Targeting Base Excision Repair in Cancer: NQO1-Bioactivatable Drugs Improve Tumor Selectivity and Reduce Treatment Toxicity Through Radiosensitization of Human Cancer

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Citation:
Starcher CL, Pay SL, Singh N, Yeh I-J, Bhandare SB, Su X, Huang X, Bey EA, Motea EA and Boothman DA (2020) Targeting Base Excision Repair in Cancer: NQO1-Bioactivatable Drugs Improve Tumor Selectivity and Reduce Treatment Toxicity Through Radiosensitization of Human Cancer. Front. Oncol. 10:1575. doi: 10.3389/fonc.2020.01575

Ionizing radiation (IR) creates lethal DNA damage that can effectively kill tumor cells. However, the high dose required for a therapeutic outcome also damages healthy tissue. Thus, a therapeutic strategy with predictive biomarkers to enhance the beneficial effects of IR allowing a dose reduction without losing efficacy is highly desirable. NAD(P)H:quinone oxidoreductase 1 (NQO1) is overexpressed in the majority of recalcitrant solid tumors in comparison with normal tissue. Studies have shown that NQO1 can bioactivate certain quinone molecules (e.g., ortho-naphthoquinone and β-lapachone) to induce a futile redox cycle leading to the formation of oxidative DNA damage, hyperactivation of poly(ADP-ribose) polymerase 1 (PARP1), and catastrophic depletion of NAD+ and ATP, which culminates in cellular lethality via NAD+-Keresis. However, NQO1-bioactivatable drugs induce methemoglobinemia and hemolytic anemia at high doses. To circumvent this, NQO1-bioactivatable agents have been shown to synergize with PARP1 inhibitors, pyrimidine radiosensitizers, and IR. This therapeutic strategy allows for a reduction in the dose of the combined agents to decrease unwanted side effects by increasing tumor selectivity. In this review, we discuss the mechanisms of radiosensitization between NQO1-bioactivatable drugs and IR with a focus on the involvement of base excision repair (BER). This combination therapeutic strategy presents a unique tumor-selective and minimally toxic approach for targeting solid tumors that overexpress NQO1.

Keywords: NQO1, PARP1 hyperactivation, ionizing radiation, base excision repair, double-strand break repair, synergy, β-lapachone, abasic sites

Abbreviations: 53BP1, tumor suppressor p53 binding protein 1; 8-oxoG, 8-Oxoguanine; AP site, apurinic/apyrimidinic site; APE1, apurinic/apyrimidinic endonuclease 1; APE2, apurinic/apyrimidinic endonuclease 2; ATP, adenosine triphosphate; BAPTA-AM, (1,2-bis(o-aminophenoxy)ethane-N,N,N',N'-tetraacetic acid); BER, base excision repair; CIP Complex, complex involved with MRN and BRCA as a scaffold; Dic, dicoumarol; DNA pol β, DNA polymerase beta; DNA-PKcs, DNA dependent protein kinase, catalytic subunit; DSβ, double-strand break; ER, endoplasmic reticulum; gH2AX, H2A histone family member X (phosphorylated Serine 139); Gy, gray of ionizing radiation; HAN, head and neck cancer; IR, ionizing radiation; Ku70/Ku80, XRCC5/XRCC6; MeOX, methoxyamine; MRN, complex of Mre11, Rad50, and Nbs1 involved in end processing; NAD+, nicotinamide adenine dinucleotide; NAMPT, nicotinamide phosphoribosyltransferase; NER, nucleotide excision repair; NHEJ, non-homologous end-joining; NQO1, NAD(P)H:quinone oxidoreductase 1; NSCLC, non-small cell lung cancer; OGG1, 8-Oxoguanine DNA glycosylase 1; PARylation, poly-ADP-ribosylation; PDAC, pancreatic ductal adenocarcinoma; ROS, reactive oxygen species; RPA, replication protein A; SSB, single-strand break; ssDNA, single-strand DNA; TNBC, triple-negative breast cancer; XRCC1, X-ray repair cross-complementing protein 1; XRCC4, X-ray repair cross-complementing protein 4.
INTRODUCTION

