Teaching new tricks to old dogs: A review of drug repositioning of disulfiram for cancer nanomedicine

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Abstract
The increasing incidence and mortality of malignant tumors have required the development of diversified innovative therapies. Compared with the new therapeutic molecule invention, it is a more economical and efficient way to reposition old drugs that have been marketed or in clinical trials for tumor treatment. Studies have been carried out for repositioning the old approval drugs that are widely applied clinically with reliable biosafety and effectiveness evidence, including aspirin, artemisinin (and its derivatives, eg, dihydroartemisinin), metformin, and disulfiram (DSF). In addition to the approved pharmacological activity, these old drugs exhibited broad tumor-suppressive effects. This review summarizes the drug repositioning strategy of DSF through nanotechnology and outlines the DSF nano-formulations for cancer targeting delivery and therapy.

KEYWORDS
combination therapy, disulfiram, drug repositioning, nanotechnology, tumor-targeting delivery

Abbreviations: 5-FU, fluorouracil; ABP, albumin binding proteins; ALDH, acetaldehyde dehydrogenase; BSA, bovine serum albumin; CMC, critical micelle concentration; CPPs, cell-penetrating peptides; CSCs, cancer stem cells; DDC, diethyldithiocarbamate; DOX, doxorubicin; DSF, disulfiram; DSF/Cu or Cu(DDC), or CuET, the chelate of diethyldithiocarbamate and copper (II); EPR, enhanced permeability retention; HA, hyaluronic acid; HMBP, hollow mesoporous Prussian blue; HMSNs, hollow mesoporous silica nanoparticles; INK, c-Jun N-terminal kinase; LBA, lactobionic acid; MAPK, mitogen-activated protein kinase; Me-DTC-SO, S-methyl-N,N-diethyldithiocarbamate sulfoxide; MMP, matrix metalloproteinases; MON, metal-organic nanoparticles; MR/CD206, mannose receptor; MSN, mesoporous silica nanoparticles; NF-kB, nuclear factor kB; NPs, nanoparticles; NSCLC, non-small cell lung cancer; OPDMA, poly[2-(N-oxide-N, N-dimethylamino)ethyl methacrylate]; P-gp, p-glycoprotein; PLGA, poly(lactic acid-co-glycolic acid); PTX, paclitaxel; Rego, regorafenib; ROS, reactive oxygen species; SOD, superoxide dismutase; SPARC, secreted protein acidic and rich in cysteine; TAMs, tumor-associated macrophages; TIR, transferrin receptors; TKI, tyrosine kinase inhibitors

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1 INTRODUCTION

Drug repositioning (also synonymous with drug repurposing, or retasking) is a novel emerged strategy for developing new uses of approved drugs beyond the scope of the original medical indications. In 2004, it was first defined as “identifying and developing the potential new uses for existing drugs.”[1] Drug repositioning is an efficient and high rewarding strategy with low cost and risk by exploiting new indications for existing drugs. Researchers have paid great attention to drug repositioning and remarkable success has been achieved in this field. A well-known case of drug repositioning is thalidomide, which was originally used as a sedative for treating morning sickness but withdrawn due to cause serious birth defects, has been re-marketed since 2006 with approval for multiple myeloma in combination with dexamethasone.[2]

Although there are many old drugs reported with new treatment potential via in vitro bioactivity screening, it is still a large challenge for their clinical translation for drug repositioning. It must bridge a huge gap between in vitro tests to in vivo efficacy. An essential issue to be addressed is drug delivery. The in vivo fate of a drug needs to be tailored for delivery to a new target for a new indication. Nanotechnology is a useful and systematic strategy for drug delivery and thus could be a powerful booster for drug repositioning. The repositioned drugs can achieve targeted delivery steered by nanotechnology with the benefit of avoiding unwanted drug exposure and reducing side effects.

Disulfiram (DSF) is a carbamate derivative for alcoholism therapy for over six decades.[3] DSF blocks the activity of acetaldehyde dehydrogenase (ALDH), which causes the accumulation of toxic acetaldehyde in the serum and a consequent unpleasant DSF-ethanol reaction such as dyspnoea, vertigo, nausea, and vomiting.[4] The pharmacokinetic profiles and safety of DSF have been well documented and therefore, repurposing DSF should be an economical and efficient avenue to develop a new therapeutic invention. Since the 1990s, cumulative evidence has revealed the tumor-inhibiting effect of DSF,[5] and there have been several clinical trials for investigating the treatment efficacy of DSF in various cancers, including non-small cell lung cancer (NCT00312819), breast cancer (NCT03223346), prostate cancer (NCT02963051), glioblastoma (NCT01907165, NCT03034135), and other solid tumors (NCT00742911).

DSF or its metabolite diethyldithiocarbamate (DDC) can strongly chelate to copper (II) with the formation of Cu(DDC)2 chelate (also written as DSF/Cu).[6] It is believed that the antitumor activity of DSF is copper ions dependent.[7] Although there is a higher level of copper in many tumors than normal tissues, DSF is difficult to accumulate in the tumors to yields Cu(DDC)2 via oral administration due to the properties of poor stability, poor bioavailability, and rapid clearance. Therefore, there is an urgent need for developing an effective method to deliver DSF for cancer therapy.

