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DNA damage response signaling pathways as important targets for combination therapy and chemotherapy sensitization in osteosarcoma

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Abstract

Osteosarcoma (OS) is the most common bone malignancy that occurs most often in young adults, and adolescents with a survival rate of 20% in its advanced stages. Nowadays, increasing the effectiveness of common treatments used in OS has become one of the main problems for clinicians due to cancer cells becoming resistant to chemotherapy. One of the most important mechanisms of resistance to chemotherapy is through increasing the ability of DNA repair because most chemotherapy drugs damage the DNA of cancer cells. DNA damage response (DDR) is a signal transduction pathway involved in preserving the genome stability upon exposure to endogenous and exogenous DNA-damaging factors such as chemotherapy agents. There is evidence that the suppression of DDR may reduce chemoresistance and increase the effectiveness of chemotherapy in OS. In this review, we aim to summarize these studies to better understand the role of DDR in OS chemoresistance in pursuit of overcoming the obstacles to the success of chemotherapy.

KEYWORDS
ATM/ATR inhibitors, bone cancer, chemoresistance, DNA-PKcs inhibitors, p53, PARP1 inhibitors

Abbreviations: ALT, alternative lengthening of telomeres; APE1, apurinic/apyrimidinic endonuclease 1; ATM, Ataxia telangiectasia mutated; ATR, Ataxia telangiectasia and Rad3 related; ATRIP, ATR interacting protein; BER, base excision repair; CDK, cyclin-dependent kinase; Chk, checkpoint kinase; DDR, DNA damage response; DNA-PKcs, DNA-dependent protein kinases; DSB, double-stranded break; EMT, epithelial-mesenchymal transition; MMP, mitochondrial membrane potential; MRE11, meiotic recombination 11; MRN, Mre11-Rad50-Nbs1; mtAPE1, mitochondrial APE1; NBS1, Nijmegen breakage syndrome 1; NER, nucleotide excision repair; NHEJ, nonhomologous end joining; NMNAT1, nicotinamide mononucleotide adenylyltransferase-1; OS, osteosarcoma; PARP1, poly (ADP-ribose) polymerase 1; piRNA, PIWI-interacting RNA; RB, retinoblastoma; RPA, replication protein A; RUNX2, Runt-related transcription factor 2; SIRT6, Sirtuin 6; ssDNA, single-strand DNA; TMZ, temozolomide; USP1, ubiquitin-specific peptidase 1.

Forough Alemi and Faezeh Malakoti contributed equally to this study.

1 | INTRODUCTION

Osteosarcoma (OS) is a prevalent bone malignancy that originated from osteoid-producing malignant cells of the mesenchymal (Malakoti et al., 2021). Despite its low incidence rate (3 per 100 million), fatality in both children and adults is high due to poor prognosis, early metastasis occurrence, and treatment limitations (Harrison et al., 2018; Y. Zhang et al., 2018). The combination of both surgery and chemotherapy is the current standard treatment for OS. However, patients are often forced to alter their chemotherapy regimens because of drug resistance (Ahmadi et al., 2020; Bazavar et al., 2020). Since the chemotherapy drugs mainly induce cancer cells apoptosis through creating DNA damage, the enhancement of DNA repair ability in these cells is one of the most critical mechanisms of chemoresistance (Harrison et al., 2018; Sadoughi et al., 2021). DNA damage response (DDR) is known as a genome preserving pathway that stabilizes DNA upon exposure to endogenous and exogenous damaging factors like chemotherapy drugs. Studies to date have shown that DDR has a fascinating association with chemoresistance in OS (Sadoughi et al., 2021). There are two subclasses of DDR signaling pathways; Ataxia-telangiectasia mutated (ATM)/Ataxia telangiectasia and Rad3 related (ATR) pathway and DNA-PKcs pathway, which conduct cells to DNA repair, cell cycle arrest, or apoptosis. Studies show that there is a strong correlation between these pathways activation and OS with higher tumor grade and shorter patient survival because cancer cells heavily rely on the protective role of DDR. There is also some evidence that the suppression of these pathways by PARP1, Chk1, ATM, ATR, and DNA-PKcs inhibitors reduce drug resistance and enhance the effectiveness of chemotherapy in OS (Biermann et al., 2013; X. Li et al., 2020; Lindsey et al., 2017; Park et al., 2018). Thus, resistance to standard therapies makes it challenging to gain absolute control over tumors progression and metastasis, and this issue highlights the necessity of improving treatment regimens and the identification of novel therapeutic targets. The current study will discuss the role of the DDR pathway in OS development and then review different DDR disorders involved in OS chemoresistance. Finally, we will summarize the present DDR inhibitors, which their effects on increasing OS sensitivity have been studied.

2 | DDR SIGNALING PATHWAYS IN OS

DDR is a multistep process that begins with proteins known as "sensors" binding to DNA lesions. Transducers and their "mediators" bridge the gap between DDR sensors and "effectors," which amplify a damage-related signal (Gorgoulis et al., 2018). Sensing kinases, including ATM, ATR, and DNA-PK (DNA-dependent protein kinase) recognize DNA damage, then orchestrate kinase cascade, which overall leads to DSB or SSB signaling amplification and DDR pathway facilitation. Therefore, the dysfunction of these proteins leads to genomic instability and OS tumorigenesis (Huen & Chen, 2008). In the coming sections, we will discuss the dysregulation of DDR's major components in OS.

