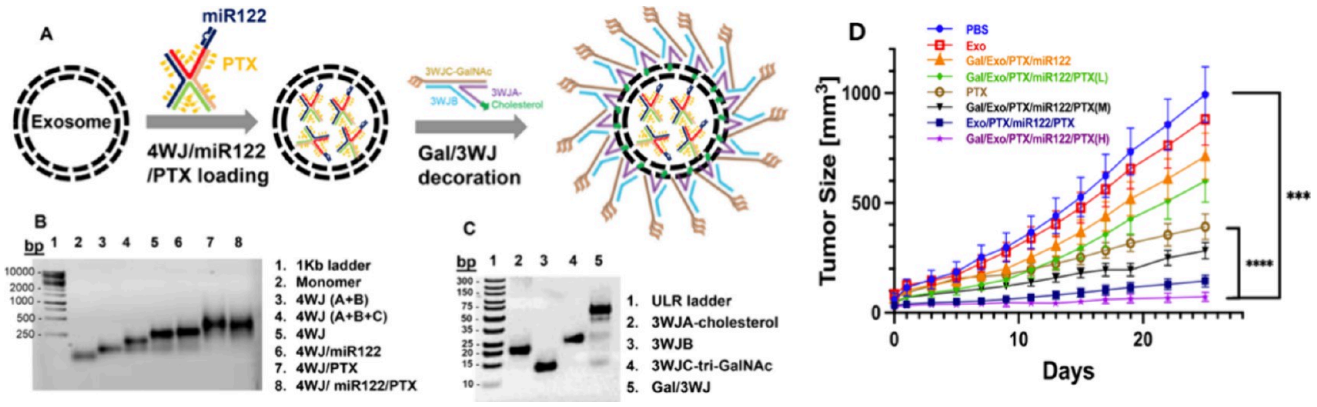

Figure 10. A) Schematic diagram of 4WJ/PTX/miR122 RNA nanoparticle loading into exosome and its decoration with Gal/3WJ (pRNA-3WJ in arrow-tail orientation) RNA nanoparticles. B) Gel-electrophoresis showing stepwise assembly of 4WJ (lane 5), 4WJ/miR122 (lane 6), 4WJ/PTX (lane 7), and 4WJ/PTX/miR122 (lane 8) RNA nanoparticle construction using the bottom-up self-assembly process. C) Gel-electrophoresis showing Gal/3WJ RNA nanoparticle construction using 3WJA-cholesterol, 3WJB, and 3WJC-tri-GalNAc. D) Tumor inhibition curves. Adapted with permission from ref 270. Copyright 2023 Elsevier.

RNA ligand on the surfaces of exosomes can enhance targeting to specific cell types or tissues. Notably, arrowtail RNA nanoparticles bearing ligands were incorporated into ginger-derived exosome-like nanovesicles (GDENs) for siRNA delivery and inhibiting tumors. The folic acid (FA) on the surface of GDENs, could effectively target siRNA to KB cancer models.[31,277]

Evidence indicated that lncRNA KCNQ1OT1 in tumor-derived exosomes could influence PD-L1 ubiquitination through the miR-30a-5p/USP22 pathway. These findings suggest that RNA ligands on exosomes could be crucial not only for drug delivery but also for shaping the tumor microenvironment to enhance treatment effectiveness.[322,311]

7.6. Clinical Translation Consideration. The clinical application of RNA-based nanodrugs for HCC has accelerated significantly following the approval of Onpattro (Patisiran), the first FDA-approved siRNA-based lipid nanoparticle therapy, developed by Alnylam Pharmaceuticals in 2018 for polyneuropathy treatment caused by hereditary transthyretin amyloidosis.[323,324] The approval of Patisiran demonstrated the feasibility of systemic RNA delivery using lipid nanoparticles and underscored the importance of robust chemistry, manufacturing, and control (CMC).[323] Its approval demonstrated the feasibility of systemic RNA delivery using lipid nanoparticles and it underscored the importance of robust chemistry, manufacturing, and controls (CMC) processes, particularly for lipid composition, particle uniformity, and siRNA encapsulation efficiency.[323] The development of *Patisiran* also emphasized the need for validated analytical methods to ensure consistency across production batches and the long-term stability of RNA-based formulations. In addition, establishing a standardized protocol for RNA-based nanodrugs characterization is needed, as well as consistent manufacturing under GMP. In clinical settings, validating analytical methods is important for quality control to address any immunogenicity issue and meet guidelines from regulatory agencies such as the FDA and EMA regarding safety, efficacy, and reproducibility of nanocarrier-based RNA therapeutics.[324,262,143]

Compared to the conventional delivery system mentioned above, RNA nanoparticles offer major advantages for HCC treatment. Structurally programmable and chemically stable, RNA nanoparticles can be engineered for precise targeting while minimizing off-target effects and immune activation.[270,276,301,278] Unlike other vectors or cationic polymers, rationally designed RNA nanoparticles exhibit low immunogenicity, thus, reducing the risk of an adverse inflammatory response. Furthermore, RNA nanoparticles can be functionalized with a ligand for active targeting, reducing nonspecific distribution and increasing the efficacy.[141] With all of these advantages, RNA nanoparticle systems must overcome key challenges, including large-scale manufacturing and long-term formulation.

