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Multidrug Resistance in Hepatocellular Carcinoma

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Abstract: Although there has been tremendous progress in the treatment of hepatocellular carcinoma over the past decades, multidrug resistance to chemotherapy and targeted therapy remains a major hindrance in its successful management. Multidrug resistance, whether intrinsic or extrinsic, is a multifactorial process that includes enhanced drug efflux, decreased drug uptake, intracellular sequestration, metabolic alterations, aberrant apoptotic and autophagic signaling, changes in tumor microenvironment, and acquisition of stem cell-like properties by the cancer cells. Although many experimental strategies have been developed to overcome drug resistance, translation of the knowledge to the clinic has not been crowned with success. This chapter provides an overview of the role of multidrug resistance in hepatocellular carcinoma and the potential approaches to overcome this obstacle.

Keywords: ATP-binding cassette transporter; drug efflux; drug sequestration; multidrug resistance; RNAi therapy

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INTRODUCTION

Hepatocellular carcinoma (HCC), the most common type of liver cancer, is increasing in prevalence with a high mortality rate. It is considered the fifth most detected cancer in men and seventh in women in the USA, and represents the third most leading cause of cancer-related death in the world. The highest incidence rate of liver cancer in the world occurs in Asia and Africa; hepatitis viruses (B and C) account for approximately 80% of all HCC cases (1). About 80% of HCC patients are currently diagnosed at advanced stages of the disease and are not suitable candidates for surgical resection of the tumor. Systemic chemotherapy with cytotoxic agents (5-Fluoracil, doxorubicin, cisplatin, and oxaliplatin) and targeted therapy with the tyrosine kinase inhibitor sorafenib are the main approaches for these patients; however, chemotherapy resistance remains a major clinical obstacle (2). In addition to drug resistance, sorafenib failed to be an optimal treatment modality for some advanced HCC patients due to adverse effects and high costs (1). Extensive studies have been carried out in the last few decades to enhance the efficacy of anticancer drugs by overcoming chemoresistance, but translating this knowledge to the clinic still represents a critical challenge. Thus, there is an urgent need to focus on elucidating the mechanisms of chemoresistance, especially multidrug resistance (MDR), and develop novel methods or tools for the treatment of HCC patients.

MECHANISMS OF MDR

MDR can be either intrinsic or acquired. In intrinsic resistance, the cancer cells are inherently resistant or unresponsive to therapeutics. In acquired resistance, cancer cells that were initially responsive become unresponsive during the course of treatment. MDR is multifactorial, and pleiotropic cellular signals are simultaneously involved in this process. These include upregulation of drug efflux, downregulation of drug uptake, sequestration of drugs, alteration in drugs metabolism, abnormal expression of non-coding RNAs, blockage of apoptotic signals, change of tumor environment, acquiring stem-cell like characteristics and autophagy (Figure 1) (3). More than one MDR mechanism can occur in a single cancer type, which pose significant challenges for a thorough understanding of the signaling network (4).

Enhanced drug efflux

Molecular pumps that transport cytotoxic drugs across the membrane of cancer cells represent a primary cause of chemotherapeutic resistance. Hyperactivation of these molecular pumps decreases intracellular drug concentrations and results in drug resistance. Permeability-glycoprotein, also referred to as P-gp, MDR-1, or ATP-binding cassette subfamily B member 1 (ABCB-1), is a well-studied 170 kDa plasma membrane drug efflux protein. It belongs to the adenosine triphosphate binding cassette (ABC) transporter superfamily, which includes MRP-1 (MDR protein), TAP1 (Transporter 1, ATP-binding cassette subfamily B member), and

