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Disulfiram: A novel repurposed drug for cancer therapy

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Abstract

Cancer is a major global health issue. Effective therapeutic strategies can prolong patients' survival and reduce the costs of treatment. Drug repurposing, which identifies new therapeutic uses for approved drugs, is a promising approach with the advantages of reducing research costs, shortening development time, and increasing efficiency and safety. Disulfiram (DSF), a Food and Drug Administration (FDA)-approved drug used to treat chronic alcoholism, has a great potential as an anticancer drug by targeting diverse human malignancies. Several studies show the antitumor effects of DSF, particularly the combination of DSF and copper (DSF/Cu), on a wide range of cancers such as glioblastoma (GBM), breast cancer, liver cancer, pancreatic cancer, and melanoma. In this review, we summarize the antitumor mechanisms of DSF/Cu, including induction of intracellular reactive oxygen species (ROS) and various cell death signaling pathways, and inhibition of proteasome activity, as well as inhibition of nuclear factor-kappa B (NF-κB) signaling. Furthermore, we highlight the ability of DSF/Cu to target cancer stem cells (CSCs), which provides a new approach to prevent tumor recurrence and metastasis. Strikingly, DSF/Cu inhibits several molecular targets associated with drug resistance, and therefore it is becoming a novel option to increase the sensitivity of chemo-resistant and radio-resistant patients. Studies of DSF/Cu may shed light on its improved application to clinical tumor treatment.

Keywords: Disulfiram; Aldehyde dehydrogenase; Reactive oxygen species; Proteasome activity; Cancer stem cells; Drug resistance

Introduction

Cancer is becoming one of the most common causes of death, and its prevalence is expected to increase worldwide.[1] Developing effective new pharmacotherapies improves survival and reduces mortality of patients with cancer. Currently, in addition to radical surgery, radiotherapy, and immunotherapy, chemotherapy that employs broad-spectrum cytotoxic drugs remains one of the most effective cancer treatments, despite having significant side effects.[2] Thus, discovering new anticancer drugs is of great importance for fulfilling a highly unmet medical need. However, developing new anticancer drugs is challenging because of high cost and being time consuming. To overcome these challenges, drug repurposing is a practical alternative strategy for using approved drugs with known toxicological and pharmacokinetic characteristics for new indications, which saves research costs and reduces the time to find new ways to treat various diseases.[3,4]

Disulfiram (DSF), the Food and Drug Administration (FDA)-approved drug to treat chronic alcoholism, has been used since 1951 and is well tolerated with minimal side effects.[5] DSF irreversibly inhibits the activity of aldehyde dehydrogenase (ALDH), leading to excessive accumulation of acetaldehyde in the body, thereby establishing the alcohol aversion reflex.[6] Recently, growing evidence shows the potential of repurposing DSF to treat various pathologies such as inflammation, Lyme disease, metabolic disorders, and cancer.[7–10] Numerous mechanistic studies reveal that DSF exhibits excellent anticancer effects such as triggering oxidative stress,[11] inhibiting proteasomes activity,[12] reducing angiogenesis,[13] arresting the cell cycle,[11,14] reducing the stemness of cancer cells,[15] reversing drug resistance,[16,17] constraining tumor metastasis,[18,19] and regulating the immune microenvironment.[20,21]

Currently, the studies on effects of DSF are progressing through several clinical trials designed to treat malig-

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nant tumors, including glioblastoma (GBM), metastatic breast cancer, and recurrent pancreatic carcinoma. Furthermore, the trace metal copper (Cu) plays a key role in potentiating the antitumor effect of disulfiram.[22] In this review, we summarize the molecular mechanism of DSF and its metabolites in the treatment of cancers, and we evaluate the contribution of DSF/Cu to enhancing drug sensitivity or reversing drug resistance, which will contribute comprehensive data for repurposing DSF in the future.

Anticancer Mechanisms of DSF/Cu

Numerous studies reveal that DSF serves as an antitumor drug. Although the anticancer mechanism of DSF is unclear, there is no doubt that the combination of DSF and Cu^{2+} achieves a better antitumor effect than DSF alone.[23–25] Strikingly, Cu^{2+} is essential for human cells as it participates in numerous processes such as mitochondrial respiration, reactive oxygen species (ROS) generation, and antioxidant/detoxification processes.[26,27] Furthermore, mounting evidence indicates that patients with malignancies have significantly higher levels of serum Cu and intracellular Cu compared with those of healthy controls.[28] Cu plays a prominent role in oncogenesis, cancer progression and severity, because Cu accumulation promotes cell proliferation, angiogenesis, and metastasis.[27,29] Elevated Cu levels in tumor cells serve as a specific target for DSF, which binds tumor cellular copper and impairs the activities of Cu-dependent enzymes, leading to inhibition of cuproplasia (Cu-dependent cellular proliferation).[30] On the other hand, a high concentration of Cu in cancer cells causes cytotoxicity through oxidative stress or by inhibiting enzyme activity to induce specific copper-dependent cell death, called cuproptosis.[31] The Cu ionophore DSF facilitates increased Cu uptake into cancer cells, enabling DSF to specifically target cancer cells while sparing normal cells.[32] Numerous studies show that the administration of DSF with Cu significantly increases anticancer activity.[33,34] Recent mechanistic studies demonstrate that $Cu(DDC)_2$ (bis-diethyldithiocarbamate-copper, also known as CuET), which is a major metabolite of DSF combined with Cu^{2+}, is the active form responsible for its tumor suppressing effects [Figure 1].[35,36] Because $Cu(DDC)_2$ is a potent anticancer agent, we focused on several targets of $Cu(DDC)_2$, including alteration of ROS levels, activation of the mitogen-activated protein kinase (MAPK) pathway, and inhibition of ubiquitin proteasome activity, as well as suppression of NF-κB signaling. Strikingly, apart from $Cu(DDC)_2$, the $Zn(DDC)_2$ complex formed by DDC binding to Zn^{2+} also represents an important antitumor activity, confirming that DSF-based tumor therapy is metal ion-dependent.[37]

