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Chapter

DNA Repair Defects in Sarcomas

Niknam Riyahi, M. Reza Saadatzadeh, Khadijeh Bijangi-Vishehsaraei, Farinaz Barghi, Pankita H. Pandya and Karen E. Pollok

Abstract

DNA repair pathway is considered to be one of the most important mechanisms that protect cells from intrinsic and extrinsic stresses. It has been established that DNA repair activity has a crucial role in the way that cancer cells respond to treatment. Sarcomas are a group of tumors with mesenchymal origin in which their association with DNA repair aberrations has been reported in numerous studies. Special attention has been focused on exploiting these alterations to improve the patient's overall survival and overcome drug resistance in cancer. While there is a large degree of heterogeneity among different types of sarcomas, DNA repair alteration is found to be a common defect in the majority of patients. In this chapter, we will introduce and review some of the most important dysregulated components involved in the DNA repair system, and discuss their association with tumorigenesis, cancer aggressiveness, drug resistance, and overall prognosis in the patients with sarcomas.

Keywords: DNA repair, Sarcoma, drug resistance, gene alteration

1. Introduction

Sarcomas represent a divergent and heterogeneous group of malignancies comprising more than 70 subtypes, with a common characteristic of being derived from mesenchymal lineages such as bone, muscle, cartilage, and fat [1]. Sarcomas are rare, accounting for less than 1% of adult cancers and approximately 15% of childhood malignancies [2]. They occur in all ages with an extensive intertumoral and intratumoral biological heterogeneity and widely varied clinical prognosis [3]. The primary standard of care approach for treatment of sarcoma patients is consist of surgery, radiation, and chemotherapy-based strategies [4]. Although the cure rate for the patients with localized sarcoma is generally more than 70%, the survival rate of metastatic and relapsed patients is still less than 30% and has not been changed in the last decades [1, 2, 5, 6]. Based on the tissue type of primary manifestation, sarcoma tumors could be categorized into two main groups: soft-tissue sarcomas (STS) and bone sarcomas. STS are more common with the incidence of approximately 13,000 reported cases versus 3000 cases of bone sarcomas each year in the United States [4]. Among STS, liposarcoma, leiomyosarcomas, and undifferentiated pleomorphic sarcomas are the most common types in adults, whereas rhabdomyosarcoma is the most common type

seen in pediatric age [7]. Osteosarcoma has the highest prevalence among bone sarcomas with a bimodal age distribution; an initial peak between the age of 10 to 20 and a second peak in incidence above the age of 60 [8]. Based on genetic criteria sarcomas can also be classified into two main groups: sarcomas with low level of genomic alterations and fairly normal karyotypes, and sarcomas with high level of genomic alterations and complex karyotypes [5]. The sarcomas found in the first group have chromosomal translocations as illustrated in **Table 1**; whereas osteosarcoma, chondrosarcoma, and liposarcoma are more genetically complex and have broader range of dysregulations resulted from copy number variations, mutations, etc. (**Table 1**) [5, 9–11].

It is well established that DNA damage response (DDR) system has a major impact on prognosis and clinical response to treatment in cancer patients [12–14]. Studies have investigated the dysregulation of different DDR pathways in various types of sarcomas and provided possible prognostic and therapeutic potentials among DDR components in order to overcome drug resistance and improve overall survival of these patients. In this chapter, we review some of the most important dysregulated DDR components which are involved in five different pathways (base excision repair (BER), nucleotide excision repair (NER), DNA mismatch repair (MMR), homologous recombination (HR), Non-homologous end joining (NHEJ), and DNA damage sensors (ATR and CHK1)) in sarcoma, and discuss the therapeutic developments and prognostic potentials in this area (**Figure 1**).

Sarcoma type	Gene translocation/inversion
Ewing sarcoma	EWSR1-FL1
	EWSR1-ERG
	EWSR1-ETV1
	EWSR1-E1AF
	EWSR1-FEV
	TLS-ERG
	EWSR1-ZSG
Synovial sarcoma	SSX-SS18
Chondrosarcoma	HEY1-NCOA2
	EWSR1-NR4A3
	TAF15-NR4A3
	TCF12-CHN
Liposarcoma	FUS-DDIT3
Rhabdomyosarcoma	PAX3-FOXO1
	PAX7-FOXO1
Fibrosarcoma	ETV6-NTRK3
	COL1A-PFGFB
	FUS-CREB3L1

References: [9, 12–14].

Table 1.
Most common chromosomal aberrations in sarcomas.

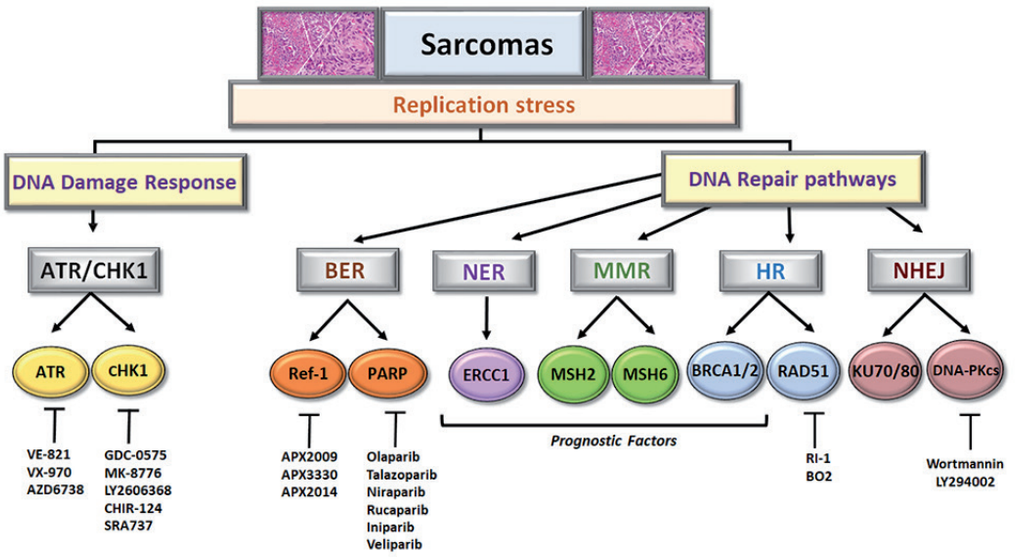

Figure 1.
Schematic summary of the most important DDR components and their respective inhibitors in sarcomas.