2.1 | ATM/ATR signaling pathway

ATM and ATR have a vulnerable cross-talk in the presence of DNA lesions. On this basis, experimental data revealed that ATM and ATR

TABLE 1 Important sensors and transducers in DNA damage response signaling pathway in osteosarcoma

	Key proteins	Roles	Reference
Sensors	MRN complex	Detection of DNA lesions, has a key role in the alternative lengthening of telomeres (ALT) process	(X.-D. Zhu et al., 2000)
	H2A-X	involved in sensing, signaling, and repairing of DNA damage	(Kopp et al., 2019)
	RPA	Detection of ssDNA and attraction of ATR	(Sadoughi et al., 2021)
	PARP-1	Poly ADP-ribosylation of proteins to act as a stress sensor	(Chaudhuri & Nussenzweig, 2017)
	Ku70/80	Detection of DSB and attraction of DNA-PKcs	(Yue et al., 2020)
Transducers and mediators	ATM	Activation of Chk-2 signaling pathway, and γH2A-X	(Sadoughi et al., 2021)
	Chk-2	Phosphorylation of downstream mediators	(Sadoughi et al., 2021)
	P53	p21synthesis	(Y. Sun et al., 2016)
	RB protein	Suppression of E2Fs and cell cycle regulation	(Nathan et al., 2009)
	ATR	Activation of Chk-1 signaling pathway	(Srinivas et al., 2019)
	Chk-1	Inactivation of CDC25C and CDC25A, activation of Wee-1	(Boudny & Trbusek, 2020)
	DNA-PKcs	Phosphorylation of downstream factors	(Yue et al., 2020)

Abbreviations: ATM, Ataxia telangiectasia mutated; ATR, Ataxia telangiectasia and Rad3 related; Chk, checkpoint kinase; MRN, Mre11-Rad50-Nbs1; PARP-1, poly (ADP-ribose) polymerase 1; RB, retinoblastoma; RPA, replication protein A.

appear to phosphorylate each other and also positively stimulate the activation and localization of each other at the site of the lesion. Both proteins involve in the phosphorylation of downstream components to regulate DNA repair or apoptosis (Jin & Oh, 2019). ATM and ATR use multiple branches and molecules to regulate the DDR signaling pathway in OS (Table 1).

2.1.1 | ATM-Chk2-p53 and Rb pathway

ATM is a serine/threonine kinase that phosphorylates checkpoint kinase 2 (Chk2) after double-strand DNA damage. ATM stimulates Chk2 activation, which induces p53 ser 15 and ser 20 phosphorylation. Now, activated p53 inhibits CDKs via p21 (CDKN1A) stimulation (Sadoughi et al., 2021). Thus, one of the most important substrates that participate in the ATM kinase pathway is p53 protein as a tumor suppressor protein. P53 genes can be mutated, and these mutations are usually rearrangements (50%), or missense mutations (22%), nonsense mutations (16%), deletions (6%) that result in p53 protein loss-of-function, which is detected in OS patients (Czarnecka et al., 2020; Thoenen et al., 2019). Therefore, it has been notified that the most frequent gene mutation in OS is p53. In addition, a considerable number of studies have revealed that p53 loss of function is associated with OS progression. Point mutations and structural variations especially intron 1 rearrangements in p53 genes are the most important victims in unrestrained cell cycle progression. Mutated-p53 cannot bind to DNA and regulate gene expression as a result of changing amino acid sequences (Adhikari & Iwakuma, 2009). Moreover, structural variations create changes in the three-dimensional structure of TP53, which negatively affects p53 DNA binding capacity (Chandar et al., 1992). In this regard, genome sequencing has shown that in 26% and 55% of OS cases, there are p53 mutations and conformational changes respectively (Chen et al., 2014). Other studies have also confirmed the involvement of p53 misfold and mutations in OS cases (Ribi et al., 2015; Sayles et al., 2019). For example, in one of these studies, an investigation on OS tumor xenografts has reported that structural variations in p53 and RB were more than other investigated genes (Sayles et al., 2019). In relation to this, encouraging evidence shows that when the mutant type of p53 is present in the cells, the total level of p53 is high. Therefore, a systematic review was conducted to investigate the relationship between p53 expression level and OS prognosis. In this study, data revealed that p53 upregulation results in a lower survival rate in OS patients. Collectively, p53 has been introduced as a valuable biomarker for evaluating OS survival rate (Fu et al., 2013).

The retinoblastoma susceptibility gene (RB1) is another tumor suppressor protein that is a downstream effector of the ATM-Chk2-p53