Cancer therapies based on nanotechnology can improve targeting delivery efficiency and the bioavailability of therapeutics.[8] Besides, the nanomedicines have been endowed with multi-features by chemical modification, such as tumor accumulation and specific microenvironment response.[9] Importantly, the tailor-made nanomedicines provide unique benefits for antitumor combination therapy, through which the drugs with different pharmacokinetic profiles can be codelivered to the same destination and exert a synergistic therapeutic efficacy.[10] Herein, we summarize the repositioned application of DSF for anticancer via various nanotechnology designs.

2 ANTICANCER EFFICACY OF DISULFIRAM

The extensive anticancer mechanisms of DSF are summarized in Figure 1, including ubiquitin-proteasome system inhibition,[7] stemness suppression of cancer cells,[11] inhibition of nuclear factor κB (NF-kB) and efflux related proteins for reversing multidrug resistance,[12,13] cancer metastasis inhibition,[14] reactive oxygen species (ROS) induction,[15] and anti-angiogenesis.[16] The anticancer effects of DSF have been actively explored. For example, it has been reported that the anti-angiogenesis of DSF was associated with the inactivation of Cu/Zn superoxide dismutase (SOD) and inhibition of matrix metalloproteinases (MMP).[16,17] DCC, the metabolite of DSF, also showed the strong inhibition of vascular SOD-1 in the isolated canine basilar arteries.[18]

The high level of copper ions in the tumors may be related to cancer progression.[19] The anticancer action of DSF requires the involvement of copper (Cu2+).[7] In vivo, DSF rapidly metabolically transforms into DDC that possesses a strong capacity of chelating metal ions (eg, copper, zinc, and iron). The thus-formed Cu(DDC)2 complex is the major active effector in DSF-based cancer therapy. Besides, the planar conformation of DSF/Cu chelate is beneficial to deactivate DNA replication enzymes, thus emerging as a potent radiotherapy sensitizer.[20]

2.1 Regulating onco-genetic signaling pathways

The cytotoxicity of disulfiram is copper-dependent. The potent chemotherapeutic effect of DSF especially DSF/Cu has been widely reported and one of the main mechanisms
FIGURE 1  Schematic illustration of the anti-cancer mechanisms of disulfiram

is that DSF/Cu suppresses AKT and cyclin D1 signaling, activates c-Jun N-terminal kinase (JNK), and inhibits NF-κB signaling, thus inducing apoptosis. [21,22] Besides, DSF was reported to inhibit tumor-initiating hepatocellular carcinoma cells by downregulating the mitogen-activated protein kinase (MAPK) signaling pathways. [23]

2.2  |  Suppression of proteasome

The ubiquitin-proteasome system is a primary mechanism for the degradation of endogenous proteins and is essential for the coordination of the cell cycle and apoptosis regulation. It has been reconciled that DSF-mediated proteasome inhibition was rather the processing of proteins ubiquitination by the p97-NPL4-UFD1 pathway than directly targeting the 20S or 26S proteasome. [6] The internalized DSF/Cu binds NPL4 and induces NPL4-p97 aggregation, and thus causes the deactivation of p97 and segregates UFD1 eventually cell death. [6] Moreover, the overexpression of p97 is related to the progression and metastasis of various kinds of cancers. [24–26] Thence, the p97-NPL4 pathway plays an essential role in DSF-based cancer therapy.
2.3 | Suppression of cancer cell stemness

Cancer stem cells (CSCs) are the hallmarks of cancer heterogeneity and malignancy relevant to tumor progression, metastasis, and drug resistance.\(^{[27]}\) The CSCs possess abnormally active acetaldehyde dehydrogenase and DSF is an efficacious inhibitor of ALDH in a Cu\(^{2+}\)-dependent manner to deplete CSCs.\(^{[28]}\) It was reported that DSF/Cu eliminated the stem cell-like ALDH\(^+\) cancer cells via ALDH1AI inhibition and Hedgehog pathway, as well as inhibited the cell stemness transcription factors Nanog and Oct-4 and thereby prevented the proliferation of multiple myeloma.\(^{[29]}\) Besides, the highly metastatic osteosarcoma cells were characterized by ALDH over-expression and were more invasive than the cells with ALDH low-expression.\(^{[30]}\) DSF, the irreversible inhibitor of ALDH, effectively blocked ALDH activity to produce fewer filopodia of metastatic osteosarcoma cells, thereby potently inhibiting metastasis.\(^{[31,32]}\)

A recent study revealed a different opinion that the real inhibitor of ALDH was a metabolite of DSF (S-methyl-N, N-diethyldithiocarbamate-sulfoxide, Me-DTC-SO), instead of DSF.\(^{[33]}\) Me-DTC-SO inhibited ALDH activity but failed to suppress the proliferation of cancer cells. Therefore, further investigation should be carried out to illustrate the details of targeting ALDH as a pathway for anticancer.

