Drug resistance in recurrent and metastatic Ewing sarcoma

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Abstract

Survival of patients with recurrent and metastatic Ewing sarcoma (ES) has not markedly improved in the last 40 years; the main reason for the poor prognosis of these patients is drug resistance. However, intrinsic and acquired resistance may occur in response to both traditional chemotherapy and targeted drugs. These complex mechanisms plausibly include instability of the genetic material, enhanced drug efflux and metabolism, positive DNA repair, inhibition of tumor cell apoptosis, miRNAs, the tumor microenvironment, cancer stem cells, autophagy, and the activation of cell proliferation pathways. The development and application of nanoparticles bring new hope for reversing drug resistance in ES, accompanied with encouraging results from preclinical trials. In this review, we elucidate the molecular mechanisms underlying drug resistance in ES and propose putative strategies to overcome this resistance to improve prognosis of patients with ES.

Drug Resistance in Recurrent and Metastatic Ewing Sarcoma

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Abbreviations	Abbreviations
ES	Ewing sarcoma
ALDH	Aldehyde dehydrogenase

CDK4/6	cyclin-dependent kinase 4/6
CT	Computed tomography
CYP450	cytochrome P450
EBP-β	enhancer binding protein beta
EFS	event-free survival
EPR	enhanced retention and permeability
ESFT	Ewing sarcoma family tumors
GSH	glutathione
GST	glutathione *S*-transferase
GST-μ	GSTM
HIF-1	Hypoxia-inducible factor 1
HSP90B	heat shock protein 90 beta
IC50	half-maximal inhibitory concentration
IGF-1R	insulin-like growth factor-1 receptor
IR	insulin receptor
MAPK	mitogen-activated protein kinase
MDR	multiple drug resistance
MGST	microsomal GST
MRI	Magnetic resonance imaging
MRP-1	motility related protein-1
MTOR	mammalian target of rapamycin
MTV	metabolic tumor volume
NBDHEX	6-(7-nitro-2,1,3-benzoxadiazol-4-ylthio)hexanol
NF-κB	Nuclear factor kappa B
NPs	nanoparticles
ORF	open reading frame
OS	overall survival
PARPi	Poly-ADP ribose polymerase inhibitor
PFS	progression-free survival
P-gp	P-glycoprotein
PI3K	phosphatidylinositol 3-kinase
PKCA	protein kinase C alpha
siRNA	small interfering RNA
STAT3	signal transducer and activator of transcription 3
SUV	standardized uptake value
TLG	total lesion glycolysis
TME	tumor microenvironment
TNF-α	tumor necrosis factor alpha
VEGF	Vascular endothelial growth factor

ABSTRACT

Survival of patients with recurrent and metastatic Ewing sarcoma (ES) has not markedly improved in the last 40 years; the main reason for the poor prognosis of these patients is drug resistance. However, intrinsic and acquired resistance may occur in response to both traditional chemotherapy and targeted drugs. These complex mechanisms plausibly include instability of the genetic material, enhanced drug efflux and metabolism, positive DNA repair, inhibition of tumor cell apoptosis, miRNAs, the tumor microenvironment, cancer stem cells, autophagy, and the activation of cell proliferation pathways. The development and application of nanoparticles bring new hope for reversing drug resistance in ES, accompanied with encouraging results from preclinical trials. In this review, we elucidate the molecular mechanisms underlying drug resistance in ES and propose putative strategies to overcome this resistance to improve prognosis of patients

with ES.

1. INTRODUCTION

Ewing sarcoma (ES) is a type of solid bone and soft tissue tumor that occurs most frequently in children, adolescents, and young adults. The peak age of onset is 15 years and the incidence is slightly higher in males than females (3:2). In Europe, there are ˜1.5 cases per million young adults and younger persons. By contrast, the incidence is lower among Asians and Africans.[1] ES has characteristic chromosome translocations, of which the most frequent (85% of all cases) is *EWSR1-FLI1* fusion. The fusion protein EWS-FLI1 is a pathogenic transcription factor that rewires the transcriptome. It creates a unique epigenetic signature by inducing *de novo* enhancers at *GGAA* microsatellites, changing the state of gene regulatory elements, and causing tumor invasion and metastasis.[1,2]