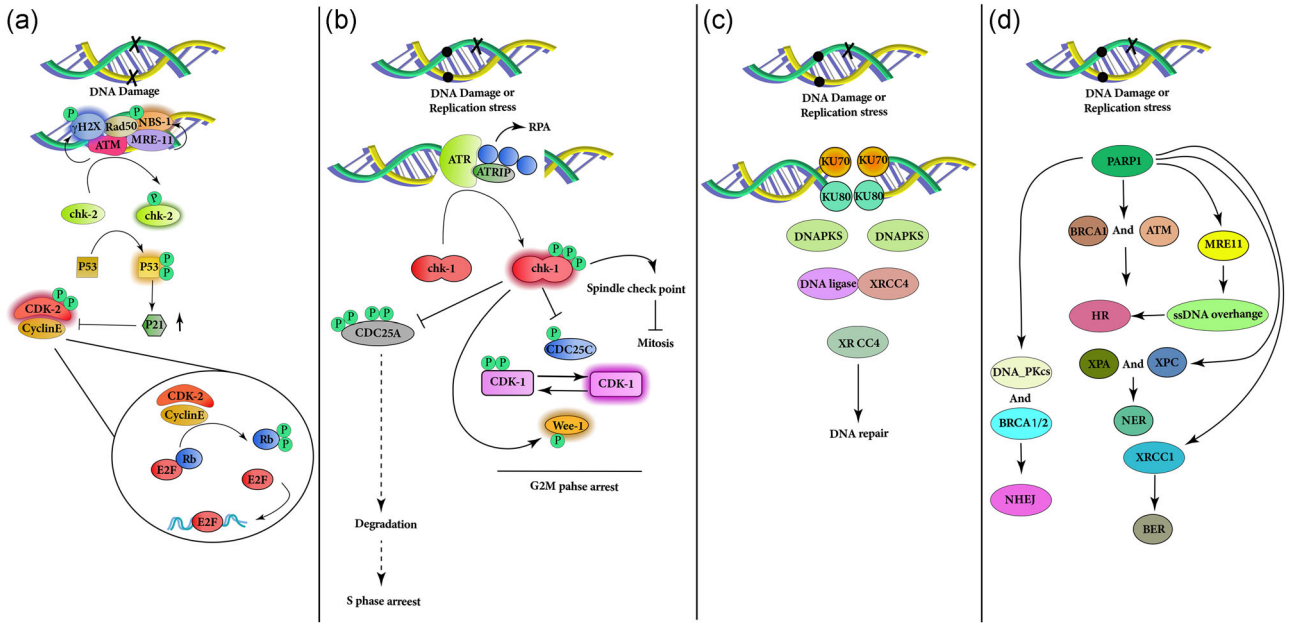

FIGURE 1 DNA damage response signaling pathways involved in osteosarcoma. (a) Ataxia telangiectasia mutated (ATM)-Chk2-p53 conducts cells to p21 activation which interacts with E/cdk2, suppresses Rb phosphorylation and E2F activation, resulting in cell cycle arrest. (b) Ataxia telangiectasia and Rad3 related-Chk1 signaling regulates three phases in cell cycle. In S phase, checkpoint kinase 1 (Chk1) inhibits CDC25A and causes cell arrest. In G2 phase, Chk1 involves in the phosphorylation and inactivation of WEE1 kinase and CDC25C phosphatase, conducting to cyclin-dependent kinase 1 (CDK1) suppression and cell arrest. In mitosis, Chk1 activates spindle checkpoints and prohibits mitosis. (c) Ku70/80 binds to double-stranded break (DSB)and recruits DNA-PKcs activates downstream effectors and causes DNA repair. (d) Poly (ADP-ribose) polymerase 1 recognizes and binds to both single-stranded break and DSB, then recruits BRCA1/2, DNA-PKcs, xeroderma pigmentosum complementation group C (XPC), XPA, XRCC1, ATM, and MRE11 to mediate nonhomologous end joining, nucleotide excision repair, base excision repair, and HR pathways

pathway and involves cell cycle regulation (Harrington et al., 1998). In detail, after p53 activation p21synthesis upregulates and binds to the cyclin E/cdk2, and consequently blocks the phosphorylation of Rb protein, leading to E2F suppression and finally cell cycle arrest. Thus, mutated p53 could not activate p21 and its downstream molecules and caused an uncontrolled cell cycle and cancer formation (Knappskog et al., 2015). Just like p53 alteration, the RB gene usually experiences structural variations and point mutations in OS cases with 30% and 10%, respectively (Tang et al., 2008). Another study has also shown that RB alterations occurred in 10 OS samples among 34 total cases (Chen et al., 2014). Although a study showed that OS is likely to be second cancer in patients with retinoblastoma (Draper et al., 1986), another study has proved that RB1 mutations are not responsible for OS initiation. This study has made it clear that RB1 mutations accelerate OS progression, not OS formation (Lin et al., 2009). Walkley et al. (2008) confirmed that the knockout of Rb and p53 genes in preosteoblastic cells in mice causes OS. Overall, it has been suggested that RB mutations are associated with OS poor prognosis and cancers development (Czarnecka et al., 2020) (Figure 1a).

2.1.2 | ATR-Chk1 pathway

ATR is a serine/threonine kinase that reacts to the single-strand DNA break (SSD), double-strand DNA (DSB) break, replicative stresses of DNA, and activates Chk1 signaling pathway (Srinivas et al., 2019). In the ATR-Chk1 signaling pathway, the RPA (replication protein A) immediately binds to single-strand DNA and attracts ATR and its regulatory unit ATRIP (ATR interacting protein) and other regulatory factors to the damage site. This complex formation stimulates ATR to phosphorylate Chk1. Then, chk1 autophosphorylates and causes the creation of the 14-3-3 proteins binding site and finally promotes its interaction with CDC25 phosphatases (K. Liu et al., 2020). Chk1 has different actions in different phases of the cell cycle. For example, in S-phase, activated Chk1 phosphorylates CDC25A, which results in CDC25A degradation. Following these steps, DNA replication stalls because of the reduction of CDK2/Cyclin complex activity. In the G2 phase, Chk1 phosphorylates WEE1 kinase and CDC25C phosphatase, which causes cyclin-dependent kinase 1 (CDK1) inhibition and cell cycle arrest (Boudny & Trbusek, 2020). Finally, Chk1 plays role in the regulation of spindle checkpoint and controlling mitosis. Therefore, Chk1 not only regulates downstream DDR effectors but also stalls the cell cycle to give enough time to cells especially cancer cells to repair damages and survive. On this basis, Chk1 depletion in OS cell lines results in the accumulation of DNA damages and polynucleated tetraploid, which is finally associated with cell death (Carrassa et al., 2009). Moreover, studies show that the stage of cancer and the life expectancy of a patient is associated with ATR expression. Cancer cells require a high level of ATR to keep them healthy from chemotherapy and radiotherapy (Gorecki et al., 2020). For example, a study in 2020 has reported that ATR expression and activation in non-survived patients was higher than this percentage in survived-OS patients. Additionally, an analysis of the experimental data in the present study showed that ATR suppression by either siRNA or berzosertib causes Chk1 inhibition, γH2AX expression, and PARP cleavage, which overall halts the DDR process, decreases metastasis in OS cell lines. As a result, ATR can be a proper biomarker to evaluate OS prognosis (X. Li et al., 2020) (Figure 1b).