Effects of DSF on ROS

Oxidative stress occurs when the accumulation of ROS exceeds the body's antioxidant capacity. Increased ROS levels are toxic by destroying cellular structures and damaging vital organs, leading to cell death.[38] DSF-mediated cytotoxicity is partially caused by increased

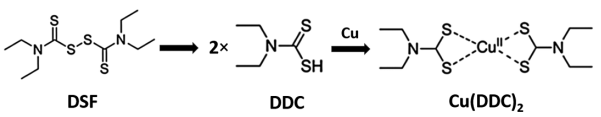

Figure 1: Chemical structure of DSF, DDC, and $Cu(DDC)_2$. $Cu(DDC)_2$: Bis-diethyldithiocarbamate-copper; DDC: Diethyldithiocarbamate; DSF: Disulfiram.

ROS production. Evidence indicates that excessive ROS exposure will exhaust cellular antioxidant capacity and selectively induce cancer cell apoptosis.[38] Accumulation of DSF, DDC, and its copper complex $Cu(DDC)_2$ in cancer cells can promote ROS generation, which eventually triggers apoptosis of cancer cells.[39,40] DSF/Cu-induced metallothionein expression results in oxidative stress and inhibits DNA replication in prostate cancer cells.[41,42] Furthermore, the reaction between DDC and Cu^{2+} reduces Cu^{2+} to Cu^{+},[43] a more toxic form of copper ion, which further reacts with O_2 and Fe^{2+} to produce highly cytotoxic ·OH through a Fenton-like reaction.[43] Moreover, the DSF/Cu complex promotes the transport of copper into inflammatory breast cancer (IBC) cells.[32] Cu accumulation causes the intercellular generation of ROS, which alters membrane permeability and further promotes copper uptake, and therefore induces oxidative stress-mediated apoptosis in multiple IBC cellular models.[32] DSF specifically transports Cu ions into tumor tissues, thus preventing Cu from interacting with non-specifically binding proteins.

Furthermore, DSF inhibits the scavenging of ROS. DSF was recently reported to downregulate glutathione peroxidase 4 (GPX4) expression to prevent ROS clearance and induce ferroptosis in GBM, which is rescued by the ferroptosis inhibitor ferrostatin-1.[44] Moreover, DSF/Cu treatment also leads to hepatocellular carcinoma (HCC) -cell death via induction of ferroptosis, associated with a compensatory activation of the transcription factor nuclear factor erythroid 2-related factor 2 (NRF2),[45] which plays a key role in counteracting oxidative stress via regulating the expression of antioxidant genes.[46] In particular, DSF, as a specific inhibitor of ALDH, prevents ROS scavenging and detoxification mediated by ALDH isozymes.[6,15] ROS are an inevitable side-product of redox reactions. Increased ROS levels and inhibition of ROS scavenging subject cells to subsequent oxidative stress and this further causes damage to DNA, lipids, and proteins, which triggers cell death.[47]

Mounting studies confirm that DSF enhances oxidative stress and induces ROS production, which is necessary for DSF to exert cytotoxicity in various malignancies, including nasopharyngeal cancer (NPC),[48] gastric cancer,[49] prostate cancer,[50] acute myeloid leukemia (AML),[11] lymphoid malignancies,[51] osteosarcoma,[52] head and neck squamous cell carcinoma (HNSC),[53] lung cancer,[54] breast cancer,[55] thyroid cancer,[56] and HCC.[57] Among them, DSF/Cu is cytotoxic for NPC[48] and HCC[57] through ROS/MAPK promoted apoptosis. Moreover, DSF/Cu is highly toxic to AML cell lines because of the alteration of the ROS balance, cell cycle

arrest, and apoptosis, as manifested by an increased rate of apoptosis of approximately 70%.[11] The c-Jun N-terminal kinase (JNK), a critical member of the MAPK family, plays a key role in apoptosis. DSF/Cu activates the ROS-JNK proapoptotic pathway in osteosarcoma cells[52] and HNSC[53] and simultaneously inhibits anti-apoptotic pathways such as those mediated by NF-κB and NRF2 signaling in malignant lymphoid cell lines and AML stem cells.[40,51] Furthermore, Xie *et al*[56] demonstrated that DSF/Cu kills thyroid cancer cells with a lower IC50 (half-maximal inhibitory concentration, 62.88 ± 0.01 nmol/L) in IHH4 cell line via inhibiting the activities of the MAPK/extracellular signal-regulated kinase (ERK) and phosphoinositide 3-kinase (PI3K)/serine/threonine kinase 1 (AKT) pathways in a ROS-dependent manner. Notably, Lu *et al*[39] compared the anticancer effects of DSF and DSF/Cu, and they found that DSF/Cu exhibits a significantly greater effect than DSF alone in A549 cells. They further revealed that DSF is metabolized to form $Cu(DDC)_2$, which accumulates in cancer cell and initiates apoptosis with overproduction of ROS and induces cell cycle arrest. To confirm the effect of ROS, several studies attempted to reverse the cytotoxicity of DSF using the ROS scavenger N-acetylcysteine (NAC).[51,58,59] These studies show a significant reversing effect of NAC on ROS induction, and the toxic effects of DSF are obviously blocked with NAC treatment. It is noteworthy that NAC contains the reactive cysteine structure, which inactivates DSF. Thus, alternatives to ROS inhibitors such as cynarin should be used to confirm ROS levels in the future. Together, the evidence shows that the anticancer effect of DSF is related to its induction of ROS and subsequent cell death [Figure 2], although further in-depth mechanistic research on the cytotoxicity of DSF should be performed.