At present, ES is treated with a combination of chemotherapy, surgery, radiotherapy, autologous stem cell transplantation, and myelotomy therapy. Chemotherapy and multidisciplinary therapy have increased the five-year overall survival rate for localized ES from 10% to [?] 70%.[3-6] However, even in localized ES, 30–40% of all patients experience relapse. The five-year survival rate after recurrence is only 15–25%, despite the administration of cytotoxic treatment regimens.[5] Over the past 40 years, there has been no significant progress in the successful treatment of metastatic and recurrent ES[4], mainly because drug efficacy is poor and the rate of tumor resistance is high. In ES, intrinsic resistance occurs before treatment and acquired resistance develops during the course of the treatment.[7] To date, the mechanisms of drug resistance have been explored mainly in breast, non-small cell lung, and ovarian cancers.[8] Such mechanisms are highly complex and involve multiple genes and factors. There is possibly a close association between the degree of resistance and the number of drug resistance mechanisms. In addition, the mechanisms of drug resistance widely differ among tumor types.[7] Currently, the mechanisms of drug resistance in ES have not been elucidated. To improve survival in patients with recurrent and metastatic ES, the mechanisms of drug resistance to traditional chemotherapy and novel targeted drugs must be identified.

2. STATUS OF DRUG RESISTANCE IN ES

The problem of drug resistance in malignant tumors has been perplexing health care practitioners and clinicians since the inception of chemotherapy.[9] The problem of multiple drug resistance (MDR) is becoming more severe. This is defined as the simultaneous resistance to several functionally and structurally unrelated chemotherapy drugs and is related to acquired resistance.[8,10] The current first-line chemotherapy regimen for ES involves alternating cycles of vincristine, doxorubicin, cyclophosphamide, ifosfamide, and etoposide.[11] The response rates of first-line chemotherapy in Ewing sarcoma family tumors (ESFT) are as high as 77%. Nevertheless, 23% of all ESFT patients present with intrinsic drug resistance.[12] In [?] 33% of all ESFT patients, drug resistance recurs during or after therapy.[6] About 60–80% of all ESFT patients die of disease progression, while $< 50\%$ of them respond to second-line therapy.[13,14]

Studies on targeted ES therapy have revealed that insulin-like growth factor-1 receptor (IGF-1R) plays an important role in downstream cell survival, angiogenesis, and metastasis mediated by EWS-FLI1. However, resistance to IGF-1R inhibitors has become a major impediment to ES treatment research progress.[4,15] In early clinical trials, IGF-1R inhibitors showed low response rates to ES. Figitumumab showed the highest objective response rate.[16,17]Partial response occurred in 15/106 (14.2%) of all enrolled patients with recurrent or metastatic ES (14.2%) (95% CI: 8.1–22.3%); the median duration of response was 4.7 months (95% CI: 3.7–6.1 months), the median PFS was 1.9 months (95% CI: 1.8–2.8 months), and the median survival was 8.9 months (95% CI: 7.2–11.1 months).[17]

YK-4-279 is a small molecule inhibitor of the EWS-FLI1 that destroys EWS-FLI1 transcriptional activity within the spliceosome by blocking the interaction between EWS-FLI1 and RNA helicase A. However, resistance to YK-4-279 owing to unknown causes has been detected in certain mouse populations.[15,18]

Cell cycle dysregulation is required for various oncogenic transformation processes. Therefore, several types of cancer cells rely on high cyclin-dependent kinase 4/6 (CDK4/6) activity.[19,20] The US-FDA has approved

the CDK4/6 inhibitors pomacillin, parbozenil, and rebozinib for breast cancer therapy.[19] However, intrinsic or acquired resistance to these drugs weakens their clinical efficacy; the mechanisms of such drug resistance have been extensively studied.[19] *CDK4* is confirmed to be an Ewing-selective dependency gene, and CDK4/6 inhibitors have proven to be efficacious against preclinical models of this invasive sarcoma.[20,21] The mechanisms of CDK4/6-inhibitor resistance must be elucidated for subsequent clinical studies.