2.2 | DNA-PKcs pathway

DNA-PKcs is a member of the PI3K-related kinase (PIKK) family and is one of the essential factors in orchestrating the nonhomologous end joining (NHEJ) pathway and repairing DNA damages to survive cells. In this regard, some studies have assessed the involvement of this protein in cancers development like OS (Toulany et al., 2017). For instance, a study confirmed that there is a positive relationship between DNA-PKcs protein expression and OS cells. Indeed, OS cells use the higher expression of DNA-PKcs to escape DNA damage accumulation and cell death (Mamo et al., 2017). DNA-PKcs overexpression is identified not only in OS cells but also in OS-treated cells by drugs. According to this claim, the experimental analysis of a study has suggested that DNA-PKcs may increase P-gp expression in OS cells by enhancing the PI3K/AKT signaling, which may lead to Chemoresistance in these cells (K. Li, Li, et al., 2016; Serej et al., 2018). Another study has illustrated that DNA-PKcs and γH2AX (DNA damage marker) are relatively low in OS cell line MG63, whereas the expression of these two components increases and decreases in OS treated cells respectively. Now, when DNA-PKcs is inhibited by 10 μM NU7026, the expression of these two factors converses, which leads to DNA damage accumulation and OS cell toxicity (Tsialikas & Romer-Seibert, 2015). Taken together, the DNA-PKcs pathway has a function in OS progression (Figure 1c).

2.3 | PARPs pathway

Poly (ADP-ribose) (PAR) polymerase-1 (PARP-1) is a member of the nuclear PARP enzyme family contributing to posttranscriptional modification of proteins through ADP-ribosylation from donor NAD + (Sadoughi et al., 2021). PARP-1 is involved in DDR in two ways; first, it acts as a sensor protein and recognizes the damages. Secondly, this protein appears to recruit DNA repair machinery to the damage site (Y. Wang, Luo, et al., 2019). In detail, PARP-1 has both direct and indirect functions in different DDR pathways. Following DNA damage, PARP-1 can detect the damage site and recruit meiotic recombination 11 homolog 1 (MRE11), which orchestrates downstream factors and consequently leads to DNA repair. In addition to MRE11 recruitment, PARP-1 is responsible to recruit XRCC1 in base excision repair (BER) and xeroderma pigmentosum complementation group A (XPA) and XPC in nucleotide excision repair (NER). When it comes to DSB, PARP-1 involves in HR repair through the recruitment of BRCA1 and ATM at the site of damage. Moreover, MRE11 recruitment by PARP-1 can create 3' single-stranded DNA (ssDNA) overhangs, which is essential during

HR initiation. Given the role of PARP-1 in NHEJ, it is associated with DNA-PKcs and BRCA1/2 functional activity (Alemi et al., 2021; Y. Wang, Luo, et al., 2019). In this regard, PARP-1 inhibition can interfere with DNA repair and the cell cycle, which is one of the suggested mechanisms for cancers treatment (Pascal, 2018). To address the low survival rate among overexpressed-PARP1 OS patients, the administration of PARP1 inhibitors have been introduced as an effective approach in OS therapy (Engert et al., 2017; Heidler et al., 2020; Kukolj et al., 2017; T. J. Liu et al., 2002; Park et al., , 2018) (Figure 1d).

3 | DDR ROLE IN OS CHEMORESISTANCE

Chemoresistance is one of the most important obstacles in the effective treatment of various cancers, including OS. Chemoresistance refers to cancer cells' resistance to chemotherapy agents that can lead to chemotherapy failure and disease recurrence. Cancer cells resist chemotherapy by a variety of mechanisms. One of these mechanisms is increasing the expression of ABC family transporters, which leads to an increase in the efflux of drugs outside the cell and a decrease in the effective concentration of chemotherapy drugs inside the cell (Vaghari-Tabari et al., 2020). Strengthen antioxidant defense and weaken the effect of chemotherapy drugs in causing ROS-induced apoptosis, enhanced antiapoptotic proteins expression such as BCL-2, and weaken proapoptotic proteins expression such as BAX and epithelial–mesenchymal transition (EMT) upregulation can be considered as other causes of the chemoresistance in malignant cells (Vaghari-Tabari et al., 2020). Damage to the DNA of cancer cells and induction of apoptosis are the main goals of chemotherapy drugs and enhancement of DNA repair ability is another most essential mechanism of chemoresistance in cancer cells. Studies to date have shown that DDR has an interesting association with chemoresistance in OS (Marchandet et al., 2021). In the following, we will briefly review the role of some components of the above DDR pathways in OS chemoresistance.