2. DNA repair machinery in Sarcoma

2.1 Base excision repair (BER) pathway

Base excision repair (BER) is a repair mechanism responsible for repairing single-strand DNA breaks (SSBs) or different types of damages including oxidization, deamination, and alkylation on a single base that do not induce significant distortion to the DNA helix [15]. Among several proteins that are involved in this pathway, APE1/Ref-1 and Poly (ADP-ribose) polymerase (PARP) are considered as the most important players in cancer progression and drug resistance [16–19].

2.1.1 APE1/Ref-1

One of the most important components of BER pathway is APE1/Ref-1 (apurinic/apyrimidinic endonuclease 1/redox factor-1). APE1/Ref-1 is a multifunctional protein involved in response to oxidative stress, cell cycle regulation, transcriptional activation, protein stability, apoptosis, and cell survival [16, 20]. The different functions of this protein can be categorized into two main activities: apurinic/apyrimidinic endonuclease activity and reduction–oxidation (redox) activity. The endonuclease activity allows APE1/Ref-1 protein to function as a DDR component in BER pathway by recognizing and cleavage of the abasic site [21]. The redox activity of APE1/Ref-1 gives it a critical transcriptional regulatory role in which enhances the activity of numerous transcription factors, including STAT3, NF-kB, HIF-1, and AP-1 [21, 22]. APE1/Ref-1 has a crucial role in maintaining cancer cells in a survival state through its DNA repair properties [23, 24]. Also, its redox function increases the activity of signaling pathways that are involved in promoting growth, migration, and survival in tumor cells as well as inflammation and angiogenesis in the tumor microenvironment [23, 25]. The overexpression of APE1/Ref-1 has been reported in many tumor types, and that change is associated with drug resistance, metastasis, cancer aggressiveness, and overall poor prognosis [16].

Several studies have shown that APE1/Ref-1 protein is overexpressed in sarcoma patients and is correlated with metastasis and lower survival rates [26–30].

The correlation between angiogenesis, as an important factor in tumor growth and metastasis, and APE1/Ref-1 in osteosarcoma was elucidated by the series of studies conducted by Wang et al. [26, 31–33]. They showed that transforming growth factor beta (TGFβ) is directly regulated by APE1/Ref-1 and its expression level was significantly reduced in APE1/Ref-1 deficient osteosarcoma cells [31]. TGFβ increases the chances of cancer metastasis through multiple mechanisms including immunosuppression, invasion, and angiogenesis [34]. They demonstrated that knocking down APE1/Ref-1 using specific siRNA in osteosarcoma led to down-regulation of TGFβ expression and suppression of angiogenesis *in vitro* based on human umbilical vein endothelial cells (HUVECs) in transwell and matrigel tube formation assays [31]. In addition, siRNA-mediated silencing of APE1/Ref-1 significantly suppressed tumor growth in xenograft mice models [31]. These experimental data indicated that APE1/Ref-1 promotes angiogenesis in osteosarcoma through a TGFβ-dependent pathway [31]. Additionally, they showed that the expression levels of vascular endothelial growth factor (VEGF) and fibroblast growth factor (FGF) are also regulated by APE1/Ref-1 [32]. However, the suppression of angiogenesis in APE1/Ref-1 knockdown cells is not dependent on their transcriptional activity [32, 33]. Wang et al., also used siRNA against APE1/Ref-1 protein to investigate its inhibition in osteosarcoma [26]. They demonstrated that the siRNA-mediated inhibition of APE1/Ref-1 sensitized the osteosarcoma cells to DNA damaging agents: methyl methanesulfonate, H2O2, ionizing radiation, and chemotherapeutic agents [26]. Another study conducted by Xiao et al., investigated the association of APE1/Ref-1 polymorphisms with osteosarcoma [35]. They performed a 2-stage case–control study in a total of 378 osteosarcoma patients and 616 normal controls and concluded that the patients who have certain APE1/Ref-1 polymorphisms have lower APE1/Ref-1 expression and higher survival rates [35]. Over the past few years, small molecule inhibitors targeting APE1/Ref-1 have been developed and showed remarkable anti-tumor effects with limited toxicity in a variety of cancers, in both *in vitro* and *in vivo* models [36–40]. However, the efficacy of these inhibitors still needs to be investigated in sarcomas.

2.1.2 PARP

Poly (ADP-ribose) polymerase (PARP) is another important DDR protein involved in cell proliferation, differentiation, and transformation [41]. PARP has the ability to covalently add poly ADP-ribose (PAR) chains to target proteins and alter their functions [42]. This enzymatic activity gives PARP the capability of being involved in diverse set of cellular processes including DNA damage repair [41, 42]. PARP inhibitors have drawn a lot of attention in cancer research community based on their remarkable anti-tumor effects in HR-deficient cancers [43]. Studies on PARP function in DNA repair system have led to development of numbers of FDA-approved inhibitors for treatment of various solid tumors [44–46].