Poly-ADP ribose polymerase inhibitor (PARPi) can block DNA repair in tumor cells.[22] Recent preclinical studies have shown that ES cells are highly sensitive to olaparib.[22,23]Several reasons have been postulated to explain this sensitivity (Fig. 1).[24] However, Phase II clinical trials demonstrated that olaparib monotherapy did not induce objective responses in 12 refractory ES patients with standard chemotherapy failure. Only four patients had stable disease and two had mild responses, with 9% and 11.7% tumor shrinkage, respectively.[24,25] The wide gap between preclinical and clinical trial results suggests that PARPi resistance has been underestimated.[24]In summary, drug resistance hinders drug development, lowers the efficacy of conventional chemotherapy, and is a major barrier to improving treatment outcomes in recurrent and metastatic ES.

3. MECHANISMS OF DRUG RESISTANCE

3.1. Increased drug efflux

Several proteins in the ATP-binding cassette transporter family participate in the development of drug resistance via reducing intracellular drug concentrations.[8,9] P-glycoprotein (P-gp)/*MDR1/ABCB1* and motility related protein-1 (MRP-1)/*ABCC1* overexpression in ES cells is an important mechanism of drug resistance.[6,26] P-gp, encoded by*ABCB1* , binds to various hydrophobic drugs including doxorubicin and vincristine that are commonly administered for ES.[8] *ABCB1* upregulation has been detected in drug-resistant ES side group cells.[27] MRP-1 has a high affinity for conjugated organic anions and mediates vincristine, doxorubicin, and antifolate efflux and transport.[9]MRP-1 expression on the ESFT cell membrane predicts overall survival (OS) and event-free survival (EFS). For ESFT, the relationship between P-gp expression and prognosis is uncertain.[12,28,29] An*in vitro* study showed that MRP-1 is the client protein of the heat shock protein 90 beta (HSP90B) family. MRP-1 is transported by HSP90B to the mitochondrial outer membrane. MRP-1 plays a role in doxorubicin-induced drug resistance in ES cell lines. HSP90 inhibitors can induce ES cell line death but do not enhance sensitivity to chemotherapy drugs. Hence, HSP90 inhibitors combined with doxorubicin are probably not efficacious for ES treatment.[14]

3.2. Increased drug metabolism

There are three phases in exogenous chemotherapy drug metabolism. Phase I is mediated by cytochrome P450 (CYP450), which catalyzes the oxidation of organic compounds. Certain gene polymorphisms in the CYP450 superfamily may cause intrinsic drug resistance by affecting protein function.[9,30] In Phase II, the drug binds glutathione, glucuronic acid, or sulfate via glutathione S -transferase (GST), UDP-glucuronosyl transferase, and sulfase catalysis, respectively and drug toxicity is attenuated.[9] In Phase III, metabolites are generated by efflux pumps such as P-gp and MRP family members.[9]The CYP3A family consists of the subtypes CYP3A4, CYP3A5, CYP3A7, and CYP3A43 and is the main member of the CYP450 family. CYP3A subtype expression maintains tumor growth by metabolically activating carcinogens and/or precarcinogens as well as protein signal kinases. CYP3A subtypes can also cause drug resistance by modulating metabolism.[31] CYP3A4 is expressed mainly in the liver and intestines and participates in most CYP3A-mediated biological processes.[31] It metabolizes various anticancer drugs administered to treat ESFT, including cyclophosphamide, doxorubicin, radiomycin, vincristine, isocyclophosphamide, topotecan, and etoposide.[31] Zia et al. collected samples and clinical data for 36 patients with ESFT. Immunocytochemistry confirmed that they presented with CYP3A4 expression upregulation that promoted metastasis and enabled the tumor cells to evade the effects of chemotherapy drugs. However, no correlation was found between CYP3A4 expression and prognosis.[31] GST comprises the subfamilies GST-α, GST-ϰ, GST-μ (GSTM), GST-ω, GST-π, GST-ϑ, GST-ζ, and microsomal GST (MGST).[32] MGST1 expression in ES was correlated with ES cell sensitivity to doxorubicin ($r = 0.98$, $P = 0.002$). Prognosis improved with decreasing MGST1 expression ($P = 0.02$).[32,33]