3.1 | ATM-Chk2-p53 and Rb pathway and chemoresistance

The relationship between ATM-Chk2-p53 and Rb pathway with chemoresistance has been shown by some studies. ATM appears to be involved in the resistance of OS cells to cisplatin (D. Wang, Qian, et al., 2019). It seems that ATM can enhance the stabilization of ZEB1, which can enhance homologous recombination-dependent DNA repair (D. Wang, Qian, et al., 2019; P. Zhang et al., 2014). In addition, increasing the expression of ZEB1 appears to attenuate the effects of miR-34 on reducing the expression of P-gp, an important ABC family transporter, in OS cells (Yan et al., 2018). Following genotoxic stress, ATM appears to be involved in enhancing Rad51 activity through PALB2 phosphorylation in OS cells. One study showed that the expression of Rad51, as a central recombinase in the HR pathway, was increased after the treatment of OS cells with epirubicin and cisplatin. This protein seems to be involved in chemoresistance by attenuating the cytotoxicity of these drugs and enhancing proliferation (Du et al., 2011). As noted in the previous section, p53 is a key player in ATM-Chk2-p53 and Rb pathway. P53 appears to be necessary for the induction of apoptosis by doxorubicin in OS cells (Y. Sun, 2016). One study has shown that transfecting wild-type p53 can significantly attenuate the resistance of OS cells to Taxol, doxorubicin, and cisplatin. It appears that p53 can increase chemosensitivity in multidrug-resistant (MDR) osteosarcoma cells, possibly through increasing p21 and BAX expression or inhibiting IGF-1r (Ye et al., 2016). The rate of p53 loss in OS seems to be significant (Kansara & Thomas, 2007) and maybe one of the main reasons for chemoresistance in OS. Some studies have also shown that Rb deficiency may be associated with methotrexate resistance in OS cells (Iida et al., 2003), suggesting a role for Rb in increasing chemosensitivity. Some proteins that affect the ATM-Chk2-p53 and Rb pathways components also have an interesting association with chemoresistance in OS. For example, Runt-related transcription factor 2 (RUNX2) is an essential protein in regulating p53-dependent DDR, which seems to cause the resistance of OS cells to adriamycin through downregulation of the TAp73, a proapoptotic protein, and p53 (Ozaki et al., 2015). Another example is WIP1. WIP1 is one of the oncogenic factors that attenuate DDR and also plays a role in strengthening DNA repair (Burdova et al., 2019; Long & Lin, 2019). One study showed that WIP1 expression increases in OS cells and can attenuate DDR and reduce the apoptotic effects of doxorubicin by inhibiting ATM/ATR/p53 signaling (Long & Lin, 2019). Given the above, it seems that the ATM-Chk2-p53 and Rb pathway can be a good target for overcoming chemoresistance in OS, which will be discussed in the next section.

3.2 | ATR-Chk1 pathway and chemoresistance

The ATR-Chk1 pathway appears to play an important role in the chemoresistance of OS. One study has shown that higher expression of ATR is associated with chemoresistance in patients with OS, and inhibition of the ATR-Chk1 pathway appears to impair DNA repair in OS (X. Li et al., 2020). The treatment of OS cells with cisplatin can enhance the activation of Chk1, which can significantly attenuate the apoptotic effects of cisplatin through cell cycle arrest and giving time to cancer cells to repair DNA damages and survive (L. Duan et al., 2014). Moreover, Wee1, a component of the ATR-Chk1 pathway, is also engaged in the chemoresistance of OS. This protein has functions in identifying and repairing DNA damage (di Rorà et al., 2020). The miR15b/Wee1 axis plays an important role in OS chemoresistance, in which miR15b can reverse this effect by inhibiting Wee1. However, the expressions of miR15b decline in OS (Z. Duan et al., 2017). In the previous section, the association of Chk1 with reduction of CDK2 activity and cessation of DNA replication was mentioned. Increased expression of CDK2 at the time of OS diagnosis is associated with a worse clinical outcome. This

enzyme can attenuate drug-induced DNA damage by enhancing DNA repair and participating in the resistance of OS cells to both doxorubicin and cisplatin (Vella et al., 2016). In general, the ATR-Chk1 pathway seems to have an interesting relationship with DNA repair and plays an important role in chemoresistance in OS, targeting this pathway may be an effective approach to overcome chemoresistance in OS. This will be discussed in the next section.

3.3 | DNA-PKcs pathway

It seems that DNA-PKcs may be involved in DNA repair following treatment of OS cells with cisplatin and etoposide, and attenuate the apoptotic effects of chemotherapy drugs on OS cells (X. Li et al., 2015). One study has shown that microtubule affinity-regulating kinases (MARKs) may play a role in OS chemoresistance by amplifying PI3K/AKT signaling and subsequently amplifying DNA-PKcs and ultimately enhancing DNA repair. PI3K/AKT signaling seems to be able to enhance the expression of DNA-PKcs in OS cells (Xu et al., 2020). Besides, the experimental analysis of a study has suggested that DNA-PKcs may increase P-gp expression in OS cells by enhancing the PI3K/AKT signaling, which may lead to chemoresistance in these cells, suggesting that there was an interesting association between PI3K/AKT, DNA-PKcs, and chemoresistance in OS cells. It should be noted that DNA-PKcs overexpression is identified not only in OS cells but also in OS-treated cells by drugs (K. Li, Li, et al., 2016; Serej et al., 2018). Therefore, it seems that the DNA-PKcs pathway is a suitable target for attenuating DNA repair following chemotherapy and increasing chemosensitivity in OS. In the next section, the effects of inhibition of this pathway on the OS chemoresistance cells will be discussed.