2.4 | Induction of reactive oxygen species

Reactive oxygen species (ROS) is at a low level at physiological conditions, and the increased intracellular ROS could activate various cell death pathways, or inhibiting cancer resistance.\(^{[34]}\) DSF alone hardly induces ROS, but the DSF/Cu complex strongly triggers intracellular ROS generation and activates its downstream JNK and p38 MAPK pathways, thereby inducing apoptosis, autophagy, or necroptosis.\(^{[23,35]}\) A recent study reported that DSF/Cu inhibited CSCs via ALDH, Sox, Nanog, and Oct signaling, and modulated intracellular ROS generation to enhance the sensitivity of breast cancer to cisplatin treatment.\(^{[36]}\)

A different conclusion was also reported that the anticancer activity of the preformed DSF/Cu was not mediated by the generation of ROS, because the results revealed that ROS generation was a by-product during the formation of DSF/Cu chelate and they also verified that no increase in ROS production was observed when cells were incubated with preformed DSF/Cu.\(^{[37]}\) Similarly, it was reported that DSF/Cu effectively killed cancer cells through paraptosis (a caspase-independent cell death) but not ROS induction.\(^{[38]}\) Therefore, the role of ROS in DSF-mediated anticancer action could vary in different cases and further works need to be conducted to depict the detailed mechanisms.

2.5 | Antiresistance

Drug resistance is a formidable bottleneck in cancer therapy and predominantly causes the failure of chemotherapy. The factors cause drug resistance, including enhanced tumor cell stemness, overexpression of drug efflux transporters, enhanced DNA damage repair, mutation of drug targets, and tumor microenvironment.\(^{[39]}\) DSF has been reported with the functions of antiresistance. For example, DSF showed the effective inhibition of P-glycoprotein (P-gp) potentially via cysteine covalent modification, thereby blocking P-gp-mediated drug efflux.\(^{[40,41]}\)

The combination therapy of DSF and fluorouracil (5-FU) caused the inhibition of NF-kB pathway and disrupted the differentiated non-stem and stem cell-like cells in pancreatic cancer patients.\(^{[42]}\) Moreover, DSF can also enhance the cytotoxicity of chemotherapeutic agents; for instance, DSF/Cu effectively reversed the resistance of gemcitabine in human colorectal cancer cells (NCI-H630) and breast cancer cells (MCF-7) by obstructing nuclear translocation of NF-kB induced by gemcitabine.\(^{[43]}\)

Targeting ALDH by DSF (DSF/Cu) is a potential method to resensitize the multidrug-resistant tumor to chemotherapeutics. For example, the non-small cell lung cancer (NSCLC) cells with resistance to cisplatin displayed stem cell-like properties with the upregulated ALDH1, and DSF/Cu re-sensitized the cells to cisplatin.\(^{[44]}\) A study revealed that DSF inhibited the NF-kB pathway and CSCs to reverse pan-chemoresistance.\(^{[13]}\) Besides, other anti-tumor activities (e.g. proteasome inhibition and tumor microenvironment regulation) mediated by DSF were reported to be effective in reversing drug resistance.\(^{[45,46]}\)

2.6 | FROUNT inhibition

FROUNT, a CCR2-binding protein, is a common regulator of chemokine receptors signaling to modulate macrophage migration and is highly expressed in tumor-promoting macrophages (M2-like).\(^{[47,48]}\) It was proposed that DSF could target FROUNT to regulate tumor-associated macrophages (TAMs), which indicated the potential of DSF in tumor microenvironment regulation.\(^{[48]}\) In this study, DSF was demonstrated to be a potent inhibitor of FROUNT by directly binding FROUNT and blocking the FROUNT-chemokine receptor interactions. The FROUNT deficiency effectively decreased the recruitment of macrophages into the tumor and abrogated the tumor-promoting activity of TAMs. Besides, the tumor cell
(BT-474) proliferation was inhibited by trastuzumab and pertuzumab plant biosimilars in combination with DSF that served as a booster and adjuvant for the therapeutic antibodies.\[49\] Therefore, DSF-based FROUNT targeting is a promising approach for cancer immunotherapy via regulating TAMs.

3 | IMPROVING DISULFIRAM-BASED CANCER THERAPY THROUGH NANOTECHNOLOGY

Although the broad anticancer activities of DSF have been well demonstrated, it is a challenge to achieve a satisfying treatment efficacy and clinical applicability. DSF is a hydrophobic compound (with an aqueous solubility of 0.2 mg/mL) and is unstable under acidic conditions (eg, stomach) and fast clearance from the bloodstream in vivo.\[50,51\] Besides, DDC, the main metabolite of DSF with potent antitumor activity, is also unstable.\[52\] Moreover, DSF medication should be avoided in patients with certain diseases, including myocardial or coronary occlusion, psychosis, and allergy to DSF or its derivatives.\[53\]