Ionizing radiation induces high levels of single-strand DNA breaks (SSBs), double-strand DNA breaks (DSBs), and oxidized bases via ROS production and DNA–protein cross-links that activate almost all DNA repair pathways (1, 2). Although effective, the toxicity of IR to healthy tissue at a therapeutic dose presents a significant limitation in the clinic (3–5). IR activates the BER pathway, in which DNA glycosylases (e.g., OGG1) create abasic sites and SSBs for base excision and replacement (6). If these SSBs persist, they are replicated through, or are within three base pairs of each other, they are converted to DSBs. The presence of one unrepaired DSB has been reported to be lethal (7, 8). Thus, combining IR with an agent that also promotes a significant increase in DNA damage through modified bases and deleterious DSBs preferentially in tumors may effectively reduce the necessary dose of IR in a clinical setting to lessen toxicity to healthy tissues and improve patient outcomes. The use of a tumor-selective drug for this purpose is an attractive possibility.

NAD(P)H:quinone oxidoreductase 1 (NQO1, also called DT-diaphorase) is a phase II two-electron redox enzyme that is highly overexpressed in most solid tumor types compared with most healthy tissues, as shown through studies by Siegel and Ross (9, 10). Ortho-napthoquinones are a unique class of quinone molecules that, unlike other quinones that are conjugated to glutathione and excreted from the cell, are bioactivated specifically by NQO1 to undergo a two-step back-reaction with oxygen (11). In this futile cycle, NQO1 continuously metabolizes the drugs and then reverts them to the parent compound (12). This process causes rapid accumulation of ROS such as superoxide radical and hydrogen peroxide (H$_2$O$_2$) that permeate the cell and nuclear membrane to cause significant numbers of oxidized bases and SSBs, which consequently lead to the formation of lethal DSBs. Poly(ADP-ribose) polymerase-1 (PARP1) is hyperactivated by this DNA damage, which rapidly depletes NAD$^+$ and ATP, causing metabolic catastrophe and cell death via programmed necrosis (termed NAD$^+-$Keresis) (13).

Base excision repair is the main repair pathway involved in activating PARP1 during the repair of SSFs and oxidized bases (14). Depleting BER enzymes, such as XRCC1, and modification of apurinic/apyrimidin (AP) sites with methoxamine (MeOX) synergizes with NQO1-bioactivatable drugs, promoting increased DSBs and rapid cell death (15). NQO1-bioactivatable drugs have long been known to synergize with halogenated pyrimidine radiosensitizers (16). More recently, synergy between PARP inhibitors (17) and IR (18, 19) has been shown. The use of NQO1-bioactivatable drugs, therefore, may be a clinically viable approach to reduce the toxicity of IR associated with high doses and also to improve the tumor selectivity of treatment. In this review, we discuss the mechanisms of radiosensitization between low doses of NQO1-bioactivatable drugs and IR—with a focus on the BER repair pathway and PARP1 hyperactivation—and present a case for combination treatment with NQO1-bioactivatable drugs and IR in the clinic.

NQO1-BIOACTIVATABLE DRUGS INDUCE A SPECIFIC FORM OF PROGRAMMED NECROSIS (NAD$^+-$KERESIS)

β-Lapachone (β-lap/ARQ761 in clinical form) is an NQO1-bioactivatable drug derived from lapachone (20), with known antimicrobial (21) and anticancer activity as a single agent (22). The futile redox cycling of β-lap by NQO1 (11) produces ROS-induced DNA damage (Figure 1A), which ultimately leads to cell death via metabolic and bioenergetic catastrophe caused by NAD$^+$ and ATP depletion following PARP1 hyperactivation (23). Boothman and colleagues have shown that within 5 min of β-lap treatment, there is a significant calcium flux from the ER to the cytosol (24). Calcium flux from the ER is necessary to activate calpain protease (24) and hyperactivate PARP1; however, the mechanistic role of calcium in PARP1 hyperactivation has yet to be firmly established (25). Within 30 min, the NAD$^+$ molecules that are produced during the futile redox cycling of β-lap by NQO1 are rapidly exhausted by hyperactivated PARP1 during the repair of ROS-induced DNA damage and SSBs (17). Depletion of NAD$^+$ consequently depletes ATP and induces a specific type of programmed necrosis, termed NAD$^+-$Keresis (26). Expression of catalase can spare cellular lethality by neutralizing the effects of hydrogen peroxide (H$_2$O$_2$) produced by β-lap (an NQO1-bioactivatable agent), confirming the role of ROS formation in toxicity (27). Inhibition of NQO1 activity with a small-molecule inhibitor (e.g., Dicoumarol) or genetically knocking out NQO1 eliminates β-lap lethality, showing the selectivity of β-lap-induced cell death to NQO1-expressing cells (17, 23). Calcium release from the ER can be blocked with the calcium chelator, BAPTA-AM, which prevents PARP1 hyperactivation and spares cancer cells from lethality, further highlighting the role of PARP1 in β-lap-induced cell death (25). When NAD$^+$ production is inhibited genetically by depleting NAMPT or pharmacologically with NAMPT inhibitors (e.g., FK866) (26) prior to β-lap treatment in NQO1-overexpressing cancer cells, a synergistic cell death due to compromised NAD$^+$ production following PARP hyperactivation highlights the critical role of catastrophic NAD$^+$ depletion in NAD$^+-$Keresis (Figure 1A) (26).