GST plays two distinct roles in drug resistance development. It deactivates anticancer drugs as substrates by catalytically binding glutathione (GSH). It also inhibits the mitogen-activated protein kinase (MAPK) pathway as well as apoptosis induced by non-GST substrates.[32] GSTM4 is a member of the GSTM family and is specifically expressed in ES. It is controlled by EWS-FLI1 through a specific regulatory element in the GSTM4 promoter and participates in ES tumorigenesis and drug resistance.[32] NBDHEX (6-(7-nitro-2,1,3-benzoxadiazol-4-ylthio)hexanol) is a GST-targeting agent that inhibits ES cells with high affinity for GSTM4. NBDHEX works synergistically with doxorubicin, vincristine, and etoposide.[32-34] Reportedly, GSTM4 expression was upregulated in ES, improved OS in xenografted mice,[32]and may be a novel therapeutic target for the treatment of ES with high GSTM4 expression. Traditional chemotherapy combined with GSTM4 inhibitors, agents activated by GSTM4, or drugs that block GSTM4/apoptosis signal regulating kinase 1 (ASK1) interactions may be more efficacious than conventional chemotherapy.[32]

3.3. Enhancement of DNA repair and inhibition of apoptosis

Most chemotherapeutics directly or indirectly damage tumor cell DNA. Cyclophosphamide and ifosfamide are alkylating agents commonly used in ES treatment. They produce interchain or intra-strand cross-links in tumor cells or transfer alkyl groups to guanines, thereby causing DNA base mismatch and preventing chain separation during DNA synthesis.[35] Doxorubicin and etoposide inhibit topoisomerase II in DNA replication and cause DNA strand breakage.[35] However, enhancement of the DNA repair mechanism in tumor cells may lead to drug resistance. When tumor cells cannot repair DNA damage caused by chemotherapeutic agents, apoptosis is activated.[9] Tumor cells inhibit apoptosis by interfering with the proapoptotic pathway and overexpressing antiapoptotic proteins.[6] Bcl-2 and Bcl-XL mediate olaparib resistance in ES. Navitoclax enhances the sensitivity of drug-resistant cell lines and patient-derived xenografts to olaparib by destroying BIM complexes. The adverse reactions it elicits are tolerable.[24] Kang et al. simulated ES growth in spheroid cultures and found that anchorage-independent ES spheroids were more resistant to chemotherapy than adherent cells. ES cells resist chemotherapeutics by inducing the *ERBB4* /PI3K/AKT pathway. Inhibiting E-cadherin adhesion or blocking *ERBBB4* may augment ES cell sensitivity to chemotherapy.[36] *CAV1* is a metastasis-related gene regulated by EWS-FLI1; it regulates tumorigenesis and numerous signaling pathways. *CAV1* increases ESFT resistance to doxorubicin- and cyclophosphamide-induced apoptosis by activating protein kinase C alpha (PKCA) phosphorylation. *CAV1*and Phospho(Thr^{638})-PKCA were co-expressed in ˜45% of all ESFT samples. Therefore, targeting*CAV1* and/or PKCA may improve treatment outcomes in patients with ESFT.[37] MiRNAs are small non-protein-encoding RNA molecules that regulate post-transcriptional gene expression. MiRNA disorders promote ES cell cycle progression, enhance apoptosis resistance, and stimulate tumor cell invasiveness and metastasis.[48] MiR-125b enhances ES cell line resistance to doxorubicin, etoposide, and vincristine by downregulating proapoptotic p53 and the homologous antagonist killer Bcl-2.[6,39]

3.4. Tumor microenvironment

The tumor microenvironment plays key roles in tumor occurrence and development and influences treatment efficacy.[40] Cell adhesion, IL-6, IGF-1, oxygen and energy supply, and hydrogen potential might be related to anticancer drug resistance.[40,41]Angiogenesis plays vital roles in tumor growth and metastasis. Unlike normal blood vessel development, tumor angiogenesis has structural and functional properties that result in retarded cell growth and hypoxic areas remote from blood vessels.[40,42] Hypoxia and slow tumor cell growth are associated with poor response to chemotherapy and rapid recurrence of tumors.[41,42] As tumor tissue lacks a lymphatic system, its interstitial fluid pressure is higher than that of normal tissue. This characteristic impedes drug delivery to the target tumor tissue. As malignant tumor cells are remote from blood vessels, various anticancer drugs are unevenly distributed in them.[42] Vascular endothelial growth factor (VEGF) is a typical angiogenic molecule in malignant tumors. Targeting VEGF can lower both interstitial fluid pressure and chemotherapy resistance. Clinical trials on bevacizumab (No. NCT 00516295), regorafenib (No. NCT02085148, No. NCT02048371, No. NCT02389244), sorafenib (No. NCT01946529, No. NCT01518413), and pazopanil (No. NCT01956669) pend results.[4,18,40,42] Hypoxia-inducible factor 1 (HIF-1) is crucial in mediating solid tumor anti-hypoxia by upregulating growth factors level, stimulating angiogenesis, preventing