Therefore, it is difficult to use the conventional oral administration of DSF to achieve sufficient efficacy. There is an urgent need to develop an effective DSF delivery strategy to maintain its stability in vivo. Recently, DSF nano-formulation has attracted great attention as a potential solution. Nanotechnology can increase the aqueous solubility of DSF and tumor-targeted delivery efficiency and thus enhance therapeutic efficacy. Various therapeutic delivery systems based on nanotechnology (eg, liposomes, micelles, and protein/lipid/polymer-based NPs) have been developed for improving the stability and delivery of DSF and potentiating its anticancer efficacy. For example, the methoxy poly(ethylene glycol)-b-poly(lactide-co-glycolide)/poly(ε-caprolactone) (mPEG-PLGA/PCL) mixed nanoparticles (NPs) encapsulated with DSF showed high stability and protected DSF from degradation, and such NPs efficiently improved the DSF bioavailability and effectively suppressed the 4T1 murine xenograft tumor.\[54\]

4 | NANOTECHNOLOGY-BASED DISULFIRAM DELIVERY FOR CANCER THERAPY

Nanocrystals are the nano-sized drug crystalline and can be simply prepared and display good stability in a nanodispersion state with a stabilizer, such as a surfactant or a polymer material.\[57\] Nanocrystallization provides a promising solution to increasing the bioavailability of insoluble drugs. Importantly, there is a minimal amount of carrier excipients used in the nanocrystalline preparation, thus yielding a very high drug loading capacity. With these advantages, nanocrystal technology has been extensively developed and oral nanocrystal formulations have been marketed for clinical application.

It was reported that the hybrid PTX-DSF nanocrystals (PTX-DSF Ns), defined as the “drug-delivering-drug (DDD)” strategy, were used to reverse multidrug-resistance in lung tumor (A549 cells with Taxol resistance) (Figure 2).\[12\] In this strategy, the cocystal of DSF/paclitaxel (PTX) was prepared and β-lactoglobulin served as a surface-coating stabilizer (Figure 2A). The β-lactoglobulin-modified nanocrystal improved the bioavailability of both PTX and DSF with the increased AUC by 77- and 31-fold than free drugs, respectively (Figure 2B,C). Besides, no significant hemolysis (less than 20%) of the PTX-DSF Ns was observed even at a high concentration (5 mg/mL). The PTX-DSF Ns were internalized through caveolae-mediated endocytosis with a 14-fold increase in cellular uptake and a fivefold enhanced apoptosis rate than PTX alone and thus overcame paclitaxel-resistance in the A549 lung tumor (Figure 2D).

4.2 | Inorganic nanocarriers

Mesoporous silica nanoparticles (MSN), as the potential drug carriers, are known for their large surface area and tunable mesoporous structure. Besides, MSNs can accommodate a large number of molecules with mono-disperisty and dispersity property.\[58\] The loaded molecules can be controlled released under specific physiological conditions. A smart strategy of Cu^{2+}/DSF in situ chelation was reported with Cu^{2+} and DSF separate
encapsulation into hollow mesoporous silica nanoparticles (HMSNs) for breast cancer (4T1) therapy (Figure 3A-E).\cite{59} The PEGylated HMSNs were Cu\textsuperscript{2+}-doped with a pH-triggered release feature and served as the carriers for DSF (DSF@PEG/Cu-HMSNs) (Figure 3B). The PEGylation prevented the nanomedicine from de-structure in the circulation and facilitated intratumoral retention. The slightly acidic tumor microenvironment cleaved the Cu–O bonds and caused the nanocarrier degradation, and the released Cu\textsuperscript{2+} and DSF developed in situ chelation. The intermediate product (Cu\textsuperscript{+}) also reacted with hydrogen peroxide (H\textsubscript{2}O\textsubscript{2}) through a Fenton-like reaction to generate the cytotoxic hydroxyl radicals (\cdotOH; Figure 3C), thus the anticancer efficacy of DSF/Cu chelation was synergistically enhanced (Figure 3D,E).\cite{59}

In another study, a multifunctional hollow mesoporous Prussian blue (HMPB) was designed for in situ chelation-activated and hyperthermia-amplified chemotherapy (Figure 3F-H).\cite{60} Prussian blue (PB), an FDA-approved antidote to remove radioactive cesium and thallium from the body, is safer than other inorganic carriers. The controlled chemical etching HMPB was doped Cu\textsuperscript{2+} with polyvinylpyrrolidone decoration (PVP/Cu-HMPB) and DSF was encapsulated into the hollow mesoporous structure (Figure 3F). After the DSF@PVP/Cu-HMPB passively accumulated in the tumor, Cu\textsuperscript{2+} and DSF were released due to the acidic condition that triggered the Cu–C≡N–Fe bonds rupture. Subsequently, the cytotoxic chelate of DSF/Cu was formed and thus suppressed tumor growth (Figure 3F-H). Notably, the photothermal properties of PB further augmented the therapeutic efficacy of DSF/Cu under NIR irradiation (Figure 3F-H).\cite{60}

An inorganic carrier was utilized for tumor delivery of DSF, and the DSF-loaded gold nanorods were developed with a high drug encapsulation (23.2%) for tumor targeting and enhanced chemotherapeutic efficacy due to the high intracellular copper concentration of breast cancer.\cite{61} This system achieved stimuli-responsive action for synergistic chemo-photothermal therapy triggered by glutathione, acid, and laser-photothermal.
4.3 | Liposomes and lipid nanoparticles

The advantages of liposomes include high drug loading capability of both hydrophobic and hydrophilic molecules, biocompatibility, and drugs-controlled release feature. Various liposomal technologies have been developed to improve bio-fate and enhance drug targeting efficiency, which includes PEGylation, co-delivery of multiple drugs, immunoliposomes and stimuli-responsive systems.