8. CONCLUDING REMARKS

The use of RNA nanotechnology as a treatment for hepatocellular carcinoma (HCC) shows great promise in changing the landscape of chemotherapy. The Christmas-Tree Circuit mechanism has shown potential in tackling drug resistance in liver cancer, presenting a significant opportunity for developing new therapies. To improve the effectiveness of RNA in HCC cells, advancements in the stabilization of RNA are necessary to prolong circulation time and enhance targeting efficiency; therefore, nucleotide modification, such as the addition of F at 2′, is necessary.

Additionally, it is important to achieve accurate targeting of hepatocytes while minimizing off-target effects, which remains crucial to ensure treatments are both safe and effective. The RNA conjugated GalNAc ligand shows promising results by inducing specific uptake of RNA by liver cancer cells through ASGPR binding. Small interfering RNAs (siRNAs) and microRNAs (miRNAs) offer a new avenue for fighting metastasis due to their ability as powerful therapeutic agents for HCC treatment. Furthermore, RNA nanotechnology, coupled with multivalent RNA molecules' flexibility and biocompatibility, allows for the fabrication of nanostructures like 4-Way Junction (4-WJ) or 6-Way Junction (6-WJ) for precise drug delivery and effective gene modulation. miR-133-directed blockade of hepatic efflux pump P-glycoprotein (P-gp) and the use of RNA nanoparticles, encompassing adherence of RNA nanoparticles to exosomes, have provided an interesting mechanism to enhance specificity in drug delivery.[262] In addition to these, it is proposed that the synergistic effect can be harnessed by incorporating multiple stimuli-controlled conjugates into RNA nanoparticles to achieve precise control over site-specific drug release for HCC treatment.

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9. FUTURE PERSPECTIVES

Looking forward, several critical challenges and opportunities will shape the future trajectory of RNA nanotechnology. The synergetic effect can be utilized by incorporating more than one stimulus-responsive moiety within RNA nanoparticles that can be controlled for site-specific drug release.[325] These moieties can be designed to respond to endogenous stimuli such as Reactive Oxygen Species (ROS), acidic pH in the tumor microenvironment, or externally applied triggers like ultrasound.[325,300] Upon reacting, these moieties can initiate cleavage that releases therapeutic cargo without compromising the RNA nanoparticles' structure.[287,326−331]

To fully utilize the modularity of RNA nanoparticles, artificial intelligence (AI) is becoming a tool in drug design, as well as delivery optimization. By using the correct software, AI can predict optimal RNA folding patterns, guide the selection of rationally designed sequences, and model the interactions with target biomolecules. These computational tools can accelerate the discovery of effective RNA nanostructures and improve the potential of RNA nanoparticles as an emerging therapy for HCC.

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Notes

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ABBREVIATIONS

ABC	ATP-Binding Cassette
AEG-1	Astrocyte Elevated Gene-1
ASGP-R	Asialoglycoprotein-Receptor
APC	Adenomatous Polyposis Coli
BCRP	Breast Cancer Resistance Protein
BM-MSC	Bone-Marrow-Derived Mesenchymal Stem Cells
CC	Cholangiocarcinoma
CNS	Central Nervous System (CNS)
C-X-C motif	Chemokine
DCCD	Dicyclohexylcarbodiimide
DOX	Doxorubicin
FCCP	Carbonyl Cyanide-p-Trifluoromethoxyphenyl-hydrazone
FNH	Focal Nodular Hyperplasia
FRS2	Fibroblast Growth Factor Substrate 2
GSTe	Glutathione S-Transferase
GPCR	G-Protein-Coupled Receptors
HAI	Hepatic Artery Infusion
HCA	Hepatocellular Adenoma
HCC	Hepatocellular Carcinoma
HGF	Hepatocyte Growth Factor
Ia CIs	Immune Checkpoint Inhibitors
KNTC2	Kinetochore-associated Protein 2
KSP	Kinesin Spindle Protein (KSP)
LCSC	Liver Cancer Stem Cell
MWA	Microwave Ablation
MDR	Multidrug Resistance
mAbs	Monoclonal Antibodies
MDR	Multidrug Resistance
PDGFR	Platelet-Derived Growth Factor Receptor
PEI	Polyethyleneimine
PD/PK	Pharmacodynamics/Pharmacokinetics
PTEN	Phosphatase and Tensin Homologue
RES	Reticuloendothelial System
RFA	Radiofrequency Ablation
ROS	Reactive Oxygen Species
RRM2	Ribonucleotide Reductase M2
SMAD7	Mothers Against Decapentaplegic Homologue 7
Sphk2	Sphingosine Kinase 2
SPIO	Superparamagnetic Iron Oxide
TMD	Transmembrane Domain
TACE	Trans-arterial Chemoembolization
TARE	Trans-arterial Radioembolization
TECs	Tumor Endothelial Cells
TLRs	Toll-like Receptors (TLRs)
TKIs	Tyrosine Kinase Inhibitors
VEGFR	Vascular Endothelial Growth Factor Receptor

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