apoptosis, and increasing anaerobic metabolism. It consists of a constitutively expressed HIF-1β subunit and an oxygen-sensitive HIF-1α subunit. The latter plays a key role in hypoxia-induced anti-apoptosis and is an important target for stimulating ES cell apoptosis.[43]The phosphatidylinositol 3-kinase (PI3K)/protein kinase B (Akt) signaling pathway participates in HIF-1α-mediated hypoxia activation and apoptosis resistance.[44] By contrast, the stroma may increase tumor cell sensitivity to specific chemotherapy agents. For example, the dissociation constant of doxorubicin is ˜9; an alkaline extracellular environment can increase doxorubicin uptake and cytotoxicity. This phenomenon is known as microenvironment-induced synthetic lethality.[41,45]

3.5. Cancer stem cells (CSCs)

CSCs occasionally occur in relative static tumors[6], can self-renew and differentiate, and promote tumor growth and invasion. CSCs have various self-protection systems including resistance to different drugs, detoxification enzymes, and DNA repair mechanisms. They are comparatively more resistant to traditional cancer therapy than ordinary tumor cells. Therefore, the successful identification, targeting, and elimination of CSCs is essential in ES treatment.[42] Aldehyde dehydrogenase (ALDH) is a marker of CSCs in breast, lung, and prostate cancers.[46] Awad et al. isolated ESFT cells with a tumor stem cell phenotype and found that they had high ALDH expression levels.[47] Thus, ALDH could serve as a CSC marker for ESFT. CSCs expressing EWS-FLI1 are relatively resistant to cytotoxic drugs, such as doxorubicin and etoposide, but are sensitive to YK-4-279. For this reason, the latter can be used to target ESFT stem cells.[47]CCAAT/enhancer binding protein beta (C/EBP-β) is a leucine-zipper transcription factor implicated in cell metabolism, differentiation, and development. C/EBP-β participates in ES carcinogenesis and is regulated by EWS-FLI1. Elevated C/EBP-β levels can increase transformation,*ALDH1A1* expression and activity, and chemotherapy resistance.[46] Bone marrow mesenchymal stem cells can upregulate the CSC transcription factor SRY-box transcription factor 2 and Nanog and octamer-binding transcription factor 4 via EWS-FLI1 and be reprogrammed into CSC cells.[48]

3.6. Autophagy

Autophagy is a self-degradation system involving lysosomes. It maintains cellular homeostasis under cytotoxic drug stress.[49,50]Autophagy can inhibit tumor growth and development, but may also promote tumor growth and chemotherapy drug resistance under other conditions.[50,51] EWS-FLI1 can upregulate *ATG4B*expression and inhibit autophagy and tumor cell apoptosis. The autophagy blocker 3-methyladenine enhances apoptosis in ES cell lines.[51] Moreover, upregulation of the ubiquitin E3 ligase family member TRIM can inhibit autophagy.[52,53]However, autophagy may promote apoptosis. Nuclear factor kappa B (NF-κB) belongs to a ubiquitous Rel-related transcription factor family and is activated by proinflammatory factors, oncogenes, viruses, and other stimuli. In cancer cells, NF-κB activity lowers cell sensitivity to apoptosis, thereby favoring tumor cell survival. Several antineoplastic drugs enhance NF-κB activity and consequently lose anti-tumor efficacy.[54] In ES cells, NF-κB activity inhibits autophagy mediated by tumor necrosis factor alpha (TNF-α). This mechanism is related to the NF-κB-dependent autophagy inhibitor target of the mammalian target of rapamycin (MTOR) pathway. Inhibiting autophagy by small interfering RNA (siRNA)-mediated knockout of the autophagy-related genes beclin 1 and *ATG7* mitigated apoptosis in ES cells. Inhibiting autophagy might be a mechanism of anti-apoptotic NF-κB activity. Hence, autophagy induction might prevent NF-κB from inducing anticancer drug resistance in tumor cells.[54]