Cell-penetrating peptides (CPP) are widely investigated in drug delivery because of their membrane penetration capability to enhance cellular internalization of cargos. However, the main shortcoming of CPP-mediated delivery is the non-specific uptake in normal cells; and the “smart” strategies have been developed to address this issue. A pH-sensitive lipid nanocapsule (DSF-S-LNCs) for DSF delivery was proposed, in which the protective shielding materials (PEG-PGA) were fragmented preferentially in the acidic tumor microenvironment and the TAT peptides were exposed to mediate intracellular penetration, and thereby the unwanted intracellular uptake in normal cells reduced. Subsequently, DSF was released and chelated with the abundant intracellular Cu²⁺ in the hepatic carcinoma cells, and the thus-formed DSF/Cu mediated tumor apoptosis (Figure 4A). The pharmacokinetic profile was improved with an approaching 4-fold increase in plasma AUC in the DSF-S-LNCs (Figure 4C). Besides, owing to the low pH-triggered TAT peptide exposure and the consequent enhancement of cellular internalization, the anticancer activity was higher at acidic pH than at physiological pH (Figure 4B).

Other lipid carriers have also been reported for tumor-targeted delivery of DSF. A thermo-responsive nanostructured lipid system was formulated for cancer diagnosis and therapy, which was composed of the organic phase-change materials (lauric acid and stearic acid, 4:1, w/w), and co-encapsulated CuET (DSF/Cu) and DIR (an near-infrared dye; CuET/DIR NPs), and featured by self-traceability for bioimaging and photo-responsive drug accumulation. When NIR irradiation, the nanoparticles were quickly heated by the photothermal DIR and subsequently the phase-change materials melted beyond the eutectic point, and thus caused the quick release of DSF/Cu to induce the pro-apoptotic effects.

4.4 | Polymer nanoparticles

Biodegradable polymers provide many advantages for drug delivery, including good biocompatibility, high drug loading performance, bioavailability improvement, and reduction of side toxicity of the therapeutics. For example, poly(lactic acid-co-glycolic acid) (PLGA), approved by
FDA for injection use, is widely utilized in drug delivery, tissue engineering, and medical devices.\textsuperscript{74} The PLGA-based nanoparticles have been reported for DSF antitumor therapy. The preparation processes (eg, PVA incorporation and sonication time) of the PLGA nanoparticles closely influenced the release profiles of DSF.\textsuperscript{75} For example, the increase in the amount and molecular weight of PVA (a stabilizer) and the sonication time resulted in the decreased particle size and serum stability but yielded a fast DSF release and high cytotoxicity. A polysorbate 80-stabilized PLGA nanoparticle system was prepared for DSF delivery, in which there were dual functions of polysorbate 80 modification: evading clearance of the mononuclear phagocytic system and achieving tumor-targeted delivery via EPR effect; such a DSF-based nanodrug suppressed cell proliferation by cell cycle arrest and activation of apoptotic pathways.\textsuperscript{76} In another study, a PLGA-PEG nanoparticle system was developed to deliver DSF for breast cancer therapy by targeting folate receptors.\textsuperscript{77} The PEG modification prevented premature drug release and elimination and the surface-modified folate mediated biomimetic delivery of DSF into breast cancer cells through receptor-mediated endocytosis; this delivery system thereby efficiently inhibited cell proliferation by ROS and apoptosis induction.