Effects of DSF on proteasome inhibition

The proteasome complex, which comprises a catalytic 20S core and a 19S regulator, selectively regulates and degrades ubiquitinated proteins.[60] The ubiquitin proteasome system (UPS) is critical for maintaining the balance of protein degradation as well as the physiological functions of cells. Cancer cells depend on UPS more than normal cells, indicating that UPS may serve as an attractive pharmacological target for cancer therapy.[61] DSF/Cu or $Cu(DDC)_2$ blocks the upstream p97 pathway of the proteasome, which induces the accumulation of polyubiquitinated proteins, ultimately leading to cell death, and $Cu(DDC)_2$ induces higher cytotoxicity for diverse types of cancer cells compared with DSF or DDC.[33] Mechanistically, Skrott *et al*[33] demonstrated that $Cu(DDC)_2$, with high affinity for thiol-containing proteins, induces aggregation and dysfunction of the nuclear protein localization protein 4 (NPL4), an adaptor of the p97 segregase essential for proteasome activity, which consequently blocks p97-NPL4-dependent processes, leading to accumulation of misfolded or even toxic proteins. Furthermore, inactivated p97 segregase induces endoplasmic reticulum (ER) stress and the heat-shock response (HSR), as indicated by detected biomarkers of ER stress and HSP70 after $Cu(DDC)_2$ treatment of U-2OS cells.[33,62] In a follow-up study, Majera *et al*[63] further demonstrated that disabling the vital NPL4-p97 pathway by DSF/$Cu(DDC)_2$ interferes DNA replication and causes DNA damage, enhancing replication stress.

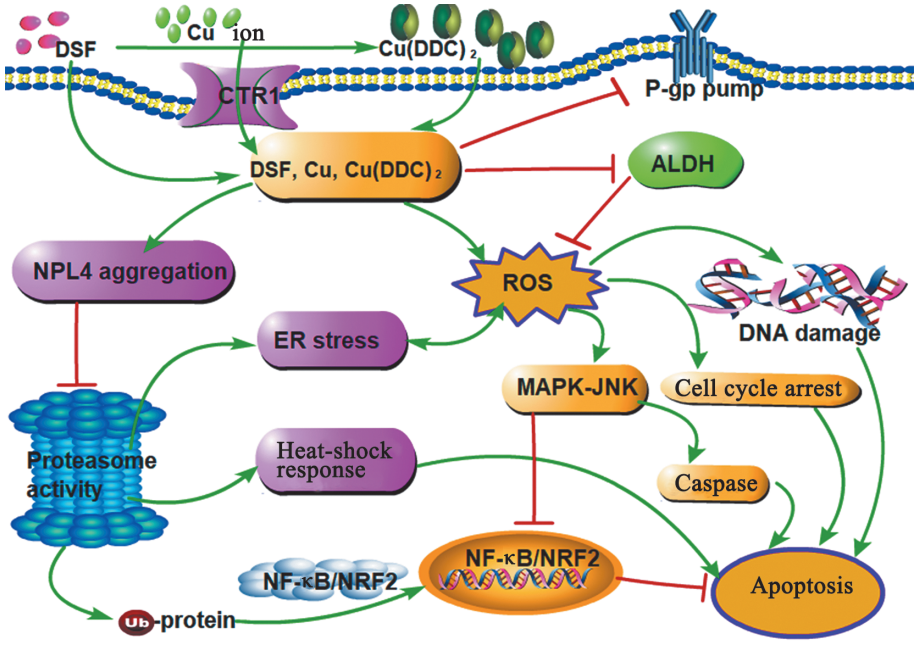

Figure 2: Anticancer mechanisms of DSF. DSF combined with Cu or $Cu(DDC)_2$ inhibits proteasome activity via NPL4 aggregation, leading to reduced NF-κB and NRF2 activity and consequently apoptosis. Furthermore, DSF, DSF/Cu, and $Cu(DDC)_2$ induce ROS production and inhibit ALDH activity to inhibit ROS scavenging, then initiating DNA damage, cell cycle arrest, and caspase pathway-mediated apoptosis. ALDH: Aldehyde dehydrogenase; $Cu(DDC)_2$: Bis-diethyldithiocarbamate-copper; CTR1: Copper-transport-related protein; DDC: Diethyldithiocarbamate; DSF: Disulfiram; ER: Edoplasmic reticulum; JNK: c-Jun N-terminal kinase; MAPK: Mitogen-activated protein kinase; NF-κB: Nuclear factor-kappa B; NPL4: Nuclear protein localization protein 4; NRF2: Nuclear factor erythroid 2-related factor 2; P-gp: P-glycoprotein; ROS: Reactive oxygen species; Ub: Ubiquitination.