TRAPPING PARP1 ON DNA SYNERGIZES WITH NQO1-BIOACTIVATABLE DRUGS

There are 17 known PARP proteins (28) that share a common catalytic domain but exhibit differential roles in DNA repair, chromatin structure and modification, transcription, and cell death. PARP proteins catalyze the transfer of one or more ADP-ribose units to substrate proteins through a process known as mono- or poly(ADP) ribosylation, respectively (29). Of particular importance to the NQO1-bioactivatable drug field is PARP1, which is required for both BER and NER to recruit and activate SSB repair proteins (30). In BER, PARP1 forms a critical complex with DNA ligase III, XRCC1, and DNA pol β (30).
In BRCA1/2-deficient breast and ovarian cancers—which are deficient in homologous recombination (HR) to repair DSBs—PARP1 inhibitors are an effective therapeutic strategy targeting repair of SSBs and BER (31). PARP trapping agents (e.g., talazoparib, rucaparib, and olaparib) are the most effective PARP1-targeting drugs, which trap PARP1/2 on the DNA by binding at the active site, preventing its interaction with NAD$^+$ and therefore preventing dissociation via the auto-PARYlation domain (32). PARP trapping prevents the recruitment of proteins needed to complete BER, leaving unrepaired SSBs that are then converted to lethal DSBs upon collision with the replication and transcription machineries (31, 32).

Recently, we reported that PARP-trapping agents Rucaparib and Talazoparib synergize with β-lap in NQO1+ lung, pancreatic, and TNBC cell lines and in vivo models of NSCLC (17). Sublethal β-lap doses showed significant synergy with non-toxic doses of PARP inhibitor Rucaparib in multiple cancer types, and up to 60 different NSCLC cell lines (17). Synergy occurred regardless of oncogenic and tumor-repressor mutations and was entirely NQO1-dependent in all cell types (17), according to the gold standard combinatorial index obtained using the Chou and Talalay method (Figure 1B) (33).

Mechanistically, the addition of non-toxic doses of PARP inhibitor (e.g., Rucaparib) to sublethal β-lap doses prevents the loss of NAD$^+$ and ATP (17). No PARYlation of PARP1 occurred in this instance; however, DSBs significantly increased, indicating a β-lap-mediated SSB-to-DSB conversion (17). NAD$^+$ and ATP sparing allows for more oxygen consumption during the futile redox cycling of NQO1-bioactivatable agents, increasing the formation of oxidized bases and unrepaired SSBs (17). This process overwhelms the DNA damage response and repair (17). ATP is then used to initiate caspase-dependent apoptosis, which is in contrast with the NAD$^+$-Keresis observed with β-lap monotherapy (17). PARP inhibitors, therefore, enhance DNA damage caused by NQO1-bioactivatable drugs and switch cell death from programmed necrosis to apoptosis (17). This is significant as necrosis may cause inflammation and lead to complications, whereas apoptosis does not. Combining β-lap with PARP1 inhibitors, therefore, reduces the toxicity of the drug in addition to enhancing its mechanism of action, making it more attractive for clinical application.