Polymer micelles are a class of biocompatible carriers self-assembled by amphiphilic polymer and are promising in drug delivery. An ideal micelle delivery system should be with low critical micelle concentration (CMC) to remain stable during dilution in biological fluids and possess sustained drug release.\textsuperscript{78,79} The synthesized amphiphilic poly (ε-caprolactone)-b-poly (L-glutamic acid)-g-methoxy poly (ethylene glycol) copolymer (PCL-b-PGlu-g-mPEG) was utilized to prepare the core-shell-corona multilayered nanoparticles, in which PCL and DSF formed the hydrophobic core and the anionic poly (glutamic acid) shell facilitated the encapsulation of cationic DOX (Figure 5A-C).\textsuperscript{80} The PEG corona contributed to maintaining the stability of micelles, with an adequate CMC of $3.98 \times 10^{-4}$ mg/mL (Figure 5B). DSF and DOX were released in the acidic tumor microenvironment and...
carried out a synergistic antitumor effect to effectively suppress the breast tumor growth (Figure 5A,C). Besides, a smart polymer-drug conjugate (SMA-ADH-DOX, SAD) was synthesized, which was composed of doxorubicin (DOX) and poly(styrene-co-maleic anhydride) (SMA) derivative with adipic dihydrazide (ADH) conjugation by an acid-sensitive hydrazone bond, and could self-assemble into the pH-response micelles with encapsulation of DSF (as a P-gp inhibitor) for combination therapy against the drug-resistant breast cancer (MCF-7/ADR) (Figure 5D-F).[81] This codelivery micelle exhibited several advantages, including long circulation, increased tumor accumulation, and temporal release in the tumor site (Figure 5E,F). The SAD was cleaved in the lysosome for sustained release of DOX but a fast release of DSF. In this manner, the DSF in burst release could inactivate P-gp and trigger the apoptotic pathways, thus effectively increasing the intracellular DOX accumulation and arresting the growth of the drug-resistant breast tumor.

A composite polymeric nanogel was developed to deliver copper ions and trapping DSF to enhance antitumor efficacy.[82] Poly[2-(N-oxide-N,N-dimethylamino)ethyl methacrylate] (OPDMA) is a water-soluble tertiary amine oxide-based zwitterionic polymer and can enter the cancer cells via macrocytosis and accumulate into the mitochondria.[83] OPDMA can bind with copper ions to form the OPDMA/Cu nanogels. The OPDMA/Cu nanogels accumulated to tumors after intravenous injection, and subsequently, oral administration of DSF was carried out. The intratumorally released copper ions captured DSF to form CuET in situ, thereby eliciting the potent antitumor effect.[82] Besides, a redox-responsive DDC-polymer conjugate, composed of poly[(2-(pyridine-2-ylsulfanyl) ethyl acrylate)-co-[poly(ethylene glycol)] (PDA-PEG), lactobionic acid (LBA), and DSF, was used for preparing the polymer-prodrug nanoparticles (LBA-PDA-PEG-DSF NP, LDNP) for metastatic ovarian cancer therapy.[84] The LBA ligand can mediate targeting delivery
to the ovarian cancer cells with β-D-galactose receptor overexpression. Subsequently, the high-level intracellular glutathione cleaved the disulfide bonds to release DSF.

4.5 | Biomimetic delivery

The biomimetic drug delivery systems have been widely applied for targeted delivery and attracted significant attention in recent years. The common overexpression of nutrients transporters in cancer cells to meet their energy and material requirements. Therefore, nutrient transporter-mediated tumor biomimetic targeting delivery has become a useful strategy and a variety of nutrient transporters have been exploited for this purpose, including albumin binding proteins (ABP), transferrin receptors (TfR), and low-density lipoprotein receptor-related protein 1 (LRP-1).[85]

Albumin is the most abundant plasma protein and is a multifunctional protein with the capacity for cargo transportation. ABP (eg, secreted protein acidic and rich in cysteine [SPARC] and albondin [gp60]) are overexpressed in malignant tumor cells and its surrounding stromal cells associated with neoplasia and invasiveness.[86] Albumin-based drug delivery can reduce systemic clearance, although the denatured or structurally altered nanoparticles could be cleared away faster than natural albumin that has an approaching half-life of 19 days).[87] Besides, evidence has been shown that the overexpression of SPARC on cancer cells can facilitate albumin binding and enhance the intratumor accumulation of albumin-based drug delivery systems.[88,89] A clinical trial showed that Abraxane® (albumin-bound paclitaxel) yielded improved clinical outcomes in SPARC-positive patients.[89] Based on these benefits, attempts at using albumin as a DSF carrier have been executed.

The albumin-corona camouflaged strategy is widely employed. The disguised albumin-corona can prolong the blood circulation of nanoparticles by suppressing the opsonization process and thus reducing the interplay of IgG and nanoparticles, complement activation, and macrophage clearance.[90] For instance, the DSF-encapsulated cationic chitosan nanoparticles were coated with anionic albumin, and the albumin-corona reduced the nonspecific cellular uptake and autoimmune.[91] In the slightly acidic tumor microenvironment, the coating albumin dissociated from the nanoparticles due to the diminished charge interaction, and the cationic chitosan nanoparticles mediated efficient cell penetration for eliminating the colon cancer stem cells.

A novel stabilized metal ion ligand complex (SMILE) method was developed via 3D-printed microfluidic technology for preparing the biomimetic metal-organic nanoparticles (MON).[92] The MON was composed of the Cu(DDC)₂ core and the bovine serum albumin (BSA) shell. The BSA shell effectively protected Cu(DDC)₂ MONs from aggregations by coordination interactions between Cu²⁺ and the amino acid residues of BSA (eg, Lys 199) to form a protective layer. The BSA-Cu(DDC)₂ MONs displayed a high tumor inhibition activity in an orthotopic 4T1 breast tumor model due to the effective tumor accumulation through EPR effects and the SPARC receptor-mediated targeting delivery. Furthermore, the SMILE and 3D-printed microfluidic technology could precisely control the mixing process of DDC and Cu²⁺, and was promising in clinical translation.