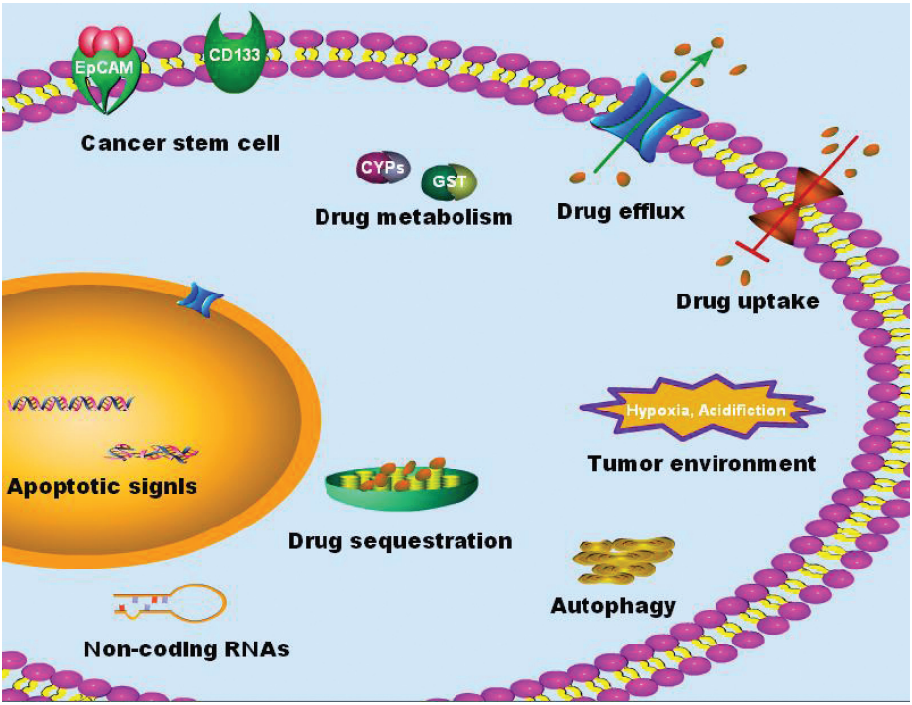

Figure 1 Multiple mechanisms of MDR in HCC. Multidrug resistance is a multifactorial process. Some of these include enhanced drug efflux, reduced drug intake, alterations in tumor microenvironment, impaired autophagy and apoptotic signals, lysosomal sequestrations, non-coding RNAs, alterations in drug metabolism, and acquisition of cancer stem cell-like phenotypes. CYPs, cytochrome P450 enzymes; GST, glutathione-S-transferase.

BCRP (breast cancer resistance protein) (5). ABC transporters are members of a conserved family of transmembrane proteins that utilize ATP as energy source to transport various substances, such as metabolic products, sterols, lipids, and drugs, across cellular membranes. The ABC proteins are comprised of cytosolic and transmembrane domains (6), and are essential for normal cellular functions. However, overexpression of ABC proteins in cancer cells usually leads to insufficient intracellular concentration and bioavailability of cytotoxic drugs as well as their metabolites (7). From a pharmacological point of view, although drug molecule–ABC interactions are very specific, one drug moiety can be a substrate of several ABC pumps (8). ABC proteins play a major role in the MDR of HCC (9, 10). The drug-resistant HCC cell line Bel7402/5-FU, developed by exposure to increasing concentrations of 5-FU, displays a higher expression of P-gp when compared with the parental cell line Bel7402. These cells are resistant not only to 5-FU but also to epirubicin (11). Kong et al. showed that P-gp and BCRP were highly expressed in the MDR HCC cells HepG2, which was induced by TGF-β1 via the SMAD4/HOTAIR/MiR-145 axis. As a result, the concentration of imatinib in HepG2 cells was significantly decreased (12). Compared with parental cells, P-gp is significantly overexpressed in the sorafenib-resistant HCC cells, HepG2

and Huh7. This was partially due to epithelial-mesenchymal transition (EMT) and AKT activation. Treatment with the novel allosteric AKT inhibitor MK-2206 reversed P-gp-mediated MDR via downregulation of phosphorylated AKT (13).

Reduced drug uptake

Drugs are transported across the cells by several mechanisms including passive diffusion and facilitated transport. The plasma membrane is an important barrier that limits drugs from reaching intracellular compartments. Passive transporters, ion-coupled transporters, and exchangers are encoded by genes of the solute carrier (SLC) family, which comprises approximately 360 uptake transporters in the cell membrane. Factors that downregulate or block the transporters can lead to drug resistance through decreased drug uptake or defective endocytic processes (14). Compared with non-tumor adjacent tissues, SLC46A3 was downregulated in 83.2% of human HCC tissues, and low expression was associated with a more aggressive phenotype. Conversely, overexpression of SLC46A3 was demonstrated to ameliorate sorafenib resistance, thereby improving the drug response, both in vitro and in vivo (15). SLCO1B3 is involved in the uptake of a number of chemotherapeutic agents, and its expression is significantly elevated in HCC patients with intratumoral cholestasis (16, 17). As a direct target of miRNA122, SLC7A1 is upregulated in miR122-silenced HCC cells, which is related to sorafenib resistance. Overexpression of miR122 can suppress SLC7A1 levels and render HCC cells more sensitive to sorafenib (18). Gao et al. analyzed SLC family genes using qPCR array and identified 11 downregulated and 3 upregulated genes in HCC specimens, compared with the para-carcinoma tissues from HCC patients who underwent surgery. In addition, they found that SLC29A1 was the only gene that correlated with poor prognosis and that it was significantly elevated in human HCC cell lines and tissues. Knockdown of SLC29A1 decreased the sensitivity of HCC cells to 5-FU, cisplatin, and sorafenib in vitro (19).