**BER IS THE MAJOR DNA REPAIR PATHWAY INVOLVED IN THE NQO1-BIOACTIVATABLE DRUG MECHANISM OF ACTION**

Base excision repair resolves non-distorting DNA lesions resulting from alkylation, oxidation, depurine/pyrimidination, and deamination, which can be drug-induced or occur from exposure to environmental toxins. There are two types of BER: short patch that repairs a single damaged base and long patch that repairs up to three damaged bases (34). The typical mammalian BER pathway occurs as follows: DNA glycosylases detect damaged bases and cleave the glycosidic bond holding the damaged base to the DNA backbone, creating an apurinic/apyrimidinic site (AP site). AP sites are cleaved by AP endonucleases (APE1/APE2), allowing DNA pol β to fill the site with the appropriate base...
(35, 36). Mechanistically, APE1 provides a significant portion of the endonuclease activity, while APE2 provides some endonuclease activity and a large portion of exonuclease activity (34). Both APE1 and APE2 provide proofreading capabilities for pol β to reduce error rates (37). DNA ligase then seals up this stretch of DNA to finalize the DNA repair (35).

Hydrogen peroxide induced by β-lap permeates the nucleus and oxidizes nucleotides, particularly guanine bases (e.g., 8-oxo-guanine or 8-oxoG) (15). Oxidized guanine (8-oxoG) formed during treatment with β-lap recruits DNA glycosylase OGG1, which, combined with APE1/2, produces a SSB that activates PARP1 during BER (15). OGG1 recognizes the oxidized lesion, cleaves at the 3’ end, and removes the lesion, in a reaction that is catalyzed by ATP (38). It has been shown that silencing OGG1 prevents 8-oxoG recognition and increases the overall amount of 8-oxoG incorporated into DNA (32). This prevents PARP1 hyperactivation, thus abrogating NAD+/ATP loss and β-lap-mediated lethality (15). This is an important finding and a potential route of resistance in the clinic to NQO1-bioactivatable drugs.

Silencing the key BER protein, XRCC1, synergizes with NQO1-bioactivatable drugs in PDAC cell lines, further indicating that BER inactivation plays a critical role in β-lap toxicity (15). XRCC1 is a scaffolding protein required for clearing oxidized bases (39). PARylated-PARP1 bound to SSBs recruits XRCC1 through the BRCT 1 domain and forms a complex consisting of XRCC1 (40), DNA pol β (41), DNA Ligase IIIα (42), and APE1 (43). Without XRCC1, DNA base lesions and SSBs cannot be repaired. In addition to mediating synthetic lethality, XRCC1 knockdown also depletes NAD+ levels at a higher rate than with β-lap alone (15). XRCC1 knockdown is currently known to be embryonic lethal and essential for mouse development (44). NQO1-bioactivatable drug synergy with BER deficiencies in PDAC cells indicates a potential for these drugs to be beneficial in targeting pancreatic cancer.

Silencing OGG1 spared β-lap-mediated lethality in PDAC, compared with XRC1 knockdown (15). It is thought that knockdown of OGG1 glycosylase protects PDAC cells from death because BER is not activated if the scanning glycosylase is non-functional. This suggests an important role for different proteins in BER with regard to solid tumors. Alteration of expression or mutation of different proteins in the BER pathway can either sensitize or protect cancer cells from NQO1-bioactivatable drug-mediated lethality.

Methoxyamine is an AP site modifier (Figure 2) used to sensitize temozolomide-resistant glioblastoma (45) and ovarian cancer (46). MeOX modification of AP sites prevents their degradation, thus mitigating sodium hydroxide-mediated hydrolysis of the DNA backbone, preventing AP site cleavage, blocking AP endonuclease action, and preventing BER, resulting in cell death (47). MeOX synergizes with β-lap, increasing the number and persistence of AP sites, PARylation, and DSBs in PDAC. This is specific to β-lap, as co-treatment with NQO1-inhibitor dicoumarol abrogates AP site formation. β-lap and MeOX were also shown to synergize and ultimately reduce tumor volume in 33% of PDAC murine xenografts (15).

**DOUBLE-STRAND BREAK AND BER PLAY A CRITICAL ROLE IN TUMOR RESPONSE TO IR**

Ionizing radiation is one of the most common and effective methods for treating solid tumors in cancer patients. IR damages DNA directly by causing ionization in DNA itself or indirectly by ionizing the surrounding water resulting in aqueous free radicals that can react with DNA. Inducing significant DNA damage by IR over several treatments results in cancer cell death; however, there are significant drawbacks to this approach, including limitations on the number of IR dose a person can receive in a lifetime, costs, the need for special diets, and serious side effects arising from healthy tissue damage (48). IR produces 1000 SSBs, 40 DSBs, 700 altered thymine bases, 700 8-oxoG base alterations, and 150 DNA–protein cross-links per gray (Gy) (49, 50). The resistance of cancer cells is considered to be determined by the efficacy of DSB repair (51, 52).

