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Cancer Drug Resistance

Review

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Exploiting DNA repair pathways for tumor sensitization, mitigation of resistance, and normal tissue protection in radiotherapy

Jac A. Nickoloff, Lynn Taylor, Neelam Sharma, Takamitsu A. Kato

Department of Environmental and Radiological Health Sciences, Colorado State University, Ft. Collins, CO 80523, USA.

Correspondence to: Dr. Jac A. Nickoloff, Department of Environmental and Radiological Health Sciences, Colorado State University, 1681 Campus Delivery, Ft. Collins, CO 80523-1681, USA. E-mail: J.Nickoloff@colostate.edu

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Abstract

More than half of cancer patients are treated with radiotherapy, which kills tumor cells by directly and indirectly inducing DNA damage, including cytotoxic DNA double-strand breaks (DSBs). Tumor cells respond to these threats by activating a complex signaling network termed the DNA damage response (DDR). The DDR arrests the cell cycle, upregulates DNA repair, and triggers apoptosis when damage is excessive. The DDR signaling and DNA repair pathways are fertile terrain for therapeutic intervention. This review highlights strategies to improve therapeutic gain by targeting DDR and DNA repair pathways to radiosensitize tumor cells, overcome intrinsic and acquired tumor radioresistance, and protect normal tissue. Many biological and environmental factors determine tumor and normal cell responses to ionizing radiation and genotoxic chemotherapeutics. These include cell type and cell cycle phase distribution; tissue/tumor microenvironment and oxygen levels; DNA damage load and quality; DNA repair capacity; and susceptibility to apoptosis or other active or passive cell death pathways. We provide an overview of radiobiological parameters associated with X-ray, proton, and carbon ion radiotherapy; DNA repair and DNA damage signaling pathways; and other factors that regulate tumor and normal cell responses to radiation. We then focus on recent studies exploiting DSB repair pathways to enhance radiotherapy therapeutic gain.

Keywords: DNA repair, DNA double-strand break repair, non-homologous end-joining, homologous recombination, radiosensitization, radioprotection, cancer therapy

INTRODUCTION

Ionizing radiation has been used to treat cancer for more than 120 years, and radiotherapy is widely used to treat many types of cancer. More than half of cancer patients receive radiation as monotherapy or in combination with surgery, genotoxic chemotherapy, and targeted therapy. Radiation is usually delivered with external beams, but radioactive implants (brachytherapy) are used to treat prostate, head and neck, breast, eye, and other cancers[1]. Regardless of the mode of delivery, ionizing radiation is effective because it causes cytotoxic DNA damage (i.e., it is genotoxic), and in this way it is similar to genotoxic chemotherapy. However, radiotherapy is only effective for local tumor control and isolated metastases, whereas genotoxic chemotherapy, delivered systemically, can also treat widespread metastatic disease. There is evidence that radiotherapy may be effective against distant disease, through immune-mediated, non-targeted abscopal effects, but this approach is currently limited to pre-clinical studies[2]. Radiotherapy has several benefits for patients: It is non-invasive, painless, and has low rates of severe side-effects, highlighting another difference from systemic, genotoxic chemotherapy which often causes side effects that compromise patient quality of life. Although metastatic disease is ultimately responsible for most cancer deaths, the importance of local tumor control should not be underestimated. As noted in a widely used radiation oncology textbook, "…for tumors with high metastatic potential, such as breast, prostate, and lung…improved locoregional control by radiotherapy with or without chemotherapy enhances overall [patient] survival"[3]. Among the ongoing challenges in the radiotherapy field are the adverse effects of radiation on sensitive, normal tissues adjacent to tumors, in particular brain, spinal cord, and heart. In contrast, systemic genotoxins cause widespread damage, in particular to proliferative normal tissues including gastrointestinal lining and bone marrow, causing nausea and anemia, as well as non-proliferating brain tissue, causing chemotherapy-induced cognitive impairment or "chemo-brain"[4]. For both genotoxic chemotherapeutics and radiation, there is great interest in understanding mechanisms of intrinsic and acquired tumor cell resistance to these agents[5-8].

The goal of radiotherapy is to completely eradicate tumor cells while sparing nearby normal tissue. The efficacy of radiotherapy has greatly improved with the development of advanced techniques for diagnostic imaging, beam-focusing, and beam-shaping[9,10], and treatment outcomes continue to improve as combination therapeutic strategies mature[11]. Two ways that combination therapies can improve therapeutic gain are to radiosensitize tumor cells, especially those with high intrinsic or acquired radioresistance, and protect normal tissue. There are many biological parameters that modulate tumor and normal cell responses to radiation, such as cell type, cell cycle phase, tissue/tumor microenvironment, oxygen levels, DNA repair capacity, and others. We begin with a synopsis of radiation damage to cellular components; cellular responses to radiation damage; environmental and cellular factors that determine normal and tumor cell radiosensitivity; and strategies used to counter tumor radioresistance or protect normal tissue from radiation damage. We then discuss how DNA repair and DNA damage response (DDR) pathways can be exploited to radiosensitize tumor cells and protect normal tissue during radiotherapy.