Strikingly, $Cu(DDC)_2$ treatment also impairs the replication protein A (RPA)–ataxia telangiectasia and Rad3 related-interacting protein (ATRIP)–ATR–checkpoint kinase (CHK1) signaling cascade that is critical for prosurvival responses to replication stress, thus collectively provoking a toxic scenario in cancer cells.[63] NF-κB is well known for its antiapoptotic role, and aberrant NF-κB activation is involved in the pathogenesis of many malignant tumors.[64] The activity of proteasomes is critical for activating the NF-κB pathway, because proteasomes cleave the inhibitor-κB molecule (IκB), thereby releasing the NF-κB p50/p65 heterodimer from the inhibitory complex to translocate into the nucleus, leading to gene transcription.[64] Thus, proteasome inhibition leads to the inhibition of NF-κB signaling and cancer cell death. Recently, growing evidence shows that DSF inhibits cancer cell proliferation and promotes apoptosis *in vitro* and *in vivo* by increasing NPL4 aggregation, confirming that the p97/NPL4 pathway is a promising therapeutic target of DSF in oncology.[65–69] Notably, the DSF/Cu complex potently inhibits the proteasomal activity of cancer cells, but not that of normal or immortalized cells in *in vitro* and *in vivo* experiments.[68] As mentioned above, selective induction of apoptosis of tumor cells is associated with elevated copper levels and these are more dependent on proteasome activity for their survival [Figure 2], further suggesting DSF as an ideal antitumor drug.

DSF targets cancer stem cells (CSCs)

CSCs, which comprise a small population of quiescent cancer cells capable of self-renewal and differentiation, play a critical role in tumor initiation, progression, relapse, metastasis, and resistance to therapy.[70] As a result, targeting CSCs may serve as a promising strategy to improve cancer therapeutics in the future.[70,71] ALDH, as a typical marker of CSC as well as an enzyme required for the stemness of CSCs during oncogenesis, is irreversibly inhibited by DSF.[71] Data from recent studies show that DSF potently inhibits CSCs in various cancers, including AML, breast cancer, and ovarian cancer (OC) owing to the inhibitory effect of DSF on ALDH through diverse mechanisms.[15,40,55,72] For example, DSF/Cu targets aldehyde dehydrogenase isoform-1A1 (ALDH1A1) to inhibit non-small cell lung cancer (NSCLC) growth and recurrence[34] and to overcome cisplatin resistance in breast cancer[72] via inhibition of stemness-related transcription factor expression in ALDH-positive CSCs. Notably, a high-throughput drug screen (HTS) identified DSF as one of the most potent anti-OC compounds. Under CSC-enriching conditions, DSF treatment efficiently inhibits ALDH activity and represses sphere formation, suggesting DSF is able to inhibit CSCs formation in OC cells. Moreover, DSF decreases CSCs populations and reduces relapse in an *in vitro* model, and DSF also shows efficacy in an *in vivo* model of post-surgery, postchemotherapy OC relapse, demonstrating that targeting CSCs prevents OC recurrence.[73] Likewise, using HTS, researchers tested the sensitivity of glioma stem cells (GSCs) to 2000 compounds, among which DSF significantly inhibits the proliferation of GSCs. Notably, DSF toxicity for cancers is enhanced by Cu, which significantly increases CSC death via inactivation of the ubiquitin-proteasome pathway.[74]

However, recent evidence seems to challenge the notion that DSF-induced CSC toxicity is attributed to ALDH inhibition. A recent study demonstrates that repurposing DSF modulates the cell cycle distribution and significantly decreases clonogenic survival of GBM stem cells independent of ALDH3 expression.[75] Skrott *et al*[66] found that inhibition of ALDH is only secondary to membrane damage and cell death, rather than the preferential cytotoxicity of DSF/Cu. Wang *et al*[76] revealed that DSF/Cu complexes block the formation of breast cancer CSCs by downregulating the NF-κB-stemness gene pathway. Correspondingly, *in vivo*, combined treatment of radiotherapy and DSF significantly inhibited mammary primary tumor growth and spontaneous lung metastasis compared with olive oil-treated mice (vehicle control).[76] As expected, DSF increased DNA damage, and induced apoptosis and autophagy as well as cell cycle arrest in irradiated CSCs of an atypical teratoid /rhabdoid tumor.[77] Concurrently, DSF combined with radiotherapy significantly potentiates the anticancer effects of radiotherapy manifested by inhibited tumor growth and prolonged survival in atypical teratoid /rhabdoid tumor mouse models.[77] More recently, Sun *et al*[59] revealed that DSF/Cu induces robust antitumor immune responses, triggering a higher level of immunogenic cell death (ICD) of breast cancer CSCs, partly caused by ROS generation and ER stress. Notably, DDC binding to Zn^{2+} also suppresses the stem cell properties of lung cancer cells.[78] Cui *et al*[79] found that DSF/Zn nanoparticles significantly inhibit the growth of CSCs and tumors without damaging non-involved organs during oral cancer therapy. Collectively, studies in the past five years demonstrate that DSF exerts strong cytotoxicity upon various CSCs through diverse mechanisms. The research on DSF targeting CSCs is listed in Table 1.[40,54,59,69,72,73,75,79–87]