3.4 | PARPs pathway

Poly (ADP-ribose) polymerase 1 (PARP1) is another important factor involved in DDR that appears to be involved in the OS chemoresistance (Huber et al., 2004). PARP-1 may increase the cisplatin resistance through enhancing the ERK1/2 signaling pathway. The knockdown of PARP1 decreases the expression of Bcl-2 and cyclin D1 and increases the expression of caspase 3 and Bax in OS cells, which clearly shows the role of PARP-1 in attenuating apoptosis (S. Li, Cui, et al., 2016). Another study has also proved that PARP1 involves the resistance of OS cells to doxorubicin (Park et al., 2018). Furthermore, Sirtuin6 (SIRT6) is an important protein in DDR and involves PARP1 activation and base excision repair (Lombard et al., 2008; Onn et al., 2020). A recent study has shown that this protein is interconnected with reducing the effect of drugs on OS cells. SIRT6 can attenuate the antiproliferative and apoptotic effects of doxorubicin on OS cells by increasing the expression of BCL2, as an antiapoptotic protein, and decreasing the expression of proapoptotic proteins such as BAX and cleaved caspase 3. This study also announced that DNA repair pathway suppression in OS cells overexpressing SIRT6 could significantly increase the cytotoxic effects of doxorubicin (Z. Zhang et al., 2020), indicating the role of SIRT6 and DDR in the chemoresistance of OS cells. According to these findings, inhibition of PARP1 or the proteins involved in its activation could be a useful approach to increase chemosensitivity in OS. All of the findings reviewed in the above sections suggest that important components involved in DDR play a significant role in OS chemoresistance and can be considered as potential therapeutic targets, which will be discussed in the next section (Figure 2).

4 | INCREASING CHEMOSENSITIVITY IN OS BY THE COMBINATION OF CHEMOTHERAPY AND DDR INHIBITORS

The use of DDR inhibitors as an adjunct can increase the effectiveness of chemotherapy by reducing cancer cells' chemoresistance. Numerous inhibitors have been identified for various DDR pathway proteins. The combination of these inhibitors with routine chemotherapy agents has been studied both in vivo and in vitro in OS. In this section, we review the current achievements in this field.

4.1 | PARP1 inhibitors

PARP1 as a damage sensor takes part in three DDR pathways including BER, NHEJ, and HR (Ray Chaudhuri & Nussenzweig, 2017). Accordingly, cancer cells are positively interrelated with PARP1 expression and combating cell death. In a huge number of studies, PARP1 inhibitors are the most common DDR inhibitors widely used in combination with chemotherapy for treating many cancers including ovarian cancer, breast cancer, and pancreatic cancer (Appleman et al., 2019; Hurley et al., 2019; Oza et al., 2015; Pothuri et al., 2020; Sandhu et al., 2013; Tuli et al., 2019). Induced apoptosis and reduced cell growth and invasion are the consequences of using these inhibitors in combination with chemotherapeutic drugs (Zheng et al., 2011). Regarding Ewing sarcoma, PARP inhibitors such as Olaparib are approved to be effective in combination with chemotherapy (Kiss et al., 2020; Ordóñez et al., 2015; Pignochino et al., 2017). Using this agent with Trabectedin increases DDR process dysregulation and increases the amounts of DNA fragmentations as a result of increased DNA damage (Ordóñez et al., 2015). Along with Trabectedin and cisplatin, doxorubicin-sensitizing is also possible through the usage of Olaparib (Park, 2018). 3-aminobenzamide is another useful PARP inhibitor that can elevate the sensitivity of OS and ovarian tumor cells to cisplatin in a time- and dose-dependent manner (J. Zhang et al., 2013; Zheng et al., 2011). Talazoparib accompanied by temozolomide (TMZ) displayed interesting results in lung cancer (Lok et al., 2017), glioblastoma (Kizilbash et al., 2017), and Ewing sarcoma (Schafer et al., 2020). This combination in OS cooperated to induce apoptosis by BAX, BAK,