PARP protein is found to be playing a key role in sarcoma as well, highlighted by several preclinical and clinical studies conducted in recent years. A study designed by Park et al., underscored the association between PARP activity and poor prognosis in osteosarcoma patients and showed the efficacy of PARP inhibition in combination with chemotherapy in this disease [47]. They evaluated the expression level of DNA damage molecules in 35 osteosarcoma patients and found that the expression levels of PARP1, γH2AX, and The Breast Cancer Susceptibility genes (BRCA1 and BRCA2) are accompanied with shorter overall survival in these patients [47]. *In vitro* experiments on osteosarcoma cell lines demonstrated that the PARP inhibitor olaparib as a single agent could inhibit cell proliferation in a dose- and time-dependent manner [47]. Moreover, the combination of olaparib with doxorubicin

showed significant synergistic effects in osteosarcoma cells [47]. The *in vivo* experiments also validated the growth-suppressive effects of individual and co-treatment of olaparib and doxorubicin in orthotopic osteosarcoma mice models [47]. In osteosarcoma cells treated with the combination treatment of olaparib plus doxorubicin, flow cytometry analysis showed increased apoptosis as evident by increased expression levels of cleaved caspase 3, cleaved PARP1, BAX, and decreased levels of BCL2 [47]. The sensitivity of HR-deficient osteosarcoma cells to PARP inhibition is demonstrated in a study performed by Engert et al. [48]. They treated a panel of osteosarcoma cell lines with PARP inhibitor talazoparib alone and in combination with chemotherapeutic drugs (temozolomide (TMZ), SN-38, doxorubicin, cisplatin, methotrexate (MTX), etoposide/carboplatin) [48]. They found a direct correlation between HR repair deficiency and increased sensitivity of osteosarcoma cells to PARP inhibition [48]. All osteosarcoma cell lines harboring BRCA1/2 mutation for both alleles (so-called "BRCAness") have shown a significant reduction in cell growth following treatment with talazoparib (MG63, ZK-58, Saos-2, and MNNG-HOS) [48]. However, U2OS (osteosarcoma cells) that are heterozygous for BRCA2 mutation and carry one intact allele were resistant to PARP inhibition [48]. Furthermore, TMZ showed the highest anti-proliferative synergistic effect with the PARP inhibitor among other chemotherapeutic drugs, and this effect induced through apoptosis pathway as indicated by caspase activation, increased expression level of BAX and BAK, DNA fragmentation, and loss of mitochondrial membrane potential [48]. These findings suggested a promising potential for development of novel therapeutic strategies using PARP inhibitors in combination with conventional treatments in osteosarcoma patients with features of BRCAness (more discussed in Section 3.4.1). Likewise, a large number of preclinical studies on other types of sarcomas including Ewing sarcoma, chondrosarcoma, rhabdomyosarcoma, and other STS emphasized the effectiveness of PARP inhibition in combination with chemotherapy as a promising therapeutic strategy for treatment of sarcoma patients [49–53]. In a study conducted by Laroche et al., the anti-tumor effect of PARP inhibitor rucaparib in combination with a chemotherapy drug trabectedin was explored in a panel of STS cell lines and a mouse model of liposarcoma [54]. The data obtained from this study demonstrated that the combination of rucaparib and trabectedin synergistically inhibited cell growth and induced G2M cell cycle arrest, γH2AX intranuclear accumulation, and apoptosis *in vitro* [54]. They also carried out *in vivo* experiments and showed that this combination significantly suppressed tumor growth, increased the progression-free survival, and elevated the percentage of tumor necrosis in the xenograft mice model [54].

Although preclinical studies have presented PARP inhibition as an effective treatment option in sarcoma, clinical trials have failed to demonstrate a promising clinical outcome in patients so far [55, 56]. Schafer et al. conducted a phase I/II clinical trial of PARP inhibitor talazoparib in combination with low-dose temozolomide in patients with refractory/recurrent solid tumors including sarcoma [55]. From April 2014 to January 2018, 40 patients (including 15 Ewing sarcoma, 4 osteosarcomas, 2 synovial sarcomas, and one rhabdomyosarcoma) were enrolled in this study and treated with talazoparib and temozolomide [55]. The data showed that this combination therapy was well tolerated; reversible neutropenia and thrombocytopenia were the primary dose-limiting toxicities (DLTs) [55]. However, no significant anti-tumor activity was observed in sarcoma patients [55]. Similarly, a phase II clinical trial of PARP inhibitor (olaparib) in refractory Ewing sarcoma patients has also failed to demonstrate a promising clinical outcome [56]. One possible explanation for this direct contrast between preclinical and clinical studies is that PARP inhibition could induce anti-tumor effects in *de novo* Ewing sarcoma but not in pretreated, chemoresistant patients [55, 56]. However, the limited number of

completed clinical trials of PARP inhibitors in sarcoma compared to other tumors hinders us from making a definite conclusion (**Table 2**). Future preclinical and clinical studies will shed more lights on the effectiveness of PARP inhibition as a possible treatment approach for sarcoma patients.

2.2 Nucleotide excision repair (NER) pathway

NER is a DDR pathway responsible for repairing bulky DNA lesions induced by ultraviolet irradiation, carcinogens, and some chemotherapeutic agents such as cisplatin [57]. The involvement of NER pathway in DNA damage induced by chemotherapeutic drugs attracted researchers to investigate the association of NER activity with the response to these cytotoxic agents in various cancers. Although there are some controversies regarding the role of NER pathway in cancer, some studies showed direct correlations between NER activity and increased response to chemotherapy [15, 57]. Recent efforts in whole-genome sequencing and data analysis of The Cancer Genome Atlas have led to a better understanding of the roles of the molecules involved in this pathway and introduced NER genes as prognostic biomarkers of response to various DNA damaging chemotherapeutic in different types of cancers [15, 57–60].