3.7. Mechanism of drug resistance of IGF-1R and CDK4/6 inhibitors

IGF mediates ES growth, differentiation, and apoptosis inhibition by autophosphorylating IGF-2R and especially IGF-1R. IGF activates the MAPK/ERK, PI3K/AKT/MTOR, NF-κB, and VEGF cell proliferation pathways.[6] The IGF-1R pathway mediates tumor progression and drug resistance[55] Several hypotheses have been proposed regarding the mechanism of drug resistance of IGF-1R inhibitors including IGF-2 mRNA level upregulation, IGF-1R expression downregulation, upregulation of the insulin receptor (IR) expression receptive to the IGF-1 signal, enhanced phosphorylation of the intracellular signal transduction pathways, IGF-2 and IR-A level upregulation, and compensatory proliferation pathway activation.[6] Three-dimensional tumor models were constructed to study the interactions between mechanical stimulation and drug resistance

to IGF-1R inhibitors in the ES microenvironment. The shear force induced by blood perfusion upregulates IGF-1 in the mesenchymal stem cells of the ES microenvironment that, in turn, mediates the resistance of ES cells to IGF-1R inhibitors by promoting IGF-1R activation. Shear force also upregulates IL-6 level in the ES microenvironment and acts on signal transducer and activator of transcription 3 (STAT3) to protect ES cells from apoptosis and promote their proliferation.[55] The IGF-1/IGF-1R and IL-6/STAT3 pathways can interact with each other, and their combination may augment the anti-tumor efficacy of IGF-1R inhibitors.[55] The tumor suppressor *PTEN* attenuates signal transduction of the PI3K proliferation pathway by dephosphorylating it. *PTEN* diminishes the sensitivity of ES to IGF-1R inhibitors but augments its sensitivity to the MTOR inhibitor temsirolimus. Identification of the *PTEN* status of patients with recurrent tumors helps guide treatment options.[56] In ESFT cells, MAPK signaling is a compensatory mechanism after IGF1R suppression. It may lead to the upregulation of distal signaling pathway components such as MTOR and ribosomal protein S6.[6] MTOR pathway inhibition activates rapamycin via an IGF-R-dependent negative feedback mechanism.[57] Combinations of IGF-1R and MTOR inhibitors may overcome the issue of drug resistance of monotherapy.[16,57] Guenther et al. performed a genome-scale open reading frame (ORF) screen to identify genes mediating CDK4/6 inhibitor resistance and found that activation of IGF-1R receptor phosphorylation regulates this process. Synergy was detected between CDK4/6 and IGF-1R inhibitors. Compared with any monotherapy, a combination of both inhibitors enhanced inhibited the cell cycle and the PI3K/MTOR signaling pathway, prolonged survival, and slowed tumor progression in a patient-derived xenograft. Hence, CDK4/6-IGF-1R co-administration could be feasible for clinical application.[21] A Phase 2 study of palbociclib and ganitumab in patients with relapsed or refractory ES is underway (No. NCT04129151).

4. DRUG RESISTANCE REVERSAL AGENTS

P-gp inhibitors affect a major MDR mechanism and their application for the treatment of solid tumors has been widely researched. However, several generations of P-gp inhibitors have failed in clinical trials[58] as they have nonspecific toxicity, which affects anticancer drug pharmacokinetics and the multifactorial characteristics of MDR. When one mode of action is destroyed, the other compensates to maintain the original defense response.[8,58] GSH/GST and PKC inhibitors, epigenetic drugs, and natural herbal constituents have been tested in the attempt to reverse drug resistance;[58,59] they were administered in clinical trials on breast, non-small cell lung, and ovarian cancers.[8]