Drug sequestration

Sequestration of drugs in cellular compartments is an important mechanism of chemotherapy resistance. Since drugs used in chemotherapy generally target molecules in the nucleus and other subcellular compartments, they must be able to achieve sufficient concentrations in these compartments and their microenvironments (20). Intracellular conditions such as intralumenal pH, electrochemical potential, lipid compositions, and resident proteins can influence the intracellular localization of drugs. Multiple drug sequestration mechanisms may be involved in a single MDR cancer cell line, and the phenomena of drug sequestration may be more complex than originally thought (21). MDR cell lines show an increased capacity to sequester drugs into cytoplasmic compartments, resulting in decreased interactions of the drug with its nuclear targets. Colombo et al. (22) demonstrated P-gp expression not only on the cell membrane but also on lysosomes of six HCC cell lines and reported that cell lines with giant lysosomes were more resistant to sorafenib than those with small lysosomes. They concluded that lysosome-associated drug sequestration plays an important role in MDR in HCC cells (22). Metallothionein also plays a role in sequestering drugs

within a cell. Sorafenib remarkably induces the expression of metallothionein-1G, which is a critical factor for sorafenib resistance in HCC. Inhibition of metallothionein-1G enhances the anticancer activity of sorafenib in vitro and in tumor xenograft models (23).

Cellular metabolism

The response to cytotoxic drugs often depends on the metabolic state of the cancer cells, and these cells rewire the metabolism of anticancer drugs. Metabolic alterations can be influenced by various factors such as oncogenes or tumor suppressor genes and the tumor microenvironment (24–29). Cancer cells that are resistant to cisplatin have high levels of reactive oxygen species (30), glutathione (GSH), and glutamate–cysteine ligase catalytic subunit (GCLC) (31, 32). Downstream of survival signaling pathways, the Warburg effect, which refers to the increased rate of glycolysis in tumorigenic cells, can be observed even in conditions of normal oxygen levels. In c-Myc-driven HCC, glucose catabolism through glycolysis is elevated via the activation of pyruvate kinase (33). Inhibition of glycolysis and increase in oxidative phosphorylation can re-sensitize HCC cells to chemotherapeutics such as sorafenib, cisplatin, and isoliensinine (34). HIF1α activates the transcription of genes encoding angiogenic cytokines, for example, VEGF, and glycolytic enzymes, such as hexokinase 1, hexokinase 2, glyceraldehyde-3-phosphate dehydrogenase, and pyruvate kinase. These enzymes rewire the metabolism of cancer cells and induce MDR in HCC (35–38). Wu et al. showed that ADRB2 pathway regulation leads to HIF1α stabilization, reprogramming of glucose metabolism, and resistance of HCC cells to sorafenib (39). Drug metabolism enzymes are also involved in the MDR. This process includes phase I and phase II enzymes. Phase I of oxidative metabolism is mediated mainly by cytochrome P450 enzymes (CYPs) and epoxide hydrolases. Phase II enzymes are involved in conjugation reactions, including glutathionylation, glucuronidation, and sulfation. These enzymes include glutathione-S-transferase (GST), UDP-glucuronosyltransferases (UGT), sulfotransferases, and arylamine N-acetyltansferases (NAT), which transform the reactive species into hydrophilic nontoxic metabolite conjugates. Therapeutic drugs are metabolized by CYPs and epoxide hydrolases, which are further conjugated by the phase II enzymes and then, in phase III, effluxed by transporters such as the members of the ABC transporter family (14). Meena et al. reported that CYP450 and fatty acid synthase protein levels were elevated in multidrug-resistant HCC cells, and downregulation of these molecules by siRNAs or cerulenin resensitized the cells to paclitaxel (40). Further, ciplatin-resistant HCC cell lines have a higher expression of GST, which can protect cancer cells from being inhibited by anticancer drugs (41).