2.2.1 ERCC1

ERCC1 is the key component of NER pathway that has been investigated in a large number of studies due to its prognostic properties in cancer treatment [61–63]. The association between the expression of ERCC1 and response to trabectedin in STS was investigated in a recent translational study designed by Moura et al. [64]. Expression levels were evaluated using qRT-PCR in 66 patients with advanced STS who were treated with trabectedin. The results showed that the expression level of ERCC1 is correlated with patients' progression-free survival [PFS (the length of time during and after treatment that the disease does not get worse)] and overall survival. Patients who had higher expression levels of ERCC1 showed better responses to the trabectedin and had longer PFS rates [64]. Similarly, ERCC1 expression has reported to be associated with treatment response in other sarcomas such as osteosarcoma and leiomyosarcoma, highlighting the importance of this key NER protein as a predictable biomarker in sarcoma [65, 66]. Polymorphism of NER genes and the relation of different alleles with the treatment response has also been investigated in osteosarcoma, indicating the association of some polymorphisms with a higher risk of osteosarcoma development [67]. A study conducted by Obiedat et al., investigated the relationship between polymorphisms of ERCC1 and ERCC2 and response to cisplatin-based chemotherapy and clinical outcomes in osteosarcoma patients [68]. They analyzed the association between ERCC1

Compound	Phase	Cancer type and trial details	Clinical trail identifier
Olaparib	II	Adult participants with recurrent/metastatic Ewing sarcoma	NCT01583543
Talazoparib	I	Advanced or recurrent solid tumors (including Ewing sarcoma)	NCT01286987
Iniparib	II	Advanced, persistent, or recurrent uterine carcinosarcoma	NCT00687687

Table 2.
Sarcoma clinical trials of PARP inhibitors.

(C118T (rs11615) and C8092A (rs3212986)) and ERCC2 (A751C (rs171140) and G312A (rs1799793)) polymorphisms and clinical parameters including event-free survival (EFS) (the length of time after treatment that a patient lives without any complications or event that the treatment intended to prevent or delay) rates in 44 patients with osteosarcoma who were treated with cisplatin-based neoadjuvant chemotherapy [68]. The findings illustrated that there is a significant positive correlation between ERCC1 C8092 A genotypes and median EFS rate. In other words, the patients who carried allele C (CC & CA) had longer EFS rates than patients with AA genotype, highlighting the importance of ERCC1 polymorphism in osteosarcoma [68]. Taken together, these studies suggested that ERCC1 could be considered as a reliable predictive factor of the effectiveness of some DNA-damaging chemotherapeutic drugs in sarcoma patients, and different polymorphisms could be used as prognostic biomarkers for designing the best treatment strategy.

2.3 DNA mismatch repair (MMR) pathway

2.3.1 MSH2-MSH6 (MutSα)

MMR pathway is responsible for repairing base mismatches, insertions and deletions arise from DNA replication, genomic recombination, and other error-prone DNA repair systems [69]. MSH2-MSH6 (MutSα) complex plays an important role in this pathway by recognizing the mismatched bases and starting the MMR process [69]. Different polymorphisms and expression levels of MMR components have shown to be associated with prognosis and survival in cancer patients [70–72].

A study conducted by Li et al., emphasized the importance of MMR pathway in Ewing sarcoma and showed that the expression levels of MSH2 and MSH6 is correlated with an increased chance of metastasis and poor prognosis in these patients [73]. They used the GEO database to investigate the correlation of the key dysregulated genes and pathways with prognosis information and metastasis status of the Ewing sarcoma patients [73]. The findings highlighted the MMR pathway as the most significantly enriched KEGG pathway in EWS patients [73]. The expression levels of key MMR components including MSH2 and MSH6 are found to be significantly associated with metastasis, shorter EFS, and overall poor prognosis in Ewing sarcoma patients [73]. Several studies have investigated the role of MMR pathway in osteosarcoma. Liu et al., investigated the growth-suppressive effects of MSH6 gene silencing in combination with cisplatin in osteosarcoma [74]. Microarray-based gene expression analysis of samples obtained from 67 osteosarcoma patients along with 24 normal patients demonstrated that MSH6 is significantly up-regulated in osteosarcoma patients [74]. Then, they evaluated cell proliferation, cell cycle distribution, gene and protein expression, and apoptosis of osteosarcoma cell line MG63 after co-treatment with cisplatin and siRNA targeting MSH6 [74]. The data showed that silencing MSH6 in combination with cisplatin reduced expression levels c-Myc, cyclin D1, Bcl-2, Stathmin, and PCNA and increased BAX expression in osteosarcoma cells [74]. This combination treatment also induced significant anti-proliferative effects, indicating that MSH6 could be considered as a potential therapeutic target for treatment of osteosarcoma patients [74]. In another study, proteomic analysis for identification of proteins that are differentially expressed between osteosarcoma and normal osteoblastic cells revealed that chromosome segregation 1-like (CSE1L) protein is significantly associated with the growth of osteosarcoma cells [75]. Co-immunoprecipitation and RNA-seq analysis in this study showed that CSE1L acts as a positive regulator of MSH6 in osteosarcoma cells [75]. In addition, they knocked down CSE1L protein in osteosarcoma cells and found significant growth suppression [75]. Furthermore, to investigate the role of MSH6, they overexpressed MSH6 in