DSF Reverses Drug Resistance

Drug resistance, either intrinsic or acquired, is a serious problem associated with the treatment of malignant tumors, which is mainly caused by factors such as hypoxia, preexisting CSC populations, enhanced drug efflux pumps, and activation of NF-κB.[16,54,88,89] Hypoxic cells and CSCs represent two key factors contributing to failure of treatment for NSCLC. DSF/Cu lengthens survival and decreases the progression of NSCLC.[54] Mechanistically, DSF induces superoxide production and mitochondrial stress, which significantly decrease the viability of hypoxic cells, mitigating resistance to radiation and chemotherapy *in vitro* and *in vivo*.[54] As mentioned above, the presence of CSCs has profound implications for drug resistance. DSF-Cu complex reverses the Taxol-resistant (A549/Taxol) cells and vincristine-resistant cells (KB/VCR cells) via decreasing the expression of ALDH2 and stem cell transcription factors.[88] On the other hand, cancer cells exert multidrug resistance (MDR) by accelerating efflux or blocking the influx of drugs through various membrane transporters such as P-glycoprotein (P-gp), multidrug resistance-associated

Table 1: Disulfiram plays a critical role in inhibiting various CSCs *in vitro* and *in vivo*.

Type of cancer	Cell line	*In vitro*	Mechanisms	*In vivo*	Administration/dose	Efficacy	References
Glioblastoma	LK17	DSF/Cu (0.1/0.1 μmol/L)	DSF/Cu inhibits clonogenic survival of glioblastoma CSCs, independent of ALDH1A3 expression.	NA	NA	NA	[75]
Glioblastoma	U87MG, U251MG, U373MG	DSF/Cu (1/10 μmol/L)	DSF inhibits hypoxia-induced GSC and EMT phenotypes by inhibiting NF-κB-p65 protein expression.	BALB/c Nu/Nu mice xenografts	DSF-PLGA (10 mg/kg) CuGlu (6 mg/kg), 3 days/week for 4 weeks	DSF-PLGA/Cu significantly reduced the intracranial and subcutaneous tumor size and tumor weight in mice.	[80]
Breast cancer	MCF-7, SKBR3, MDA-MB-435S	DSF (1 μmol/L)	DSF inhibits ALDH activity and inhibits Sox, Nanog, and Oct expression in CSCs, and modulates ROS generation.	NA	NA	NA	[72]
Breast cancer	MDA-MB-231, UACC-812	DSF/Cu (0.15/1 μmol/L)	DSF/Cu induces ICD in breast CSCs partially by ROS generation and IRE1α/XBP1 pathway activation.	NA	NA	NA	[59]
Breast cancer	MCF-7, BT549, MDA-MB-231	DSF (0.5–15 μmol/L)	DSF suppresses EMT and CSC by inhibiting *SOX4*, which is induced by upregulating miR-30a expression.	NA	NA	NA	[81]
NSCLC	H292	DSF/Cu (0.05–0.15/15 μmol/L)	DSF/Cu complex induces oxidative stress, including superoxide, peroxide, lipid peroxidation, and mitochondrial damage.	Athymic nude mice xenografts	DSF 100 mg·kg^{-1}·day^{-1} by oral gavage for 25 days.	DSF decreased xenograft tumor growth and exerted chemo- and radio-therapy-sensitizing effects.	[54]
AML	KG1α, Kasumi-1	DSF/Cu (0.5/1 μmol/L)	DSF/Cu induces ROS-JNK pathway and inhibites pro-survival NRF2 and NF-κB pathways to kill CSCs.	NOD/SCID xenograft models	DSF (3 mg·20 g^{-1}·day^{-1}) Cu (0.03 mg·20g^{-1}·day^{-1}) for 2 weeks	DSF/Cu also significantly inhibited tumor growth and reduced tumor burden.	[40]
AML	THP1, UT7	DSF (0.9 μmol/L)	DSF in combination with Ara-c suppresses P65 expression and increases intracellular γ-H2AX formation in CSCs.	NOD/SCID mice xenograft model	3 mg·20g^{-1}·day^{-1} DSF for 4 consecutive days	DSF eliminated the ALDH high leukemia cells and enhanced sensitivity to Ara-c in transplanted mice.	[82]
DTCs	K1, WRO	DSF/Cu (0.1/1 μmol/L)	DSF/Cu targets CSCs in DTCs by inhibiting c-Myc- or E2F1-mediated BMI1 expression.	NA	NA	NA	[83]
Cervical cancer	SiHa, HeLa	DSF/Cu (0.1/0.01 μmol/L)	DSF/Cu inhibites the expression of stemness markers (ALDH, CD49f) and reduces the LGR5$^+$CSCs.	BALB/c-nude mice xenograft models	DSF (30 mg/kg), $CuCl_2$ (1.5 mg/kg) twice per week for the experiment	DSF/Cu complex inhibited tumor growth and had the greater antitumor efficacy on cervical cancer than cisplatin *in vivo*.	[84]
Medulloblastoma	D425med, D341	DSF/Cu (0.1/0.01 μmol/L)	DSF/Cu reduces ALDH activity and CD133 expression.	Athymic Nu/Nu mice xenografts	DSF (150 mg·kg^{-1}·day^{-1}), Cu^{2+} (2 mg·kg^{-1}·day^{-1}) of 5 days/week for 3 weeks	DSF/Cu inhibited tumor growth and prolonged survival *in vivo*.	[69]
Ovarian cancer	IGROV1, SKOV3 SKOV3IP1	DSF/Cu (1/1 μmol/L)	DSF/Cu increases intracellular ROS levels triggering apoptosis of ovarian CSC.	NA	NA	NA	[85]
Ovarian cancer	OV90, OVCAR8	DSF (0.25 μmol/L, OV90), DSF (0.5 μmol/L, OVCAR8)	DSF promotes ROS generation and enhances oxidative stress in CSCs, thus increasing cell death.	Athymic Nu/Nu mice xenografts mice	DSF (10 mg/kg) three times per week for 3 weeks	DSF was effective in a post-surgery, post-chemotherapy ovarian cancer relapse model *in vivo*.	[73]
Chondrosarcoma	SW1353, CS-1	DSF/Cu (0.05/1 μmol/L)	DSF/Cu decreases NF-κB-stemness pathway in CSCs.	Xenograft nude or NSG mouse	DSF (50 mg·kg^{-1}·day^{-1}), Cu (0.03 mg·kg^{-1}·day^{-1}) for 7 days	DSF/Cu inhibited tumor growth and prolonged survival *in vivo*.	[86]
Multiple myeloma	NCI-H929	DSF/Cu (0.1/1 μmol/L)	DSF/Cu can inhibit the ALDH+ stem cells through suppressing ALDH1A1 and Hedgehog pathway.	NOD/SCID xenograft mouse model	DSF (150 mg·kg^{-1}·day^{-1}), Cu (2 mg·kg^{-1}·day^{-1}) for 3 weeks	DSF/Cu reduced the tumor growth and inhibited stemness of multiple myeloma in xenograft model.	[87]
Oral carcinoma	Cal27	DSF (25 mg/g IRMOF3), IRMOF3 (100 μg/mL), Zn (100 μmol/L)	Folic acid-modified DSF/Zn-IRMOF3 nanoparticles could inhibit ALDH1$^+$ CSCs by downregulating the expressions of ALDH1A1, Nanog, OCT4, and SOX2.	BALB/c mouse xenograft model	IRMOF3-DSF-FA every 3 days for 30 days	IRMOF3-DSF-FA could inhibit tumor growth and had a good tumor-targeting ability *in vivo*.	[79]