Ionizing radiation-induced DSBs activate DSB repair via NHEJ and HR (Figure 3A). NHEJ occurs in all phases of the cell cycle (53), which is a quick and easy way to fix massive levels of dsDNA breaks, and is utilized for V(D)J recombination for the human immune system (52). During this process, Ku70 and Ku80 heterodimers bind the end of the double-stranded DNA breaks and form a complex to protect and recruit DNA-PKcs (54) to the site of the damage. XRC4 binds to the Ku dimers through Ku70 mediating the attachment of other proteins necessary to fix
FIGURE 3 | Ionizing radiation induces a wide variety of DNA damage. (A) IR causes dsDNA breaks that are repaired by HR (in S/G2 phase) or NHEJ (all phases of cell cycle). In HR, the RPA complex and BRC proteins form a scaffold complex with the sister chromatid and use it as a template to correct damage without error. NHEJ utilizes the Ku70/80/DNA-PKcs complex to quickly combine and ligate double-strand breaks. (B) IR-mediated radiolysis of water leads to ROS formation, which then creates base damage through oxidation. BER then repairs these lesions through the use of a type II DNA glycosylase (OGG1), AP endonuclease (APE1/APE2), PARP1, DNA polymerase, and ligase. This occurs through either short patch or long patch BER.

FIGURE 4 | Proposed mechanism of IR and β-lap radiosensitization. NSCLC tumors contain high levels of NQO1 compared to normal tissues. In the presence of NQO1, β-lap causes ROS-induced oxidative base DNA damage, which eventually leads to the formation of SSBS that activate PARP1. IR induces massive SSBS through contact with DNA and oxidized bases due to water radiolysis that require PARP1 and BER to resolve. This combination therapy pushes cumulative amount of DNA damage high enough that overwhelms and hyperactivates PARP1 during DNA damage response and repair, leading to programmed necrosis. Thus, NQO1 may be used as a predictive biomarker for selective targeting of NQO1-overexpressing cancers with low-dose IR in combination with NQO1-bioactivatable agents as radiosensitizers.

SUBLETHAL DOSES OF β-LAP RADIOSENSITIZE NSCLC CELLS TO LOW-DOSE RADIATION THERAPY

In A549 and H1650 NSCLC cell lines, a sublethal dose of β-lap causes significant sensitization to low-dose radiation therapy,
leading to a remarkable increase in cell death. Monotherapy with sublethal β-lap induces minimal DSBs, and low-dose radiation monotherapy induces characteristic increase in DSBs followed by efficient repair (18). Combination therapy with low doses of β-lap and radiation therapy, however, promotes rapid and sustained 53BP1 and gamma-H2AX foci formation that is consistent with DSBR formation and compromised DSBR repair (18). In NQO1+ NSCLC luciferase murine models, β-lap and IR combination therapy reduces tumor volume and increases survival up to 70% in comparison with either agents alone. Tumors tissues from mice treated with IR and β-lap demonstrated enhanced PAR and gamma-H2AX (pS139-H2AX, surrogate marker for DSBs) level compared with monotherapy, as well as decreased NAD\(^+\) and ATP levels (18). Normal tissues, which generally overexpress catalase and lack NQO1, were unaffected by co-treatment. The BER pathway may play a crucial role in this sensitization since β-lap treatment engages BER and PARP hyperactivation, and IR also activates BER. Mechanistically, we hypothesize that the combination of IR and β-lap treatment creates cumulative clusters of oxidative DNA damage and SSBS that result in severe PARP1 hyperactivation (inactive form of PARP1), which compromises BER and SSB repair. SSBS that are unrepaired are eventually converted to lethal unrepaired DSBs due to the lack of NAD\(^+\) and ATP molecules available to activate the efficient repair of SSBS and DSBs (Figure 4). Since most solid tumors overexpress NQO1, combining low-dose radiation with a sublethal concentration of β-lap may enhance tumor-selective and targeted killing and improve patient safety by lowering the overall doses of both agents. Using NQO1 as a predictive biomarker, this combination treatment strategy may reduce the impact of treatment on a patient's lifetime exposure to IR, may cut the costs associated with cancer treatment, and potentially reduce the amount of time needed for therapeutic response.