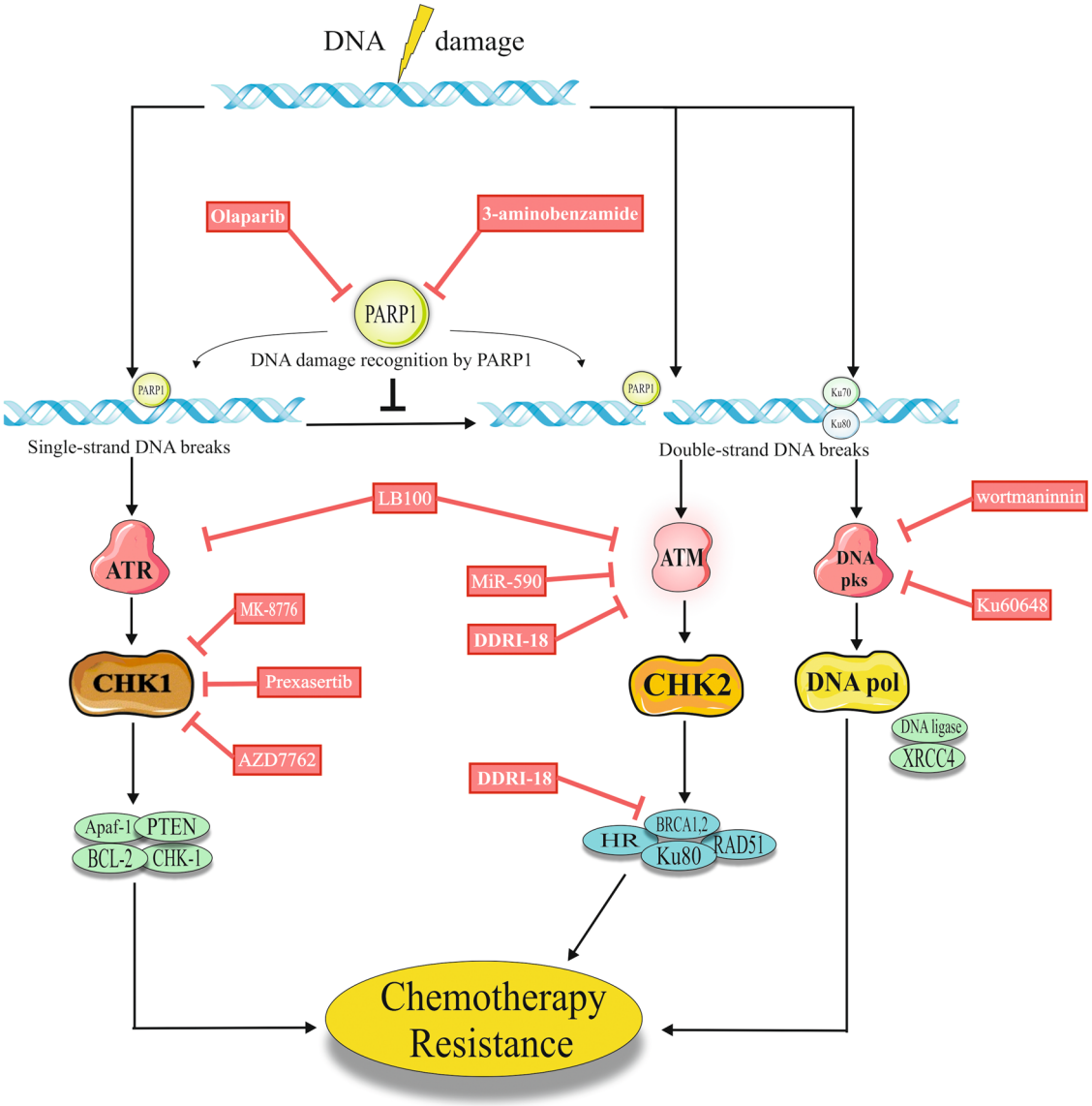

FIGURE 2 Schematic representation of increasing chemosensitivity in osteosarcoma by the combination of chemotherapy and DNA damage response (DDR) inhibitors. Several types of inhibitors have been identified that can reduce chemotherapy resistance through inhibition of DDR pathway proteins when combined with standard treatment

and caspase activation as well as decreasing the mitochondrial membrane potential (MMP), and fragmentation of the DNA (Engert et al., 2017). However, along with these effects of talazoparib, it cannot be neglected that only HR- and/or BRCA1/2-deficient OS are responsive to this agent (Engert et al., 2017). This limitation should be considered before decision-making for OS patients. Nicotinamide mononucleotide adenylyltransferase-1 (NMNAT1) is another enzyme employed in DDR, and studies have shown that it is effective on U-2OS OS cells (Kiss et al., 2020). Cisplatin and doxorubicin have more efficacies on OS cells when used with NMNAT1 inhibitors because of their effects on limiting the function of PARP1. However, impaired cellular bioenergetics are indispensable parts of the NMNAT1's effects (Kiss et al., 2020). To sum up, PARP1 inhibitors can use to increase the effectiveness of therapy in OS patients.

4.2 | CHK inhibitor

As we discussed before, CHK is one of the key components in DDR and its inhibition by different inhibitors has been evaluated in cancer therapy (Endersby et al., 2021; Thompson, Meuth, et al., 2012; Zhou et al., 2017). For example, MK-8776 is a Chk1 inhibitor that is used for sensitizing lung cancer and breast cancer (Grabauskiene et al., 2013; Zhou et al., 2017). AZD7762 is another CHK inhibitor that is demonstrated to increase γH2AX expression, apoptosis, and mitotic catastrophe when combined with cisplatin in OS cells (J. Zhu et al., 2019). However, this result should be confirmed by more clinical trials. Prexasertib is another Chk1 inhibitor that might also be competent for increasing cisplatin potency on OS cells (Heidler et al., 2020). In this study, Heidler et al. used Prexasertib, cisplatin,

and talazoparib to reduce clonogenic survival by inducing apoptosis in OS cells. For using Chk1 inhibitors in clinics, Massey (2016) showed that the combination of Chk1 and ATR inhibitors would be more effective in increasing the general levels of replication stress; however, using this combination as a clinical procedure for OS cases requires further investigations. Concerning increasing the clinical outcome and therapy's efficacy, detecting the nuclease activity of Mre11 is another strategy that might be useful. Thompson et al. combined a specific Mre11 inhibitor with MK-8776 (Chk1 inhibitor) and identified resistance afterward. They suggested that Mre11 can be used for determining whether a tumor is sensitive to MK-8776 or not (Thompson, Montano, et al., 2012).