IONIZING RADIATION DAMEGE TO CELLULAR COMPONENTS AND CELL RESPONSES

Genotoxic chemotherapeutics and ionizing radiation kill cells by directly or indirectly damaging DNA or interfering with DNA metabolism (DNA polymerases, topoisomerases, or chromosome segregation machinery). Ionizing radiation, whether delivered by X-rays, protons, or carbon ions, causes damage to cellular components through direct energy absorption or indirectly by ionizing water to generate reactive oxygen species (ROS), including hydroxyl radicals, superoxide, and hydrogen peroxide[12]. ROS are highly reactive and interact almost immediately with cellular components, causing oxidative and other damage to proteins, nucleic acids, and membrane components. ROS are also generated during normal cell metabolism, primarily from mitochondrial function[13,14]. Cells survive and thrive despite > 100,000 spontaneous DNA lesions/cell/day, including ~10,000 single-strand breaks and ~50 DNA double-strand breaks (DSBs)[15-17].

Nearly all DNA lesions block DNA replication, although some can be bypassed by error-prone translesion DNA polymerases[18]. The ability of cells to manage this remarkable daily lesion load is a reflection of the high efficiency of DNA repair systems. That said, DNA damage can cause mutations, chromosome structural alterations, cell cycle arrest, senescence, and cell death. Among the hundreds of types of DNA lesions, DSBs are among the most cytotoxic, and the cytotoxicity of genotoxic chemicals and ionizing radiation is largely due to DSBs[19,20]. Other double-strand lesions, such as inter-strand crosslinks, are also highly cytotoxic[21].

Cells respond to DNA damage by activating checkpoint signaling and DNA repair pathways, collectively termed the DDR. DDR promotes cell survival and suppresses cancer by promoting genome stability, but it also triggers programmed cell death when damage is excessive. Altered expression or mutation of DDR proteins predispose to cancer, determine tumor response to chemo- and radiotherapy, and underlie several congenital conditions including multiple types of Seckel syndrome, primordial dwarfism, and premature aging syndromes[22-24]. The DDR is a major determinant of cancer cell responses to chemo- and radiotherapy, and is thus an enticing target to augment cancer therapy[25-30]. DDR components are often defective in cancer, but because the DDR is a complex network of interacting/cross-talking pathways, cells can respond to alterations in one pathway with compensatory changes in other pathways. Compensatory pathways within the DDR network represent formidable obstacles to successful cancer treatment. A better understanding of DDR pathways can reveal synthetic lethal relationships that can be exploited to augment cancer therapy in general, and to develop personalized therapies[31-35].

The DDR includes two checkpoint signaling pathways, one centered on ataxia telangiectasia mutated (ATM), a kinase that responds to DSBs and one centered on ataxia telangiectasia and Rad3 related (ATR) kinase that is triggered by single-stranded DNA (ssDNA) generated by 5'-3' resection of DSB ends and by decoupling of the replication machinery from MCM helicase at stalled replication forks[36-39]. ATM and ATR, along with DNA-PKcs, are PI3 kinase-like kinases (PIKKs) that are "early responders" to DSBs and replication stress. PIKKs phosphorylate large networks of proteins[40-42] including the downstream effector kinases Chk1 and Chk2 that phosphorylate p53 and other targets to arrest the cell cycle in response to damage, promote DNA repair, and promote programmed cell death pathways when damage exceeds a threshold[43-46] [Figure 1]. The DDR thus presents two broad targets to manipulate for therapeutic gain: inhibiting DNA repair sensitizes cells to damage and inhibiting checkpoint signaling prevents cell cycle arrest in response to damage, increasing replication stress, fork collapse to DSBs, genome instability, and cell death[20,47-50].

DSBs are repaired by error-prone non-homologous end-joining (NHEJ) or by homologous recombination (HR) repair [Figure 2][51,52], templated from sister chromatids (restricted to S/G2 phases), homologous chromosomes, or short sequence repeats if the double-strand damage occurs within or nearby repeated sequences - not uncommon given the human genome comprises > 50% repetitive elements (Alu, MIRs, SINEs, LINEs, *etc.*)[53]. HR is generally accurate, but it does pose risks of genome rearrangements including large-scale loss of heterozygosity and translocations that can initiate tumorigenesis and drive tumor progression[27,54,55]. When the primary NHEJ or HR pathways fail, even more error-prone DSB repair pathways serve as back-up, including alternative (microhomology-mediated) NHEJ, single-strand annealing, and break-induced replication[56-62].