Non-coding RNAs

The term “non-coding RNAs” (ncRNAs) refers to RNAs that do not encode proteins. These include miRNAs, long ncRNAs (lncRNAs), and circular RNAs (circRNA) (42). ncRNAs are involved in multiple cellular processes, such as proliferation, migration, apoptosis, angiogenesis, and immune responses (43).

A number of studies have highlighted the key roles of ncRNAs in the evolution and progression of drug resistance in cancers. They mainly modulate drug transporters, cell cycle-related proteins, apoptotic signals, and the tumor microenvironment (44). While all ncRNAs potentially play roles in drug resistance in a context-specific manner, the major role is played by miRNAs and lncRNAs (45, 46). The miRNAs are small (~20 bp) non-coding RNAs, which target specific mRNA sequences and inhibit protein translation (47).

One of the most abundantly expressed miRNA in the liver is miR-122, which plays a major role in basic liver function and homeostasis (48, 49). The loss of miR-122 is attributed to dysregulation of hepatocyte differentiation, poor prognosis, and metastasis of liver cancer. Restoration of miR-122 increased the sensitivity of drug-resistant HCC cells to cytotoxic agents through downregulation of MDR-related genes, and inhibition of cell growth by cell cycle arrest at G0/G1 phase (50). Moreover, miRNA microarray data indicate that miR-122 is decreased in sorafenib-resistant HCC cells. miR-122 downregulation-mediated activation of insulin-like growth factor 1 and subsequent activation of the RAS/RAF/ERK pathway are thought to be the major mechanisms of resistance (51). He and colleagues found that miR-21 was overexpressed in sorafenib-resistant HCC cells, and inhibition of miR-21 with oligonucleotides resensitized these cells to sorafenib (52). They concluded that miR-21 participated in the acquired resistance of sorafenib by suppressing autophagy through the Akt/PTEN pathway (52). Multidrug-resistant Huh-7 cell lines, developed with increasing concentrations of doxorubicin, cisplatin, carboplatin, mitomycin C, and vincristine, demonstrated a significant differential profile of miRNAs when compared with the parental cell line. miR-27b, miR-181a, miR-146b-5p, miR-181d, and miR-146a were the most differentially expressed, and they are thought to play critical roles in the acquisition of MDR by regulating PTEN, P53, and KRAS (53).

Apoptotic signals

Apoptosis is involved in the regulation of many physiological and pathological processes (54). Disruption of apoptotic signals, one of the hallmarks of cancer, is a major obstacle in the success of chemotherapy. In general, there are two apoptotic pathways: (i) the intrinsic pathway involving the release of cytochrome c from mitochondria and (ii) the extrinsic pathway with the activation of death receptors. The initiation of these pathways results in the activation of caspases, which mediate the cleavage of cellular substrates, leading to morphological and biochemical changes that accompany apoptosis (55). DNA damage and oncogene activation either induce the accumulation of p53, which causes cell cycle arrest in the G1 phase, or trigger apoptosis, depending on the extent of DNA damage. Mutation or inactivation of p53 can result in chemotherapy resistance in cancer via suppression of apoptotic pathways (10). Zhang et al. reported that cisplatin reversed tumor necrosis factor (TNF)-related apoptosis-inducing ligand (TRAIL) resistance in HCC cells, dependent on the status of p53 (56). Modulating the expression of p53 and BCL-2 using long interspersed nuclear element-1 ORF-1 protein led to the resistance of HepG2 cells to cisplatin and epirubicin in vitro (57). The BCL-2 family, including pro-apoptotic proteins (BAX, BAK, BID, BAD, and PUMA) and anti-apoptotic proteins (BCL-2, BCL-xl, and MCL-1), can

regulate apoptosis induced by wild-type p53 in response to stress. Mitochondrial pathway-associated chemotherapy resistance is mainly regulated by the BCL-2 family (14). BCL-2 plays a pivotal role in the glycochenodeoxycholate (GCDA)-induced chemoresistance, while suppressing the GCDA-stimulated phosphorylation of BCL-2 significantly attenuates the survival and drug resistance in HCC cells (58). Sorafenib-resistant HCC cell lines, including HepG2R and Hep3BR, exhibit altered expression of BCL-2 and MCL-1. Navitoclax, an inhibitor of BCL-2, can restore the anticancer activity of sorafenib and regorafenib via a mitochondrial caspase-dependent mechanism in vitro and in vivo (59).