4.3 | ATM and ATR inhibitors

ATM and ATR are two well-studied factors in the DDR process. Encouraging evidence showed that the inhibition of ATM and ATR is associated with chemosensitivity. The inhibition of ATM and ATR has been reported to be effective in glioblastoma and esophageal squamous cell carcinoma sensitization (S. Liu et al., 2015; Shi et al., 2018). LB100 is a protein phosphatase 2A inhibitor whose main effect is not on ATM or ATR, but it is approved to affect these protein kinases among all its target proteins, as well (C. Zhang et al., 2015). In addition to that, influencing p53 is also helping this agent's effectiveness in increasing OS cell death and decreasing metastasis after cisplatin treatment (C. Zhang et al., 2015). DDRI-18 or 3,3′-(1H,3′H-5,5′-bibenzo[d]imidazole-2,2′-diyl) dianiline is another DDR inhibitor whose function is not specific to ATM and affects plenty of DDR-related proteins (Jun et al., 2012). DDRI-18 increases the sensitivity to doxorubicin, etoposide, bleomycin, and camptothecin due to its broad range of effects but its mechanism of action is still not clear (Jun et al., 2012). Administering noncoding RNAs, which has attracted a lot of attention, is also examined for chemosensitizing OS cells. MiR-590 is one of the microRNAs that researchers utilized for inducing the response of these cells to doxorubicin. This study also found that this miRNA does this function through the ATM-p53 signaling pathway (Long & Lin, 2019). Just like ATM, targeting ATR is associated with the suppression of DDR and cell proliferation in OS. Berzosertib (ATR inhibitor) inhibits ATR-Chk1 signaling in dose-dependent manner. For example, 10 μM of Berzosertib showed OS cells toxicity (X. Li et al., 2020). According to these findings, ATM and ATR inhibitors can improve the effects of therapies in OS cases.

4.4 | DNA-PKcs inhibitors

DNA-PKcs knockdown is a practical approach to improve DNA damage accumulation and radiosensitization or chemosensitization in a variety of cancers like glioma, breast cancer, and OS (Lan et al., 2016; G. Sun et al., 2017). The expression level of DNA-PKcs is high when OS MG63 cell line exposes to cisplatin and etoposide. DNA-PKcs suppression sensitizes this cell line to chemotherapy drugs in two ways. First, DNA-PKcs knockdown is associated with increasing apoptotic factors like caspase-3 and caspase-10, which develops cells apoptosis. Secondly, DNA-PKcs inhibition results in cyclinD1 and CDK4 reduction, G1 arrest, and finally drug sensitization. In this study, data showed that after DNA-PKcs downregulation, G1 arrest in OS cells treated by cisplatin or etoposide increased by approximately 20% in experimental groups, compared to control groups. In conclusion, the IC50 in OS cells cotreated by DNA-PKcs inhibitors and chemotherapeutic drugs is significantly low. This cooperation can be a useful method to improve the efficacy of OS treatment (Tsialikas & Romer-Seibert, 2015). As an example, it has been suggested that KU60648 plays an essential role in increasing OS radiosensitization through DNA-PKcs blocking. In other word, the combination of KU60648 and radiotherapy is a positive method to improve the efficacy of OS therapy (Mamo et al., 2017). As we mentioned before, DNA-PKcs is a member of the PIKK family and there is a homology between PI3-kinase catalytic domain and DNA-PKcs. So, it is not surprising that wortmannin as a PI3-kinase inhibitor can negatively affect DNA-PKcs activity in OS cells (Kubota et al., 1998). Collectively, radiation or chemotherapy sensitization and effectiveness can develop through DNA-PKcs repression.

4.5 | Other inhibitors

Except for the mentioned inhibitors, we only found another research regarding an inhibitor that can affect other parts of the DDR process. As reported by Jun et al. (2012) DDRI-18 can affect many proteins and thereby sensitize OS cells to four chemotherapeutic drugs, which are mentioned before. γH2AX histone protein, NHEJ process, and BRCA1 are prone to be affected by this agent (Jun et al., 2012). Taken together, PARP1, ATM/ATR, and CHK inhibitors are the most common agents examined on OS cells. Despite the exciting results of utilizing these inhibitors, still, there is a lot of room for more investigations on OS chemoresistance. For instance, in 2013, Dai et al. (2013) indicated that APE1 expression regulates the expression of many genes (via miRNAs) and signaling pathways such as p53. The ability of RAD51 in radio-sensitizing is also approved, and other studies show chemosensitizing is also another feature of this essential protein (Huang et al., 2013; D. Wang et al., 2020). Hence, we suggest that APE1 and RAD51 inhibitors would also make great candidates for OS chemosensitizing.

5 | CONCLUSION

DDR is a well-conserved complex network required for DNA damage detection and repair, which is activated by DNA SSB and DSB. ATM/ATR, DNA-PKcs, PARP1, and Chk1 are the most important members of DDR, which their dysregulation are known in OS. Overexpression of these factors is accompanied by chemoresistance of OS to the routine chemotherapy regimens such as cisplatin, etoposide,

camptothecin, and doxorubicin. Targeting DDR proteins by specific inhibitors in combination with chemotherapies can increase the efficacy of OS treatment. In this regard, PARP1 inhibitors such as Olaparib, Chk1 inhibitors like AZD7762, and Prexasertibare, ATM/ATR inhibitors including LB100 and MiR-590 are the most common DDR inhibitors, which have the potential to use with chemotherapy in OS cases. Taken together, DDR inhibitors have shown a promising role in overcoming OS chemoresistant, and some essential advances have already been achieved in this regard. However, future studies are needed to find more effective DDR inhibitors to increase the success of existing OS treatments.

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CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

AUTHOR CONTRIBUTIONS

Forough Alemi: writing—original draft (lead); conceptualization (lead) writing—review and editing (equal). **Faezeh Malakoti**: writing—original draft (equal); writing—review and editing (equal). **Mostafa Vaghari-Tabari**: writing—original draft (equal). **Jafar Soleimanpour**: writing—review and editing (equal). **Nazila Shabestani**: writing—original draft (equal). **Aydin R. Sadigh**: visualization (lead). **Nafiseh Khelghati**: writing—review and editing (equal). **Zatollah Asemi**: writing—review and editing (equal). **Yasin Ahmadi**: Conceptualization (equal). **Bahman Yousefi**: project administration (lead); supervision (lead).

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