OUTLOOK/FUTURE

Further work is required to fully determine the critical role of BER in IR and β-lap combination therapy. We have previously shown that loss of specific BER factors potentiates the lethality of an NQO1-bioactivatable agent, β-lap, selectively in NQO1-overexpressing solid tumors. In fact, inhibition of PARP1—a critical factor involved in DNA damage response and repair of modified DNA bases and SSBS—prior to treatment with NQO1-bioactivatable drug causes a synergistic cancer cell death. Thus, we hypothesize that PARP1-mediated BER and SSB repair are the main DNA repair pathways that are activated by β-lap, which promotes severe PARP1 hyperactivation and subsequent lethality at high doses. PARP inhibitors in combination with IR and NQO1-bioactivatable drugs may further enhance synergy seen previously (6); however, three-drug combinations are currently rare. Overall, combining low-dose radiation therapy with NQO1-bioactivatable drugs may be a viable, less toxic, and more tumor-selective strategy for treatment of various solid tumors that overexpress a predictive biomarker, NQO1.

AUTHOR CONTRIBUTIONS

DB, prior to his death, directed the review’s thematic points regarding the synergistic value of IR or PARP inhibitors in combination with NQO1-bioactivatable therapies in the treatment of various NQO1-positive cancers. Following DB’s death, CS (DB’s graduate student), EB, EM, and XH (DB’s previous postdoctoral fellows, now faculty at IU School of Medicine) worked to write, edit, and review the manuscript. CS and EM took the lead in writing and made all the figures. EB, XH, NS, SB, SP, I-JY, and XS contributed to the editing of the review article. All authors contributed to the article and approved the submitted version.

FUNDING

This work was supported in part by NIH/NCI R01 CA210489 and NIH/NCI R01 CA224493. The content of the article is solely the responsibility of the authors and does not necessarily represent the official views of the funders.

ACKNOWLEDGMENTS

This review article is dedicated to the memory of our mentor, DB, who contributed significantly to the studies on the mechanism of action of NQO1-bioactivatable drugs. He will be greatly missed.

REFERENCES

1. Nickoloff JA, Boss MK, Allen CP, LaRue SM. Translational research in radiation-induced DNA damage signaling and repair. Transl Cancer Res. (2017) 6:8575–91.
2. Toulany M. Targeting DNA double-strand break repair pathways to improve radiotherapy response. Genes (Basel). (2019) 10:25. doi: 10.3390/genes10010025
3. Barnett GC, West CM, Dunning AM, Elliott RM, Coles CE, Pharoah PD, et al. Normal tissue reactions to radiotherapy: towards tailoring treatment dose by genotype. Nat Rev Cancer. (2009) 9:134–42. doi: 10.1038/nrc2587
4. Ryan JL. Ionizing radiation: the good, the bad, and the ugly. J Invest Dermatol. (2012) 132:985–93. doi: 10.1038/jid.2011.411
5. Hubenak JR, Zhang Q, Branch CD, Kronowitz SJ. Mechanisms of injury to normal tissue after radiotherapy: a review. Plast Reconstr Surg. (2014) 133:49–56.e. doi: 10.1097/PS.0000440818.23647.0b
6. Pachkowski BF, Tano K, Afonin V, Elder RH, Takeda S, Watanabe M, et al. Cells deficient in PARP-1 show an accelerated accumulation of DNA single strand breaks, but not AP sites, over the PARP-1-proficient cells exposed to MMS. Mutat Res. (2009) 671:93–9. doi: 10.1016/j.mrfmmm.2009.09.006
7. Khanna KK, Jackson SP. DNA double-strand breaks: signaling, repair and the cancer connection. Nat Genet. (2001) 27:247–54. doi: 10.1038/85798
8. Frankenberger-Schwager M, Frankenberg D, Harbich R. Potentially lethal damage, sublethal damage and DNA double strand breaks. Radiat Protect Dosimetry. (1985) 13:171–4. doi: 10.1093/oxfordjournals.rpd.a079571
9. Siegel D, Ross D. Immunodetection of NAD(P)H:quinone oxidoreductase 1 (NQO1) in human tissues. Free Radic BiolMed. (2000) 29:246–53.
28. Ame JC, Spenlehauer C, De Murcia G. The PARP superfamily.