Hyaluronic acid (HA), a natural, biocompatible polymer with a targetability to the cluster of differentiation-44 (CD44) receptors, has also been widely used in cancer-targeting biomimetic delivery. CD44 is overexpressed in a variety of solid tumors and is involved in tumor cell migration and adhesion, tumor microenvironment signal regulation, tumor cell stemness.[93] The biomimetic HA-modified metal (Cu³⁺) coordination DSF nanoparticles (CuET@HA NPs) with dual pH- and redox-responsive delivery were prepared for cancer therapy through the p97-NPL4-UFD1 pathway intervention.[94] The HA decoration not only reinforced the stability of the nanoparticles and prolonged their blood circulation but also enhanced the accumulation of the CuET@HA NPs in the CD44-overexpressed cancer cells.

There was another interesting case that could marginally fit for this category. A Cu²⁺ nanoparticle-induced DSF capture strategy in the tumor was proposed.[95] The nanoparticles were prepared by covalently cross-linking ferritin and albumin, then the copper ions were encapsulated into the ferritin nanocage to form ferritin–albumin–Cu nanoparticle (FHC NP). The i.v. injected FHC NP was in combination with oral administration of DSF, and the treatment showed desired tumor accumulation and enhanced anti-tumor efficacy.

5 | DSF-BASED COMBINATION THERAPY VIA NANOTECHNOLOGY

Combination therapy can enhance efficacy minimize the adverse reactions in cancer treatment due to the synergistic therapeutic effect. Effective combination therapy is based on the optimum synergistic ratio of multiple drugs to maximize the therapeutic effect. However, it is difficult to synchronize drug exposure in tumor tissues due to the different pharmacokinetics between the combined drugs. Ideally, combination therapy should follow the “3R” delivery principle — effective delivery of multiple drugs to the Right place with the Right dose at the Right time
FIGURE 6  A schematic illustration of pharmacotherapy that is characterized by 3R delivery principle (effective delivery of multiple drugs to the Right place with the Right dose at the Right time). Reproduced with permission under the terms of the Creative Commons license.

— to optimize cancer synergistic therapy (Figure 6).

Nanotechnology-based combination therapy provides a useful tool to approach this goal. By co-encapsulation of two drugs into the same carrier, it can achieve the synchronized delivery at the desired site with a fixed-dose ratio. The co-encapsulation nanotechnology contributes to synchronize the pharmacokinetic behavior of the combined drugs. For example, the synergistic ratios of cytarabine and daunorubicin could be maintained for over 24 h in the plasma after co-encapsulated in a liposome (CPX-351).

5.1  DSF-based combination therapy for overcoming drug resistance

Due to the antiresistance potential of DSF, it has been widely utilized in combination therapy against drug resistance. It was reported that DSF could target CSCs and thus enhanced the sensitivity of paclitaxel in drug-resistant triple-negative breast cancer cells via a mechanism of suppressing the NF-κB-related stemness gene pathway. A DSF and DOX codelivery liposomal was developed for reversing multidrug resistance in breast cancer. These PEGylated liposomes were long-circulating and intracellularly released DSF to suppress the P-gp in the resistant cancer cells via sulfhydration of P-gp and its ubiquitination, and re-sensitized the resistant cancer cells to the subsequently reversed DOX.

In another study, the Cu(DDC)₂ nanoparticles were prepared by the SMILE method with a high drug-loading capacity for overcoming drug-resistant prostate cancer. The SMILE technology is a simple and green method for Cu(DDC)₂ NPs preparation, without the involvement of a complicated process, toxic organic solvents, and purification process. The core-shell-structured PEG-PLA/Cu(DDC)₂ NPs were formed by incorporating methoxy PEG 5000-b-poly(L-lactide) 5000 (PEG–PLA), and the NPs efficiently inhibited drug-resistant prostate cancer cells (DU145-TXR) proliferation through paraptosis.

The albumin nanoparticle co-encapsulated with regorafenib (Rego) and DSF/Cu also attained an improved therapeutic effect in reversing multidrug resistance in colorectal cancer therapy. The DSF-based albumin nanoparticle was prepared by a mild urea/NaBH₄ denaturation method with hydrophobic drug-induced self-assembly (Figure 7A). The HCT8/ADR cells and tumor-associated macrophages (TAMs) were overexpression of both SPARC and mannose receptor (MR, or CD206) (Figure 7B). Therefore, the albumin nanoparticle (Man-BSA NP) with surface modification with mannose simultaneously targeted the tumor cells and TAMs and via a so-called “two-bird-one-stone” delivery and therapeutic strategy for reversal of treatment resistance (Figure 7B–E). TAMs are a major population of immune cells in the tumor microenvironment and highly plastic and can be switch between the phenotypes of protumor M2 and antitumor M1. TAMs are closely associated with therapeutic resistance. Furthermore, a similar strategy was developed by using the TRR-binding T12 peptide (THRPPMWSPVWP) modification for preparing the T12 decorated albumin nanoparticles (T12-BSA NPs) coloaded with Rego and DSF/Cu for treating osimertinib-resistant NSCLC and its brain metastases. EGFR tyrosine kinase inhibitors (TKI)-based therapy has been used as first-line therapy in NSCLC with EGFR mutation, represented by the third-generation TKI osimertinib treatment. But there still is a formidable challenge that the rapid development of TKI resistance and high incidence of brain metastasis cause high mortality of NSCLC. The T12-BSA NPs effectively repolarized the tumor-promoting CD206hi TGF-β1+ TAMs via inhibition of FROUNT and thus remodeled tumor immune microenvironment for overcoming osimertinib-resistant NSCLC and its brain metastases.