There is active current research on drug delivery systems. The use of nanoparticles (NPs) to overcome multidrug resistance is promising.[42,58] NPs may be polymers, metals, solid lipids, liposomes, quantum dots, dendrimers, or micelles. The clinically approved drug albumin paclitaxel is a type of polymer nanoparticle.[42] Compared with traditional small molecular drugs, macromolecular nanodrugs have the following advantages: (i) higher solubility longer half-life; (ii) targeted transport through cellular barriers; (iii) co-delivery of two or more drugs; and (iv) "passive targeting" because of enhanced permeability and retention (EPR) effects.[49] Subr et al. showed that water-soluble *N* -(2-hydroxypropyl) methacrylamide copolymer (P(HPMA)) conjugate transferred P-gp inhibitors to a doxorubicin-resistant murine monocytic leukemia P388/MDR subline and increased its drug sensitivity by 50×. Furthermore, the half-maximal inhibitory concentration (IC50) of simultaneous doxorubicin/P-gp inhibitor transport was 10–30× lower than that of either doxorubicin or P-gp inhibitors alone.[60]Nanocarriers have been used to carry traditional anticancer drugs such as doxorubicin and methotrexate to treat osteosarcoma and ES.[61] Claveau et al. successfully transferred siRNAs targeting EWS-FLI1 to a mouse ES model using nanodiamonds of detonation origin. This approach protected the transmitted siRNA from degradation, nuclease attack, and reticuloendothelial cell clearance.[62] Susa et al. loaded doxorubicin onto biocompatible, lipid-modified dextran polymer NPs and demonstrated that they induced apoptosis to the same degree in both doxorubicin-resistant and doxorubicin-sensitive osteosarcoma cells.[63] The same authors used lipid-modified dextran polymer NPs to transfer siRNAs to osteosarcoma cells expressing multidrug resistance protein 1 (ABCB1).[64] The siRNA NP treatment downregulated P-pg in drug-resistant osteosarcoma cells and inhibited their growth by 100× more than doxorubicin administration alone.[64]Although nanodrugs have a passive targeting effect that enhances their permeability (owing to the abnormal vascular structure of tumors) and retention (owing to poor lymphatic drainage of tumors), they

are nonetheless distributed in healthy tissues and cause side effects.[61] Hence, it is essential to improve active targeting using nanodrugs and attenuate drug resistance in malignant tumors such as ES.[42] Active targeting may be achieved by combining nanostructures with surface ligands, monoclonal antibodies, and molecules such as mannose, folic acid and ferritin.[61]

5. CONCLUSIONS

The prognosis of patients with metastatic and recurrent ES is poor and the efficacy of therapeutic agents is low because of drug resistance in the tumors. ES may exhibit resistance both to traditional chemotherapeutic agents and targeted drugs. ES has displayed preclinical and clinical resistance to EWS-FIL1, IGF-R, PARP, and CDK4/6 inhibitors. The mechanisms of drug resistance are highly complex and influenced by numerous and diverse intracellular and extracellular factors. P-gp inhibitors are new research hotspots in the field of drug resistance reversal; however, their clinical efficacy has been lower than expected. The use of nanocarriers to deliver drugs has shown high potential in preclinical experiments. Future research should target NPs to tumor tissues to mitigate distribution of the drugs in healthy tissues. To the best of our knowledge, very few studies have investigated the application of nanodrugs to reverse chemotherapy resistance in ES. Other research objectives going forward should include ways to improve survival in patients with recurrent and metastatic ES.

CONFLICT OF INTEREST STATEMENT

Both authors declare they have no conflicts of interest.

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AUTHOR CONTRIBUTIONS

Lili Zhang wrote the article, and Yonghua Yu revised and improved it. Both authors reviewed and agreed upon the manuscript content.

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FIGURE LEGEND

Fig. 1. Ewing sarcoma sensitivity to PARP inhibitors. (i) EWS-FLI1 upregulates *PARP1* expression and triggers PARP capture (PARP trapping). (ii) EWS-FLI1 causes R-loop aggregation and inhibits *BRCA1* -mediated DNA repair (synthetic lethality). (iii) EWS-FLI1 upregulates the Schlafen gene 11 (*SLFN11*) expression, which potentiates DNA-damaging agents (synergistic effect). (iv) EWS-FLI1 induces double-stranded DNA (dsDNA) breaks (more dsDNA breaks).

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