ALDH: Aldehyde dehydrogenase; ALDH1A3: Aldehyde dehydrogenase isoform-1A3; AML: Acute myeloid leukemia; Ara-C: Arabinocytidine; BCSCs: Breast cancer stem cells; BMI1: B lymphoma Mo-MLV insertion region 1 homolog; c-Myc: cellular-myelocytomatosis viral oncogene; CSCs: Cancer stem cells; CuGlu: Copper gluconate; DSF: Disulfiram; DSF/Cu: Combination of DSF and copper; DTCs: Differentiated thyroid carcinomas; E2F 1: Early 2 factor transcription factor 1; EMT: Epithelial mesenchymal transition; GSC: Glioma stem cell; ICD: Immunogenic cell death; IRE1α: Inositol-requiring enzyme 1α; JNK: c-Jun N-terminal kinase; LGR5: Leucine-rich repeat-containing G-protein coupled receptor 5; MiR: Micro RNA; NA: Not available; NF-κB: Nuclear factor-kappa B; NOD: Non obese diabetes; NRF2: Nuclear factor erythroid 2-related factor 2; NSCLC: Non-small cell lung cancer; PLGA: Poly lactic-co-glycolic acid; ROS: Reactive oxygen species; SCID: Severe combined immune deficiency; XBP1: X-box binding protein 1; γH2AX: γ-H2A histone family member X.

Table 2: DSF/Cu-enhanced drug sensitivity by targeting specific molecules.