Tumor microenvironment

Solid tumors are heterogeneous structures. The tumor microenvironment is composed of cancer and stromal cells embedded in extracellular matrix, sustained by aberrant vasculature (60, 61). Tumor hypoperfusion, secondary to the hyperpermeability of the aberrant vasculature, along with low oxygen, depleted nutrition, low pH, and high interstitial pressure can cause chemoresistance (61, 62). Compared to normal cells, cancer cells exhibit higher glucose metabolism rates and preferentially utilize glycolysis over oxidative phosphorylation, especially in hypoxic conditions (Warburg effect). This process ultimately generates lactic acid, leading to intracellular acidification (63, 64). As a result, cancer cells may express relatively more proton pumps in order to maintain intracellular pH homeostasis, rendering the extracellular environment highly acidic. According to the ion trapping theory, weakly basic drugs, such as doxorubicin, mitoxantrone, and vincristine, are ionized extracellularly and, as a consequence, lead to chemoresistance (14). Being an anti-angiogenic agent, sorafenib treatment reduces tumor vessels, prompts hypoxia in the tumor microenvironment, and stimulates HIF-mediated cellular responses that favor the selection of chemo-resistant cells (65). Hypoxia has been shown to induce resistance to sorafenib, 5-FU, gemcitabine, cisplatin, adriamycin, and 6-thioguanine in BEL-7402, HepG2, and SMMC-7721 HCC cell lines (66).

Cancer stem cells

Cancer stem cells (CSCs) are a subpopulation of tumor cells with the capacity of self-renewal, differentiation, as well as drug resistance (14, 67, 68). CSCs in human HCC have been identified and validated through isolation and xeno-transplantation experiments in animal models. These cells have pivotal roles in the development and progression of HCC (69) as well as chemotherapy resistance (66). CSC markers of HCC include epithelial cell adhesion molecule (EpCAM), CD133, CD90, CD44, CD24, CD13, deubiquitinating enzyme ubiquitin-specific protease 22 (USP22), and oval cell marker OV6. Some of these markers have been reported to confer chemoresistance to HCC (2, 70, 71). Multi-signal pathways and their cross-talk, including EpCAM, Wnt/β-catenin, Sonic Hedgehog, and Notch, are required to maintain the stemness phenotype of HCC CSCs (67). $CD133^+$ HCC cells isolated from human HCC cell lines and xenograft mouse models were resistant to chemotherapeutics, through the activation of Akt/PKB and Bcl-2 pathways (72). Downregulation of USP22 significantly suppressed the expression of ABCC1 (MRP1) in an HCC cell line, with validation of the

relationship between USP22 and ABCC1 in clinical HCC tissue samples. These results suggest that USP22 is associated with the MDR phenotype of BEL-7402/FU (71). In addition, GSK2879552 and pargyline, inhibitors of lysine-specific histone demethylase 1A (KDM1A or LSD1), were demonstrated to alleviate acquired resistance to sorafenib through the suppression of the Wnt/β-catenin signaling pathway in HCC CSCs (73).

Autophagy

Autophagy is a highly conserved cellular "self-degradative" process, in which cytoplasmic components (long-lived or misfolded proteins, protein aggregates, and damaged organelles) are degraded and recycled to maintain homeostasis. Deficient autophagy is closely related to the development of many diseases including cancer. Autophagy occurs at a basal level in cells and can be induced by diverse signals and cellular stressors, including chemotherapeutic agents (74). In general, autophagy plays a dual role in the process of MDR in cancers. It not only contributes to the development of MDR, but also kills MDR cancer cells in which apoptosis pathways are inactive, leading to inconsistence results across studies (75, 76). Autophagy inhibitors can increase the sensitivity of HCC cells to cytotoxic agents (77). Fan et al. showed that elevated peptidylarginine deiminase IV (PADI4) was associated with chemoresistance through autophagy induction in HCC in vitro and in vivo. Inhibition of autophagy restored the sensitivity of HCC cells to chemotherapy (78). The exact relationship between autophagy and MDR in HCC remains unclear and requires further research.

STRATEGIES TO OVERCOME MDR

Extensive studies have been carried out during the last few decades to enhance the efficacy of chemotherapy by suppressing or evading the mechanisms of MDR. These approaches include the use of MDR modulators or chemosensitizers (79, 80), improved drug delivery (81, 82), RNAi therapy (83), and natural products (84).