CSE1L-knockdown osteosarcoma cells [75]. The results showed that overexpression of MSH6 significantly increased cell proliferation rate and reversed the anti-tumor effects observed in CSE1L-knockdown cells, indicating that CSE1L activity is dependent on MSH6 expression [75]. Moreover, down-regulation of MSH6 resulted in suppression of cell growth in both *in vitro* and *in vivo* experiments [75]. The prognostic potential of MSH6 and CSE1L was also explored by evaluation of the MSH6 and CSE1L expression levels in tumor samples [75]. They found a significant correlation between the expression of these two proteins and overall poor prognosis in osteosarcoma patients [75]. Similarly, another study on osteosarcoma patients showed that overexpression of MSH2 and MSH6 is significantly associated with shorter survival time, lower sensitivity to chemotherapy, and higher chances of metastasis [76]. These studies underscored the significance of MMR proteins as both prognostic biomarkers and possible therapeutic targets in sarcoma.

2.4 Homologous recombination (HR) pathway

2.4.1 BRCA1/BRCA2

HR is the major DDR mechanism responsible for repairing double-strand DNA breaks (DBSs) [69]. HR repairs DSBs in an error-free manner by using homologous sequence of sister chromatid as an undamaged template [15]. BRCA1, BRCA2 and RAD51 are the key factors involved in this DDR pathway which have shown to be dysregulated in various types of cancers [15, 77–79]. Inherited mutations of the BRCA genes predispose individuals to develop tumors in various organs including breast and ovary [80]. Moreover, the chance of developing cancer significantly increases by acquiring BRCA mutations, and these mutations are commonly seen in patients with breast and ovarian cancers [80]. However, it has been reported that BRCA mutation has potential for inducing synthetic lethality in the cancer cells [81]. PARP inhibition in BRCA-mutated cancer cells (HR-deficient) induces synthetic lethality and cell death and provides a promising opportunity to eliminate cancerous cells (**Figure 2**). Several PARP inhibitors have been approved as monotherapies in HR-deficient ovarian and metastatic breast cancers [81].

As we discussed earlier in this chapter (Section 3.1.2), studies on osteosarcoma demonstrated that BRCA is frequently mutated in osteosarcoma and PARP inhibition either as a monotherapy or in combination with chemotherapy could induce significant anti-tumor effects in BRCA-mutated osteosarcoma cells [48, 82]. Although the significance of BRCA mutation status as a prognostic factor in sarcoma has been reported in a numerous studies [83–85], more clinical trials are warranted to determine the efficacy of PARP inhibitors in BRCA-mutated sarcomas. The importance of BRCA status in sensitivity to the chemotherapeutic drug trabectedin in STS is emphasized in a review paper gathered by Monk et al., and presented that BRCA mutations are significantly associated with favorable clinical response to trabectedin [83]. The frequency of BRCA mutation in soft-tissue sarcoma though, has not found to be significantly high in a study conducted by Seligson et al. [86]. They performed DNA sequencing analysis on 1236 STS patients as well as an additional 1312 leiomyosarcoma patients [86]. The unselected STS analysis revealed that only 1% of patients had BRCA2 mutation [86]. However, subset analysis showed that BRCA2 mutation could be found in 10% of leiomyosarcoma patients [86]. The frequency of BRCA1 mutation was not significant in either analysis [86]. Furthermore, they showed that PARP inhibition demonstrates effective clinical outcomes in BRCA2 deficient leiomyosarcoma patients [86]. Consistently, another study demonstrated a significant correlation between the overexpression of BRCA1,

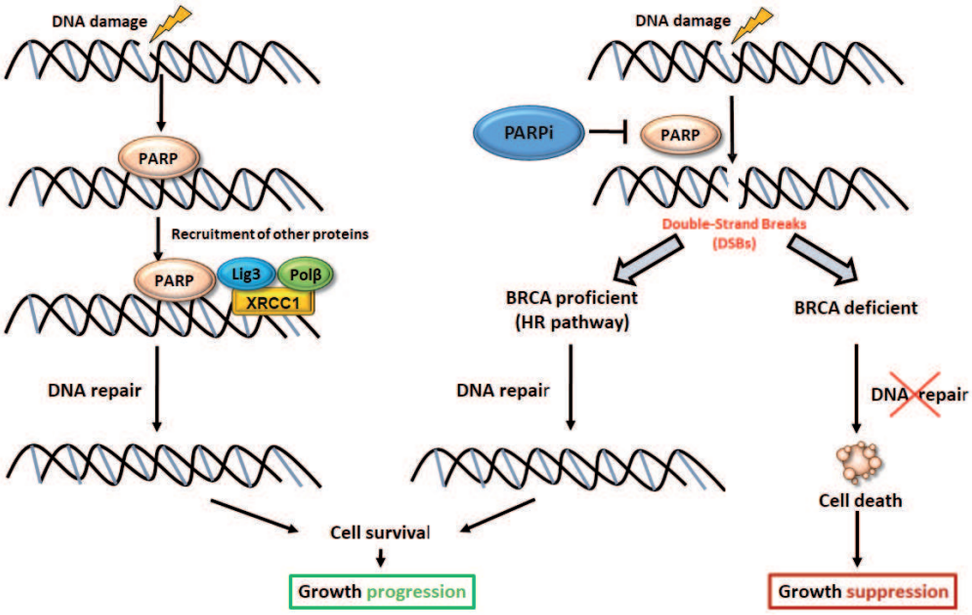

Figure 2.
Schematic role of PARP and PARP inhibition in synthetic lethality. (A) After binding to damaged DNA, PARP undergoes conformational change and poly(ADP-ribosyl)ation which results in recruitment of other DNA Damage Response (DDR) proteins, like DNA ligase 3 (Lig3), DNA polymerase β (polβ) and X-ray repair cross-complementing protein 1 (XRCC1), leading to DNA repair and cell survival. (B) PARP inhibitors block PARP activity, leading to double-strand break (DSB). The cells that have normal homologous recombination (HR) pathway are able to repair the DSBs in a error-free manner, leading to cell survival. However, BRCA-mutated cancer cells (HR-deficient) are unable to efficiently repair DSBs which ultimately results in cell death.