27. Bey EA, Reinicke KE, Srougi MC, Varnes M, Anderson VE, Pink JJ, et al. Targeting the DNA repair defect in BRCA mutant cells as a therapeutic strategy. Nature. (2005) 434:917–21. doi: 10.1038/nature03445

26. Mackey ZB, Ramos W, Levin DS, Walter CA, McCarrey JR, Tomkinson AE. XVI: NAD(P)H:quinone oxidoreductase activity is the principal determinant of beta-lapachone cytotoxicity. J Biol Chem. (2000) 275:5416–24. doi: 10.1074/jbc.275.8.5416

25. Finkielman M, Reinicke KE, Srougi MC, Varnes M, Anderson VE, Pink JJ, et al. Targeting the DNA repair defect in BRCA mutant cells as a therapeutic strategy. Nature. (2005) 434:917–21. doi: 10.1038/nature03445

24. Tagliarino C, Pink JJ, Dubyak GR, Nieminen AL, Boothman DA. Calcium is a key signaling molecule in beta-lapachone-mediated cell death. J Biol Chem. (2001) 276:19150–9. doi: 10.1074/jbc.m10730200

23. Bey EA, Bentle MS, Reinicke KE, Gao J, Ritchie JM, Bornmann W, et al. Efficacy of beta-lapachone in pancreatic cancer treatment: exploiting the novel, therapeutic target NQO1. Cancer Biol Ther. (2005) 4:95–102.

22. Caldecott KW, Aoufouchi S, Johnson P, Shall S. XRCC1 is specifically associated with poly(ADP-ribose) polymerase inhibitors. Cancer Cell. (2016) 30:940–52.

21. Park HJ, Ahn KJ, Ahn SD, Choi E, Lee SW, Williams B, et al. Requirement for the Xrcc1 DNA base excision repair gene during early mouse development. Dev Biol. (2005) 281:368–94. doi: 10.1016/j.ydbio.2004.09.018

20. Reinicke KE, Bey EA, Bentle MS, Pink JJ, Ingalls ST, Hoppel CL, et al. Development of beta-lapachone prodrugs for therapy against human cancer cells with elevated NAD(P)H:quinone oxidoreductase 1 levels. Clin Cancer Res. (2014) 20:2601–9. doi: 10.1158/1078-0432.ccr-13-2508

19. Cruiz F, Ricordi C, Iturriza GA, Flueckiger S, Hughey R, et al. Alternative splicing event which occurs in mouse pachytene spermatocytes and negatively regulates its activity following DNA damage. Mol Cell Biol. (1999) 19:14–26. doi: 10.1016/j.dnarep.2014.03.030

18. Chakrabarti G, Silvers MA, Ilcheva M, Liu Y, Moore ZR, Luo X, et al. The NQO1 bioactivatable drug, beta-lapachone, alters the redox state of NQO1+ pancreatic cancer cells, causing perturbation in central carbon metabolism. J Biol Chem. (2017) 292:18203–16. doi: 10.1074/jbc.m117.813923

17. Huang X, Motea EA, Moore ZR, Yang J, Dong Y, Chakrabarti G, et al. Leveraging an NQO1 bioactivatable drug for tumor-selective use of poly(ADP-ribose) polymerase inhibitors. Cancer Cell. (2016) 30:940–52.

16. Boothman DA, Greer S, Pardee AB. Potentiation of halogenated pyrimidine radiosensitizers in human carcinoma cells by beta-lapachone (3,4-dihydro-2,2-dimethyl-2H-naphthol(1,2-b):pyrazan-5,6-dione), a novel DNA repair inhibitor. Cancer Res. (1987) 47:3561–6.

15. Kubovics F, Szukasov C, Unk I, Haracska L. Human Ap2e protein has a 3'-5' exonuclease activity that acts preferentially on mismatched base pairs. Nucleic Acids Res. (2006) 34:2508–15. doi: 10.1093/nar/gkl259

14. Reynolds P, Cooper S, Lomax M, O'Neill P. Disruption of PARP1 function inhibits base excision repair of a sub-set of DNA lesions. Nucleic Acids Res. (2015) 43:4028–38. doi: 10.1093/nar/gkv250