MDR modulators or chemosensitizers

MDR modulators or chemosensitizers have been classified into first-generation, second-generation, and third-generation on the basis of their affinity for certain transporters and effects (5). As P-gp is the most extensively characterized transporter of the ABC superfamily, ways to modulate P-gp have been studied extensively. The first-generation P-gp modulators include verapamil, cyclosporine A, trifluoperazine, quinidine, progesterone, calmodulin antagonists, and tamoxifen. Kim et al. reported that a high dose of verapamil is required both clinically and experimentally to overcome MDR of HCC and that the combination of tamoxifen and cyclosporine A showed a significant reduction in the IC_{50} value of doxorubicin in MDR HCC cell lines (85). Due to disappointing therapeutic outcomes and high systemic toxicities, these modulators were replaced with the second-generation MDR modulators (86, 87) such as dexverapamil, valspodar,

biricodar citrate, and dexniguldipine. Valspodar was shown to improve the anticancer effect of doxorubicin by modulating P-gp in HCC and hepatoblastoma cell lines (88). Although the second generation of MDR modulators can inhibit P-gp and increase the intracellular accumulation of drugs better than the first-generation modulators, there are several disadvantages that limit their clinical application. Numerous chemotherapeutics are substrates of both P-gp and cytochrome P450. Thus, the combination of anticancer agents with the second-generation MDR modulators may lead to unpredictable pharmacokinetic or incorrect dosing of chemotherapeutics (5, 89). The third-generation MDR modulators include tariquidar, zosuquidar, laniquidar, elacridar, mitotane, diarylimidazole, and annamycin. Comparative molecular field analysis (CoMFA) and comparative molecular similarity indices analysis (CoMSIA) associated with 3D-quantitiative structure–activity relationship (3D-QSAR) studies were performed to aid the research and design of the third-generation MDR modulators (90). These modulators are about 300 times more potent than the first- and second-generation modulators. Importantly, these agents do not interact with cytochrome P450 (90, 91). Takahata et al. found that breast cancer-resistant protein (BCRP) expression correlated well with the chemo-sensitivity of irinotecan hydrochloride (CPT-11) in HCC cell lines. Elacridar, an inhibitor of BCRP, enhanced the sensitivity of CPT-11 in BCRP-overexpressing KYN-2 cells (92).

Enhanced drug delivery

Nanotechnology has the power to deliver anticancer drugs and radically change chemoresistance of cancer cells by overcoming MDR (82). There are several drug delivery systems including liposomes, dendrimers, polymeric micelles, nanoparticles, polymer–drug/protein–conjugates, and carbon nanotubes. These nanoformulations may overcome several challenges in efficient drug delivery such as solubility, pharmacokinetic profiles, cellular uptake, bio-distribution patterns, circulation times, and clearance (93). For instance, pluronic P85 can sensitize MDR tumors to many chemotherapeutic agents through various mechanisms: (i) membrane fluidization, (ii) ATP depletion, (iii) direct interaction with the ABC efflux transporter, (iv) reduction of the GSH/GST detoxification system, (v) drug release from acidic vesicles, and (vi) incorporation into the mitochondrial membrane, thereby inhibiting cellular respiration (94). Moreover, all these nanomaterial-based drug delivery systems can be conjugated with various kinds of ligands (e.g., proteins, antibodies, and small molecules) producing the so-called actively-targeted material that favors drug targeting to specific cell surfaces and thus to specific cell populations, leading to a selective and reduced toxicity (82).

Polyethylene glycol (PEG) and polyethylenimine (PEI) co-conjugated ultrasmall nano-graphene oxide (NGO) loaded with C6-ceramide (NGO-PEG-PEI/Cer) were reported to subvert MDR in HCC cells by inactivating MDR and AKT signaling. NGO-PEG-PEI/Cer combined with sorafenib represents a promising potential therapeutic strategy for the treatment of drug-resistant HCC (95). HA/anti-miR-21/PPAuNCs, a nonviral gene delivery system, which condensed anti-miR-21 into hyaluronic acid-conjugated and PEI-modified PEGylated gold nanocages (AuNCs), enhanced intracellular drug accumulation and restored sensitivity to doxorubicin in a doxorubicin-resistant HCC cell line through upregulating PTEN expression and downregulating P-gp (96). Bmi1 is essential for the survival

and proliferation of liver CSCs. Yang et al. demonstrated that Bmi1 siRNA delivered via cationic nanocapsules of cisplatin (NPC/Bmi1siR) eliminated the side population of $CD133^+$ HCC cells dramatically and overcame drug resistance of HCC (97).