BRCA2, PARP, and γH2AX and higher tumor stage, higher chances of metastasis, lower survival rates, and overall poor prognosis in STS patients [87]. These studies highlighted the significance of BRCA status in sarcoma and underscored the fact that HR mutations should be considered as predictive factors for increasing the overall survival of patients by choosing the best treatment strategy.

2.4.2 RAD51

RAD51 is another key protein in the HR pathway that is also associated with prognosis and treatment response in various cancers. A growing number of studies demonstrate that RAD51 protein is overexpressed in many cancers including breast, prostate, bladder, pancreas, and lung, and this overexpression can up-regulate HR activity and result in resistance to DNA-damaging drugs [88–91]. Increased expression of RAD51 has also been reported in sarcoma patients [92, 93]. Du et al. conducted a study to explore the relationship between RAD51 expression and resistance to radio- or chemotherapy in osteosarcoma [93]. They suppressed the expression of RAD51 using shRNA and found increased sensitivity to chemotherapy and radiation in osteosarcoma cell lines through induction of cell cycle arrest and apoptosis [93]. Hannay et al., investigated the association between RAD51 expression and resistance to chemotherapy in STS patients [92]. They evaluated the RAD51 expression in 62 human primary recurrent and metastatic STS samples [92]. Only 3 tumor samples showed no RAD51 expression, while most of them had overexpressed RAD51 expression levels [92]. They showed that siRNA-mediated RAD51 targeting resulted in STS sensitivity to doxorubicin [92]. Overall, these studies highlighted the significance of RAD51 in chemoresistance and suggested that RAD51 could be considered as a prognostic factor or even a therapeutic target for treatment of sarcoma patients.

2.5 Non-homologous end joining (NHEJ) pathway

The NHEJ pathway is another important pathway responsible for repairing DSBs [94]. Unlike HR, NHEJ directly re-ligates two broken DNA strands without requiring a homologous sequence as an undamaged template, which makes this pathway prone to making errors [94]. NHEJ is initiated by binding of KU70/80 proteins, followed by recruitment of other key factors such as DNA-dependent protein kinases (DNA-PKcs), XRCC4, XLF, LIG4, and PAXX (a newly identified NHEJ component) [95] to complete the repair process [96]. Loss of the key factors involved in this pathway is positively correlated with increased genomic instability and sensitivity to DNA damaging chemotherapy drugs [94]. However, the over-activation of NHEJ has also been reported to be associated with increased genomic instability and tumorigenesis due to error-prone and inappropriate repair [94]. Thereby, both loss and over-activation of NHEJ factors have found to be associated with increased cancer incidence [94]. Moreover, a large number of studies have shown that differential expression of key NHEJ factors has significant impacts on the treatment response and overall prognosis in different types of cancers [94, 97–101].

Several studies have shown the significance of NHEJ components in sarcomas. For example, in a study on Ewing sarcoma patients, Kyriazoglou et al., has reported that NHEJ and HR genes are significantly up-regulated in comparison with healthy blood donors [102]. They analyzed the expression levels of 15 genes in 32 cases of Ewing sarcoma using Real-time PCR. XRCC5, XRCC6, Polm, LIG4 from the NHEJ pathway and RAD51, RAD52, RAD54, BRCA2, and FRANCD from the HR pathways have found to be significantly up-regulated in Ewing sarcoma patients [102]. In another study, Ma et al. investigated the role of PAXX protein in chemoresistance in osteosarcoma [96]. They found a significant positive correlation between enhanced PAXX-KU70 interaction and NHEJ efficiency and resistance to doxorubicin and cisplatin [96]. They also showed that PAXX deficiency re-sensitizes osteosarcoma cells to the chemotherapy drugs, which provides evidence that PAXX protein could be considered as a target for treatment of chemoresistant osteosarcoma patients [96]. Additionally study conducted by Hu et al., demonstrated the significance of KU80 expression in radiosensitivity of osteosarcoma cells [103]. They have shown that shRNA-mediated suppression of KU80 protein sensitized U2OS osteosarcoma cells to radiation through shortening of telomere length [103]. Taken together, key NHEJ factors have important roles in cancer progression, drug resistance, and patient's prognosis [94], which makes them interesting for further research regarding their prognostic and therapeutic potential in sarcomas. However, targeting NHEJ remains challenging as little is known about the inhibitors. Several PI3K inhibitors such as wortmannin and LY94002 (**Figure 1**) are being used for therapeutic intervention of DNA-PKcs in the NHEJ pathway [104].