Type of cancer	Compound (dose)	Mechanisms	*In vivo*	Administration/dose	Efficacy	References
NPC	DSF/Cu + cisplatin	DSF/Cu induced NPC apoptosis by ROS/MAPK and inactive CAFs by inhibiting α-SMA.	BALB/c nude mouse 5-8F xenograft model	150 mg·kg^{-1}·day^{-1} DSF, 2 mg·kg^{-1}·day^{-1} Cu, 5 mg/kg CDDP per 3 days for 15 days.	Combined with CDDP, DSF/Cu significantly inhibited tumor growth of NPC tissues *in vivo*.	[48]
TGCTs	DSF + cisplatin	DSF sensitized resistant NTERA-2 CisR cells by decreasing ALDH activity and alteration of stemness-associated genes expression.	Balb/c-nu/nu xenograft model	50 mg·kg^{-1}·day^{-1} DSF i.p., 3 mg·kg^{-1}·day^{-1} cisplatin i.p. for 28 days.	DSF in combination with cisplatin inhibited tumor growth of NTERA-2 CisR xenografts.	[92]
ESCC	DSF/Cu + cisplatin + radiation	DSF/Cu sensitized chemo/radio-resistant ALDH1-positive ESCC cells by inhibiting ALDH1 and downregulating the PI3K/Akt pathway.	BALB/c nude mice	50 mg/kg DSF (i.p.), 0.15 mg/kg Cu (orally), radiotherapy (4 Gy)	DSF/Cu complex enhances the radiosensitivity in ESCC via inhibition of ALDH1 in tumor-initiating cells.	[93]
Breast cancer	DSF/Cu + cisplatin	DSF overcame cisplatin resistance by targeting ALDH, inhibiting the expression of *Sox*, *Nanog*, and *Oct*, and modulating ROS generation.	NA	NA	NA	[72]
Breast cancer	DSF + DOX	Lipo-DSF-DOX effectively overcame DOX resistance by inhibiting P-gp activity and its ubiquitination.	NA	NA	NA	[94]
Breast cancer	DSF/Cu + radiation	DSF/Cu induced ICD and improved the sensitivity in IR-resistant CSCs partially by ROS generation and IRE1α/XBP1 pathway.	NA	NA	NA	[59]
Breast cancer	DSF + DTX	DSF inhibited P-gp expression and increased ROS production and apoptosis.	Balb/c mice orthotopic breast cancer	73 mg/kg DSF (i.p.), 20 mg/kg DTX (i.v.), 0.085 ppm Cu in drinking water	DSF/Cu enhanced anti-tumor efficacy and prevented lung metastasis *in vivo*.	[95]
NSCLC	DSF/Cu + Taxol/VCR	DSF/Cu downregulated the expression of ALDH2 and reduced the levels of P-gp and stem cell transcription factors *in vitro*	Balb/c nude mice xenograft model	60 mg/kg DSF, 1.92 mg/kg Cu, 10 mg/kg Taxol; 30 mg/kg or 60 mg/kg DSF, 9.6 mg/kg Cu, 1 mg/kg VCR	DSF/Cu significantly inhibited tumor growth and reversed microtubule inhibitor resistance *in vivo*.	[88]
HNSCC	DSF/Cu + cisplatin + irradiation	DSF/Cu inhibited cisplatin-/IR-induced G2/M phase arrest. Triple treatment of DSF/Cu + cisplatin + IR significantly increased the cytotoxicity by enhancing the ROS accumulation.	NMRI nu/nu mice PDX model	Disulfiram (60 mg/kg, s.c.) three times a week, cisplatin (8 mg/kg, i.v.) once a week and irradiation (10 Gy)	DSF inhibited tumor growth in three different HNSCC-derived PDX models, supporting DSF as a strong radio-chemosensitizer.	[96]
NSCLC	DSF/Cu + cisplatin + irradiation	DSF/Cu enhanced radiation and chemotherapy toxicity in tumor cells dependent on ROS overproduction and Cu retention.	Athymic nude mice xenograft model	Radiation (3 × 6 Gy)+ carboplatin (2 × 15 mg/kg)+ DSF(100 mg/kg)	DSF decreased xenograft tumor growth when combined with radiation and carboplatin *in vivo*.	[54]
GBM	DSF/Cu + temozolomide	DSF/Cu impaird DNA repair and enhanced the effects of DNA alkylating agents to augment temozolomide activity.	SCID mice orthotopic transplantation	100 mg·kg^{-1}·day^{-1} DSF, 2 mg·kg^{-1}·day^{-1} Cu, 50 mg·kg^{-1}·mouse^{-1}·day^{-1} temozolomide	DSF/Cu prolonged *in vivo* survival in patient-derived BTIC models established from both newly diagnosed and recurrent tumors.	[97]
GBM	DSF + galunisertib	DSF inhibited ALDH activity and decreased TGF-β signaling.	SCID mice orthotopic xenograft model	50 mg/kg DSF, 75 mg/kg galunisertib	DSF and galunisertib suppressed therapeutic-resistant GBM growth *in vivo*.	[98]
PDAC	DSF/Cu + IR + 5-FU/ FOLFIRINOX	DSF/Cu targeted PCSCs and inhibited the NF-κB-stemness gene pathway.	C57BL/6 xenograft model	50 mg/kg DSF, 8 Gy IR,10 mg/kg 5-FU, FOLFIRINOX (mixture: 4.75 mg/kg irinotecan, 10.5 mg/kg leucovorin, 2.25 mg/kg oxaliplatin, 20 mg/kg 5-FU)	DSF/Cu combined with IR and 5-FU was more effective than either IR + 5-FU or IR + FOLFIRINOX therapy in inhibiting tumor growth of the mouse.	[99]

α-SMA: α-Smooth muscle actin; A549/Taxol cells: Taxol-resistant A549 cells; ALDH: Aldehyde dehydrogenase; BTIC: Brain tumor-initiating cells; CAFs: Cancer-associated fibroblasts; CDDP: Cisplatin; CisR: Chemoresistant; CSCs: Cancer stem cells; DOX: Doxorubicin; DSF: Disulfiram; DSF/Cu: Combination of DSF and copper; DTX: Docetaxel; ESCC: Esophageal squamous cell carcinoma; 5-FU: 5-Fluorouracil; FOLFIRINOX: Mix of 4 drugs: Irinotecan, Leucovorin, Oxaliplatin, and 5-Fluorouracil; GBM: Glioblastoma; HNSCC: Head and neck squamous cell carcinoma; ICD: Immunogenic cell death; i. p.: Intraperitoneal injection; IR: Ionizing radiation; IRE1α: Inositol-requiring enzyme 1α; i. v.: Intravenous injection; KB/VCR cells: Vincristine-resistant KB cells; MAPK: Mitogen-activated protein kinase; NA: Not applicable; NF-κB: Nuclear factor-kappa B; NPC: Nasopharyngeal cancer; NSCLC: Non-small cell lung cancer; PCSCs: Pancreatic cancer stem cells; PDAC: Pancreatic ductal adenocarcinoma; PDX: Patient-derived tumor xenograft; P-gp: P-glycoprotein; PI3K: Phosphoinositide 3-kinase; ROS: Reactive oxygen species; s.c.: Subcutaneous injection; SCID: Server combined immune-deficiency; TGCTs: Testicular germ cell tumors; TGF-β: Transforming growth factor β; VCR: Vincristine; XBP1: X-box binding protein 1.