RNAi therapy

RNA interference (RNAi) is considered a highly specific approach for gene silencing and has emerged as a novel therapeutic tool for various pathologic conditions, including cancers (98,99). RNAi molecules are a group of small regulatory RNAs that include miRNAs and small (or short) interfering RNAs (siRNAs). miRNAs are endogenous RNAs that are produced from non-coding RNAs, while siRNAs are derived from exogenous long dsRNAs (100, 101). In addition, exogenous short hairpin RNA precursors that are processed by a distinct cellular machinery to form siRNAs can also lead to effective gene silencing (101, 102). These artificially generated oligonucleotides mediate gene silencing through post-transcriptional mRNA cleavage and decomposition in the cytoplasm, resulting in the knockdown of target gene expressions (98, 103). Theoretically, RNAi-based strategies can be used in a wide variety of experimental models to target genes that are involved in disease processes (103, 104).

Enhancer of zeste homolog 2 (EZH2) is overexpressed in the MDR HCC cell line Bel/Fu, and siRNA depletion of EZH2 sensitized these cells to 5-FU by inhibiting MDR1 protein expression, promoting apoptosis, and inducing cell cycle arrest at G1/S phase (105). It has been reported that MAPK14/Atf2 signaling predicted a poor response to sorafenib in human HCC. Rudalska et al. demonstrated that silencing MAPK14 by shRNA reverted sorafenib resistance in HCC in vitro (106). Knockdown of the autophagy-related gene LC3 by RNAi significantly enhanced the sensitivity to epirubicin and inhibited proliferation of HepG2 cells (107). As silencing a single miRNA may sequentially activate other compensatory signaling pathways, a combinatorial approach modulating many miRNAs related to a signal pathway may be a promising strategy. The miRNAs miR-21, miR-153, miR-216a, miR-217, miR-494, and miR-10a-5p have been shown to be elevated in sorafenib-resistant HCC cells. Simultaneous targeting of these miRNAS using artificial long non-coding RNAs reversed sorafenib resistance in these cells both in vitro and in vivo (108). RNAi, apart from being a potential therapeutic tool, can also be used as a tool for biomarker screening for chemotherapy sensitivity. Through a high-throughput RNAi screening with 176 shRNA pools against 88 histone methyltransferases and histone demethyltransferases, Li et al. (109) found that silencing of the histone methyltransferases genes, *ASH1L, C17ORF49,* and *SETD4,* promoted the sensitivity of HepG2 cells to sorafenib.

Natural products

Natural products have attracted increasing attention as anticancer tools. A large pool of products with potential functions on reversing MDR have been identified and classified (Figure 2) (84, 110). Steroidal saponin from *Trillium tschonoskii* reversed MDR of HCC cell lines in a dose-dependent manner by inhibiting MDR-related molecules such as MRP1, MRP2, MRP3, MRP5, MVP, and GST-π (111).