2.6 ATR/CHK1 DNA damage sensors in DNA repair pathways

2.6.1 ATR

Ataxia-telangiectasia and Rad3-related protein (ATR) is one of the most upstream DDR kinases and belongs to the phosphatidylinositol 3-kinase-related kinase (PIKK) protein family [105]. ATR is a serine/threonine-protein kinase activated in response to a broad spectrum of DNA damage, including DSBs and various DNA lesions that interfere with replication [106]. In response to DNA damage, several proteins are phosphorylated at Ser/Thr-Glu motifs and additional sites in response to DNA damage by ATR [107]. ATR phosphorylates its major downstream effector checkpoint kinase 1 (CHK1) and prevents the entry of cells with damaged

or incompletely replicated DNA into mitosis from the G2 phase of the cell cycle [108]. This regulation is particularly apparent in cells with a defective G1 checkpoint, a common cancer cell feature because of p53 mutations [109, 110]. ATR also suppresses replication stress (RS) by inhibition of extra origin firing, particularly in cells with activated oncogenes [111]. Therefore, ATR could be an ideal therapeutic target in cancer. Currently, ATR inhibitors have been developed and are used either as single agents or in combination with radiotherapy or chemotherapy in both preclinical and clinical studies [112].

Several preclinical studies demonstrate that ATR could be a therapeutic target in sarcomas [113–117]. Laroche-clary et al., designed a study to investigate the anti-tumor effects of ATR inhibition in STS [113]. They treated STS cell lines with ATR inhibitor VE-822 either as a single agent or in combination with gemcitabine as a chemotherapeutic drug [113]. The data demonstrated significant synergist effects between these two drugs [113]. They found considerable cell growth suppression, apoptosis induction, and increased γH2AX expression after combined treatment of STS cells with VE-822 and gemcitabine in a higher efficacy than either agent alone [113]. Furthermore, they performed *in vivo* experiments on a patient-derived xenografts (PDXs) of undifferentiated pleomorphic sarcoma and found significant tumor growth suppression and increased PFS after treatment with combination of VE-822 and gemcitabine [113]. Taken together, this study highlighted the importance of ATR in STS and showed that ATR inhibition in combination with chemotherapy is efficacious in pre-clinical models. In another study, a series of parallel high-throughput siRNA screens were performed by Jones et al., in synovial sarcoma tumor cells and the results were compared with more than 130 non-synovial sarcoma tumor cells to get better insights into genetic dependencies and potential therapeutic targets in synovial sarcoma [114]. The analysis revealed a significant reliance of synovial sarcoma tumor cells on ATR protein kinase activity [114]. Furthermore, they showed that ATR inhibition will result in significant anti-tumor effects in synovial sarcoma *in vitro* and *in vivo* [114]. They also performed combination treatments with cisplatin and PARP inhibitors and found higher tumor-suppressive effects than either agent alone [114]. In summary, this study presented ATR protein alteration as a key factor in synovial sarcoma progression and proposed a novel therapeutic potential for synovial sarcoma patients [114]. The role of ATR protein was also demonstrated in Ewing sarcoma [115]. Nieto-Soler et al., designed a study to investigate the importance of ATR pathway in Ewing sarcoma [115]. They showed that Ewing sarcoma tumors that have high levels of RS are significantly dependent on ATR pathway [115]. Furthermore, they treated Ewing sarcoma cell lines and mice models with two independent ATR inhibitors and found considerable anti-tumor effects both *in vitro* and *in vivo* [115]. Collectively, this study highlighted the dependency of Ewing sarcoma to ATR pathway and identified ATR inhibition as a promising therapeutic strategy in Ewing sarcoma with high levels of RS [115]. Future preclinical studies and subsequent clinical trials will provide with additional reliable data on the effectiveness of ATR inhibition in sarcoma to translate this therapeutic approach into clinic as a possible treatment approach for sarcoma patients.

2.6.2 CHK1

As mentioned above, checkpoint kinase 1 (CHK1) is the major downstream effector of ATR [108]. CHK1, a serine/threonine-specific protein kinase, plays an essential role in preventing cell cycle progression when damaged DNA is being repaired [118, 119]. DNA damage is sensed by ATR, activated ATR phosphorylates and activates CHK1. CHK1 has several targets which all act to regulate cell cycle

arrest [118]. Phosphorylation of the CDC25 dual specificity phosphatase family mediated by CHK1 causes phosphatase degradation, resulting in increased phosphorylation and inhibition of multiple cyclin dependent kinase (CDK) proteins, positive regulators of the cell cycle [119]. In addition to CDC25 phosphatases, WEE1 kinase is phosphorylated and activated by CHK1, subsequently leading to the inhibitory phosphorylation of CDK1 [120]. It is therefore logical that inhibitors of CHK1 in cancer treatment could facilitate cell cycle progression with damaged DNA and induce apoptosis [118].

Several preclinical studies and a few clinical studies demonstrate that CHK1 could be a therapeutic target in sarcoma treatment [115, 116, 121–124]. Larocheclary et al., conducted a study to investigate the role of CHK1 protein kinase in p53-mutant and wild-type STS [122]. They performed a systematic screening of a panel of 10 STS cell lines after combination treatment of CHK1 inhibitor (GDC-0575) with gemcitabine [122]. They showed that GDC-0575 induced apoptosis by abrogating DNA damage-induced S and G2–M checkpoints [122]. Moreover, they observed a synergistic or additive effect of GDC-0575 in combination with gemcitabine *in vitro* and *in vivo* in TP53-proficient but not in TP53-deficient sarcoma models [122]. Before conducting the mentioned study, they had analyzed the expression profile of a series of 339 complex genomics sarcomas and 108 translocation-related sarcomas, they showed that CHK1 expression is significantly associated with poor prognosis in sarcoma patients [125]. Moreover, they evaluated the efficacy of CHK1 inhibition in STS patients in a phase 1 clinical study with 3 STS patients (two with p53 mutation and one without p53 mutation) [122]. Two STS patients who had p53 mutation demonstrated promising response to the combination of gemcitabine and GDC-0575, while the other patient displayed no clinical benefit [122]. In conclusion, they provided pre-clinical and clinical evidence of the significance of CHK1 activity in STS and revealed that combination of CHK1 inhibitors with chemotherapy could be a promising treatment strategy for p53-mutant STS patients [122, 125]. There are also numbers of studies which have highlighted the important role of CHK1 activity in osteosarcoma progression and drug resistance and showed that CHK1 inhibitors either as a single agent or in combination with other drugs could be considered as a promising therapeutic target for treatment of osteosarcoma patients [126–129]. Regarding the role of CHK1 in Ewing sarcoma progression, some studies demonstrated that CHK1 protein is over-activated in Ewing sarcoma and showed that Ewing sarcoma cells are sensitive to CHK1 inhibitors either as a single agent or in combination with other drugs *in vivo* and *in vitro* [116, 121, 130, 131]. Further clinical investigations are needed to confirm whether treatment of sarcoma with CHK1 inhibition is efficacious therapeutic approach to improve sarcoma patient outcomes at a higher level of evidence.