13. Tagliarino C, Silvers MA, Ilcheva M, Liu Y, Moore ZR, Luo X, et al. The PARP superfamily.

12. Silvers MA, Deja S, Singh N, Egnatchik RA, Sudderth J, Luo X, et al. The NQO1 bioactivatable drug, beta-lapachone, alters the redox state of NQO1+ pancreatic cancer cells, causing perturbation in central carbon metabolism. J Biol Chem. (2017) 292:18203–16. doi: 10.1074/jbc.m117.813923

11. Tagliarino C, Silvers MA, Ilcheva M, Liu Y, Moore ZR, Luo X, et al. The PARP superfamily.

10. Ross D, Siegel D. Functions of NQO1 in cellular protection and CoQ10 metabolism and its potential role as a redox sensitive molecular switch. Front Physiol. (2017) 8:955.
51. Mladenov E, Magin S, Soni A, Iliakis G. DNA double-strand break repair as determinant of cellular radiosensitivity to killing and target in radiation therapy. *Front Oncol.* (2013) 3:113.

52. Borrego-Soto G, Ortiz-Lopez R, Rojas-Martinez A. Ionizing radiation-induced DNA injury and damage detection in patients with breast cancer. *Genet Mol Biol.* (2015) 38:420–32. doi: 10.1590/s1415-47573820150019

53. Deckbar D, Jeggo PA, Lobrich M. Understanding the limitations of radiation-induced cell cycle checkpoints. *Crit Rev Biochem Mol Biol.* (2011) 46:271–83. doi: 10.3109/10409238.2011.575764

54. Williams GJ, Hammel M, Radhakrishnan SK, Ramsden D, Lees-Miller SP, Tainer JA. Structural insights into NHEJ: building up an integrated picture of the dynamic DSB repair super complex, one component and interaction at a time. *DNA Repair (Amst).* (2014) 17:110–20. doi: 10.1016/j.dnarep.2014.02.009

55. Ochi T, Wu Q, Blundell TL. The spatial organization of non-homologous end joining: from bridging to end joining. *DNA Repair (Amst).* (2014) 17:98–109. doi: 10.1016/j.dnarep.2014.02.010

56. Roberts SA, Strande N, Burkhalter MD, Strom C, Havener JM, Hasty P, et al. Ku is a 5'-dRP/AP lyase that excises nucleotide damage near broken ends. *Nature.* (2010) 464:1214–7. doi: 10.1038/nature08926

57. Gu J, Lu H, Tippin B, Shimazaki N, Goodman MF, Lieber MR. XRCC4-DNA ligase IV can ligate incompatible DNA ends and can ligate across gaps. *Embo J.* (2007) 26:1010–23. doi: 10.1038/sj.emboj.7601559

58. Krejci L, Altmanova V, Spirek M, Zhao X. Homologous recombination and its regulation. *Nucleic Acids Res.* (2012) 40:5795–818.

59. Limbo O, Chahwan C, Yamada Y, de Bruin RAM, Wittenberg C, Russell P. Ctp1 is a cell-cycle-regulated protein that functions with Mre11 complex to control double-strand break repair by homologous recombination. *Molecular Cell.* (2007) 28:134–46. doi: 10.1016/j.molcel.2007.09.009

60. West SC. Molecular views of recombination proteins and their control. *Nat Rev Mol Cell Biol.* (2003) 4:435–45. doi: 10.1038/nrm1127

61. Masson JY, Tarsounas MC, Stasiak AZ, Stasiak A, Shah R, McIlwraith MJ, et al. Identification and purification of two distinct complexes containing the five RAD51 paralogs. *Genes Dev.* (2001) 15:3296–307. doi: 10.1101/gad.947001

62. Constantinou A, Chen X-B, McGowan CH, West SC. Holliday junction resolution in human cells: two junction endonucleases with distinct substrate specificities. *EMBO J.* (2002) 21:5577–85. doi: 10.1093/emboj/cdf554

63. Matsos J, West SC. Holliday junction resolution: regulation in space and time. *DNA Repair (Amst).* (2014) 19:176–81. doi: 10.1016/j.dnarep.2014.03.013

64. Ward JF. Biochemistry of DNA lesions. *Radiat Res Suppl.* (1985) 8:S103–11.

65. Blaisdell JO, Wallace SS. Abortive base-excision repair of radiation-induced clustered DNA lesions in *Escherichia coli*. *Proc Natl Acad Sci USA.* (2001) 98:7426–30. doi: 10.1073/pnas.131077798

**Conflict of Interest:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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