protein 1 (MRP1), and ATPase copper transporting proteins alpha and beta (ATP7A and ATP7B). DSF inhibits ATP7A expression, which increases the levels of platinum–DNA adducts as well as apoptosis of human urothelial carcinoma (UC) cells, indicating that DSF confers UC cells higher sensitivity to chemotherapy.[16] Furthermore, chemoresistance is closely related to the activation of NF-κB in cancer cells.[89] Liu *et al*[90] found that administration of liposome-encapsulated DSF reverses the chemoresistance of breast cancer cells by targeting the NF-κB pathway *in vitro* and *in vivo*. Collectively, these data support the conclusion that DSF serves as an adjuvant strategy for overcoming drug resistance. Moreover, DSF significantly increases the sensitivity to ionizing radiation of pancreatic cancer cells and xenografted nude mice by aggravating DNA damage as well as inducing cell cycle arrest and apoptosis.[14] Similarly, DSF improves T-cell-mediated antitumor immunity via directly activating T-cell antigen receptor (TCR) signaling. Furthermore, DSF-induced antitumor immunity against colon cancer and melanoma in mouse models is further enhanced when combined with anti-programed cell death-1 (PD-1).[91] However, Zirjacks *et al*[75] demonstrated that DSF/Cu does not improve the treatment response to radiotherapy and temozolomide in mesenchymal GBM cells. And the further mechanism research found that temozolomide can reduce the effect of DSF on clonogenic survival, likely caused by pharmacological interactions between DSF and temozolomide. Here, the effects of DSF/Cu-based therapy on improving radio/chemo-sensitivity are summarized in Table 2,[48,54,59,69,72,88,92–99] which will hopefully provide insights and inspirations for researchers in this field.

Improved Drug Delivery System for DSF

Although DSF/Cu exhibits high toxicity in various cancer cells, clinical studies of DSF/Cu in cancer patients are not satisfactory. These inconsistent outcomes may be attributed to the rapid degradation of DSF or the unwanted modification of its metabolite DDC in the liver. Consequently, DDC loses its functional sulfhydryl groups, resulting in reduced chelation between DDC and Cu, which deceases the levels of the active $Cu(DDC)_2$ complex.[58] To overcome its instability and to realize the maximal therapeutic efficacy of DSF, various combination therapies and drug delivery systems (DDSs) have been extensively explored. For example, the use of nano-encapsulation technologies such as liposomes, polymers, polymeric micelles, or protein (albumin) particles encapsulating the DSF/DDC to protect the functional thiol groups of DDC has been widely applied to the treatment of various cancers.[90,94,100–103] Furthermore, nanoparticles are easily captured by tumor cells, increasing drug concentrations at the lesion site, which alleviates cytotoxicity.[104] In contrast, the codelivery systems for DSF and other chemotherapeutics may efficiently overcome drug resistance, promoting a synergistic effect.[105–107] Collectively, recent DSF-based treatment strategies have undergone dramatic development with huge potential for the treatment of cancer, which are well summarized in a recent review by Lu *et al*.[108]

Challenges and Perspectives

DSF is a first-line anti-alcoholism drug with good safety profiles that has been used in clinics for over 70 years. Recently, drug repurposing research has shown its great potential for developing an antitumor agent. However, the clinical trials of DSF encountered some challenges that must be addressed. First, given that DSF-induced cytotoxicity depends on Cu, supplementation with copper should be applied to patients with Cu deficiency. Notably, DSF/Cu manifested more serious and uncontrollable toxicity against cancer cells than DSF alone. Thus, considering its safety profile, balancing between DSF with slight efficacy and DSF/Cu with strong cytotoxicity should be fully explored to allow the clinical application of DSF for comprehensive cancer treatment. Second, DSF/Cu regulates the immune microenvironment and induces the death of immunogenic cells that attack cancer cells. However, further research is needed to explore how to prevent excessive cytokine release and inflammatory response during antitumor treatment. Furthermore, when DSF is used as an adjuvant therapy for cancers, the pharmacological interactions with comedications should be carefully investigated in advanced for the success of future clinical trials. Finally, DSF can also be combined with another metal, such as Zn ion which also shows anticancer activities, hence the application of DSF *in vivo* in complicated and should be further investigated in the future. Despited these challenges, we firmly believe that the prospect of DSF in serving as a treatment for cancer is promising. Further studies should focus on the in-depth mechanism of DSF as an anticancer reagent, and trials designed to bridge the gap between the laboratory and the clinic are required.

Conclusions

DSF, especially DSF/Cu, shows high anti-tumor effects on diverse cancers, which is associated with induction of the intracellular ROS, inhibition of proteasome activity, as well as inhibition of nuclear factor-kappa B (NF-κB) signaling. In addition, DSF or DSF/Cu targets CSCs and improves radio/chemo-sensitivity, which will provide a novel avenue for cancer treatment in the future.

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Conflicts of interest

None.

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