3. Implications/conclusions

Collectively, the studies summarized in this chapter indicate that it will likely take more than just targeting a particular dysregulated DNA repair pathway in the context of chemotherapy to cure many relapsed and aggressive sarcomas. As mentioned above, targeting the dysregulated process of replication stress and genomic instability which promotes tumorigenicity in many cancers such as sarcoma is an area of intense interest [132]. The use of small molecule inhibitors that block not only DNA repair mechanisms but other global networks that may be connected to or independent of DNA repair mechanisms may be key to improving clinical outcomes. As such, our group used a systems biology approach to discover risk signatures and potential biomarkers of therapeutic response in pediatric adolescent and young

adults with aggressive osteosarcoma. We found that the MYC-RAD21 copy number gain correlated with poor overall survival and was a potential marker of replication stress. We demonstrated that an increase in replication stress via a combination therapy consisting of BET and CHK1 inhibitors in xenograft models of pediatric and AYA osteosarcomas that have copy number gains of MYC and RAD21, was efficacious and well tolerated [126]. Furthermore, to obtain insight into other potential treatments where DNA repair inhibitors can be combined, numerous efforts have focused on investigating and understanding of the cross-talk between the various DNA damage-repair pathways as well as with the tumor microenvironment so that novel therapeutic combinations can be identified [133]. For instance, it has reported that hypoxic conditions within the tumor microenvironment impairs the fidelity of DNA repair pathways [133]. Furthermore, increased immune response to tumor neoepitopes have been observed in cancer with impaired/dysregulated DNA repair pathways [133]. Therefore, preclinical and clinical validation of using DNA repair inhibitors in combination with anti-hypoxic or immunomodulatory therapies warrants additional investigation. Notably, DNA repair mechanisms clearly contribute to tumor resistance [134]. In fact, one mechanism, by which tumor resistance is regulated involves cancer stem cells (CSC) which have increased DNA repair capacity [134]. Additionally, it has been reported that chromatin structure (euchromatin vs. heterochromatin) impacts the efficacy of DNA repair [135]. Thus, combination therapy targeting DNA repair pathways with agents targeting CSC or epigenetic proteins that regulate chromatin also require further evaluation. Several studies have shown associations between DNA repair pathways. With advancements in next-generation sequencing and use of precision genomics one clinical implication is that it may be possible to identify germline and/or somatic mutations involved in DNA repair proteins that could help delineate subsets of sarcoma patient-population that are predisposed to factors such as likelihood of getting the disease, onset of relapse/metastasis/recurrence, or possibility of therapeutic resistance to certain treatments [136]. Furthermore, within the patient population genetic polymorphisms associated with efficacy for DNA repair also become evident [137].

With progress in scientific technology, characterizing and profiling key components of the repair pathways is now more feasible. This results in increased preclinical validation studies using DNA repair inhibitors to improve therapeutic outcomes for otherwise therapeutically plateaued cancers like sarcomas. Development and implementation of novel therapeutic interventions involving DNA-repair proteins in combination with other targeted therapies and/or standard-of-care agents may help improve clinical outcomes in the patients. Furthermore, the role of DNA repair proteins and damaged cellular DNA are not only relevant in sarcomas but are pertinent to other cancers as well as contributing to the pathogenesis of many other diseases [138]. Therefore, identification of novel therapeutic combination involving DNA repair proteins is of high clinical value as it may be applicable for treating other human ailments.

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to fund new childhood cancer treatments and pediatric cancer research. For more information on how to help, please contact Curing Kids Cancer at 1-866-933-CURE (2873) or visit curingkidscancer.org<http://curingkidscancer.org/> to learn more.

Conflict of interest

The authors declare no conflict of interest.

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Author details

Niknam Riyahi[1,2,3], M. Reza Saadatzadeh[1,3,4,5], Khadijeh Bijangi-Vishehsaraei[1,5], Farinaz Barghi[1,3,6], Pankita H. Pandya[1,3,4,5,6] and Karen E. Pollok[1,2,3,4,5,6*]

1 Department of Pediatrics, Herman B Wells Center for Pediatric Research, United States

2 Department of Pharmacology and Toxicology, United States

3 Indiana University School of Medicine, Indiana University, Indianapolis, IN, 46202, United States

4 Indiana University Simon Comprehensive Cancer Center, United States

5 Division of Hematology/Oncology, Riley Hospital for Children, Indianapolis, IN, 46202, USA

6 Department of Medical and Molecular Genetics, United States

*Address all correspondence to: kpollok@iu.edu

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