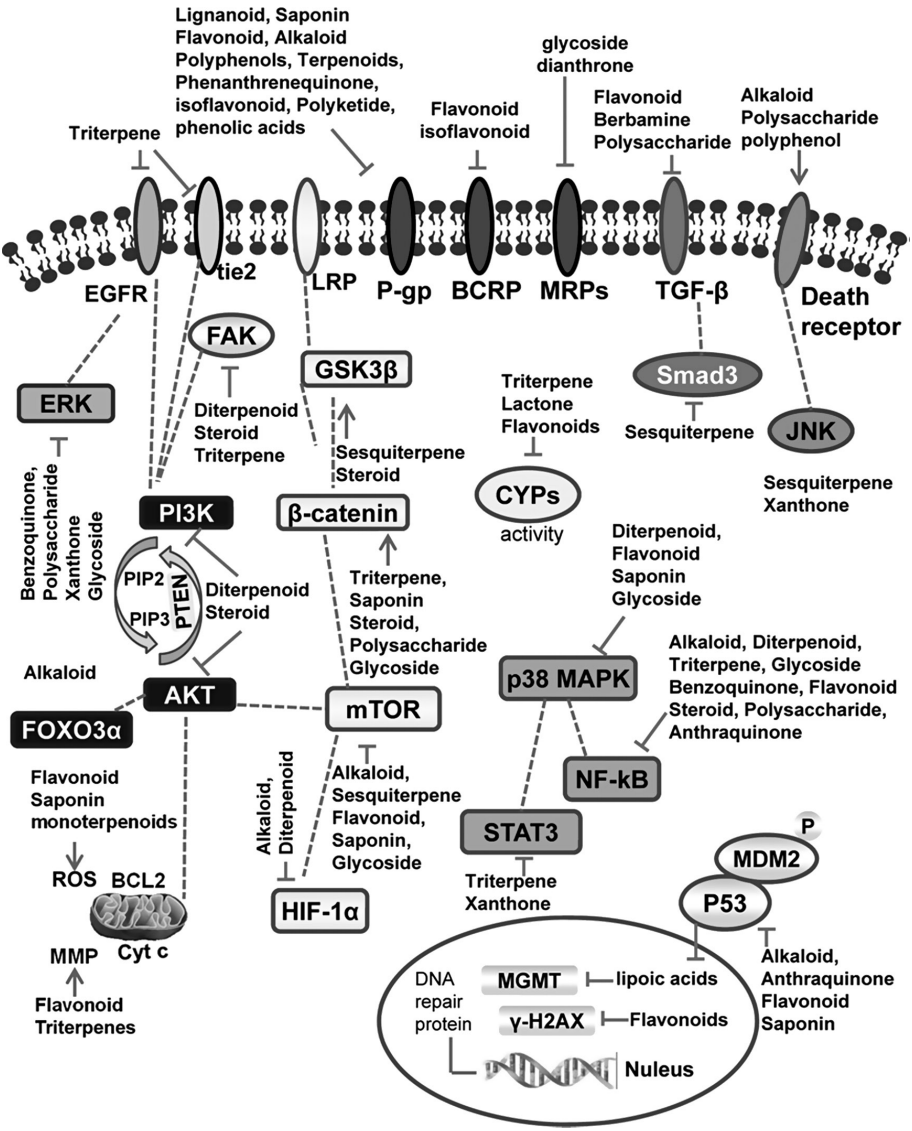

Figure 2 Natural products and their potential role in reversing MDR. Experimental data show that natural products can reverse MDR via regulating drug efflux, drug metabolism, and apoptotic pathways in cancer cells (110).

Treatment of HepG2/ADR cells with rhamnetin, derived from Persian berries, reduced the expression of Notch-1, P-gp, and BCRP and increased the susceptibility of HepG2/ADR cells to sorafenib, etoposide, and paclitaxel (112). Baicalein, isolated from *Radix scutellariae,* increased the intracellular accumulation of Rho123 and epirubicin, induced apoptosis and autophagy, decreased the expression of P-gp and Bcl-xl, and reversed MDR in Bel7402/5-FU cells (11).

Moreover, natural products can also increase the sensitivity of HCC cells to anticancer drugs by regulating cellular metabolism. Li and colleagues demonstrated that dauricine dose-dependently suppressed glucose glycolysis and increased oxidative phosphorylation by downregulating the expression of hexokinase 2 and pyruvate kinase M2, consequently increasing the sensitivities of HCC to cisplatin, sorafenib, and isoliensinie (34).

CONCLUSION

Despite a better understanding of the mechanisms of drug resistance, and the experimental approaches that have been taken to overcome drug resistance over the decades, clinical utility of these approaches has not come to fruition. To date, there is no effective tool to overcome MDR of HCC patients. Among the various strategies described to address drug resistance, nanotechnologies appear to offer particular advantages with their presumed target-specific delivery of chemotherapeutics and other conjugated agents. While RNAi can be designed for specific targets and used successfully in vitro, the in vivo silencing effects of RNAi are far from satisfactory even in highly controlled experimental conditions. Natural products can affect multiple targets and pathways with minimal side effects. However, the current literature is not sufficient to justify their use in clinical settings. Given that the liver plays a major role in drug metabolism and detoxification, and its function is already impaired in HCC patients, any drug combination that depends on normal liver metabolism is unlikely to be a successful strategy to overcome drug resistance. Taken together, continuous efforts are needed to explore the mechanisms in more detail and design novel approaches to overcome MDR to improve outcomes for HCC patients.

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