5.2  DSF-based combination therapy for modulating the tumor microenvironment

DSF/copper-based combination therapy in glioblastoma patients has been in clinical trials (NCT03363659). It was reported DSF based-combination therapy took action through not only inducing apoptosis of the cancer cells but also remodeling the tumor microenvironment; for example, a similar combination of Rego and DSF/Cu
FIGURE 7  Albumin-based dual-targeting biomimetic delivery of Rego and DSF/Cu for cancer therapy via remodeling the tumor microenvironment. (A) Schematic illustration of the biomimetic albumin nanoparticles prepared by the mild urea/NaBH₄ denaturation method. (B, F) The expression of the nutrient transporters. (C) The expression of SPARC and MR in M2 phenotype macrophages. (D) Tumor weight of HCT8/ADR xenografts after treatment. (E) The TAM (M2) population in THE tumor tissues after treatment. (G) The treatment efficacy of GL261 orthotopic glioma. (H) The expression of MR and B7-H4 in glioma tissues after treatment. (I) The M2 macrophage (marked by CD206) and M1 macrophage (marked by CD80) in orthotopic glioma tissues after treatment. (A-E) Reproduced with permission. [98] Copyright 2017, Wiley-VCH. (F-I) Reproduced with permission under the terms of the Creative Commons license. [101] Copyright 2018, Royal Society of Chemistry.
relied on the albumin codelivery nanoparticles was used for glioma therapy by remodeling the tumor microenvironment and directing macrophage-mediated immunotherapy.[101] In this study, T12 peptide and mannosene were used to modify the albumin nanoparticles (T12/Man BSA NP) for penetrating the blood-brain barrier and enhancing the glioma-targeting efficiency. After treatment with the T12/Man BSA NP, the orthotopic gliomabearing mice survived longer than any control group (Figure 7F,G).[101] In addition to the M2 macrophage repolarization effect, the T12/Man BSA NP also regulated the T cell immunity and intermingled the interplay between Treg and macrophage in the tumor microenvironment via suppressed expression of the B7-H4 (Figure 7H,I). In the tumor microenvironment, Treg induces the suppressive signal to inhibit T-cell immunity.[102]

Besides, a multitargeting liposomal system (CDX-LIPO) modified by the α7 nicotinic acetylcholine receptors (nAChRs) affinity peptide CDX (GREIRGRAERWSEKF, D-form sequence) was developed for codelivery of honokiol and DSF/Cu for glioblastoma therapy by reprogramming tumor metabolism and immune microenvironment via the regulation of mTOR (mammalian target of rapamycin) pathway.[103] mTOR signaling is closely associated with the modulation of tumor immunity and metabolism. The CDX-LIPO induced immunogenic cell death and activated dendritic cells that primed T and NK (natural killer) cells, resulting in antitumor immunity and tumor regression. In addition, the CDX-LIPO promoted M1-macrophage polarization and facilitated mTOR-mediated reprogramming of glucose metabolism in glioma.

6  |  SUMMARY AND OUTLOOK

Repositioning the approved old drugs for new indications is an efficient and economical way to develop new medicines. Nanotechnology provides a useful tool for targeted delivery and controlled release of drugs and thus can play an important role in modulating the in vivo fate of the drugs for meeting the requirements of treating the proposed indications. By using a nano-formulation, the treatment efficacy of DSF can be enhanced while reducing the unwanted side effects.

More importantly, nanotechnology offers a method for synchronized delivery to a target at a fixed-dose ratio for combination therapy and bridging the gap between in vitro tests and in vivo studies. Indeed, the combined drugs with different pharmacokinetic properties often fail to yield a synergistic effect in vivo. By using advanced delivery techniques, DSF-based nanomedicine would achieve targeted delivery and spatiotemporal controlled release, thus providing a potential solution to address the 3R principle to maximize the efficacy of combination therapy. Of note, the marketing of the combination liposome (Vyxeos®) in 2017 represents a groundbreaking advance in nanomedicine. It unveils a new realm for nontherapeutic research. Indeed, the nanotechnology application for combination therapy has still been underexplored. Single-use of DSF has already exhibited the anticancer therapeutic potential and it is expected that nanotechnology will further promote the application of DSF in combination with other anticancer drugs, eventually promoting clinical translation.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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