RADIOBIOLOGICAL PROPERTIES OF THERAPEUTIC IONIZING RADIATION

Three types of external beam radiation are used to treat cancer. X-rays and protons are low linear energy transfer (LET) radiation, although proton LET varies (see below). LET is a measure of ionization density, thus low LET X-rays (and protons for the most part) are sparsely ionizing. This means that most X-ray lesions, including DSBs, are widely dispersed. X-rays are massless photons that interact weakly with

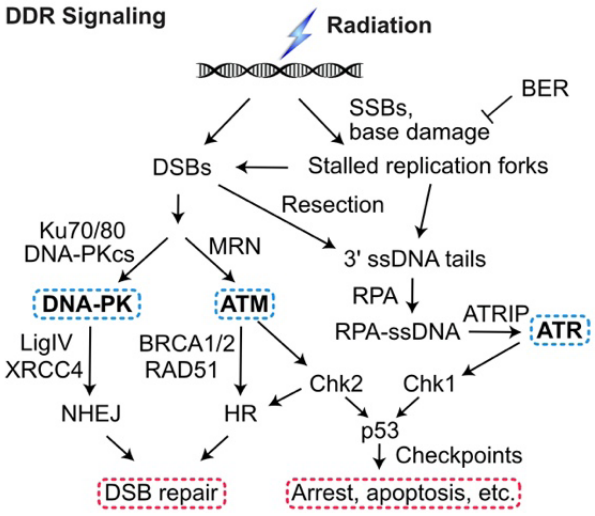

Figure 1. DDR signaling. Ionizing radiation and genotoxic chemotherapy create single- and double-strand DNA damage including DSBs that activate three PIKKs: DNA-PK, ATM, and ATR. Single-strand breaks and base damage, if not repaired by base excision repair (BER), block replication, which produces ssDNA when the replisome decouples from the MCM helicase or stalled forks are cleaved to produce DSBs, which, along with frank DSBs, are resected to 3′ single-stranded tails that are coated by RPA. This activates ATR to signal checkpoint responses through Chk1 and p53. Non-resected DSB ends are bound by the Ku70/Ku80 heterodimer, which recruits and activates DNA-PKcs in the DNA-PK holoenzyme, LigIV/XRCC4 ligates DNA ends to effect NHEJ. The competing HR pathway initiates with limited DSB end resection by MRE11/RAD50/NBS1 (MRN), more extensive resection by Exo1 and Dna2, and RAD51 binding to ssDNA (mediated by BRCA1, BRCA2, and other proteins) to yield the RAD51-ssDNA nucleoprotein filament that effects HR. DDR: DNA damage response; DSBs: DNA double-strand breaks

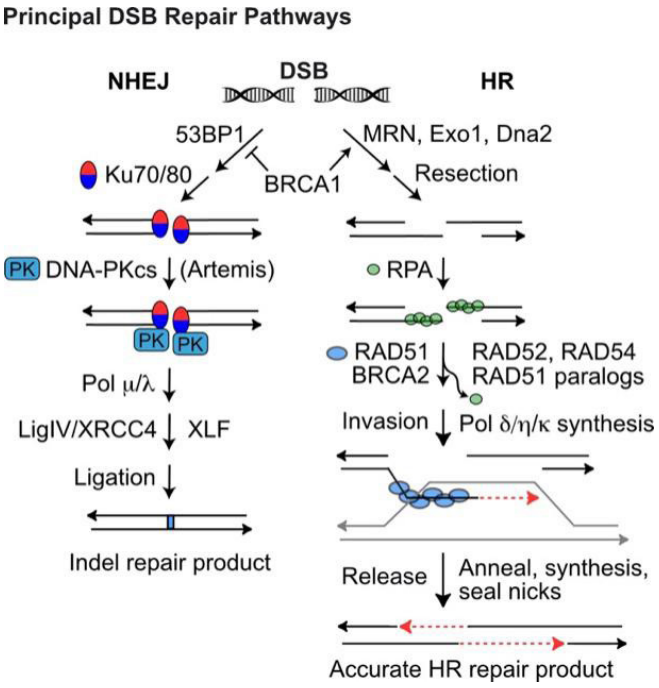

Figure 2. DSB repair by NHEJ and HR. Pathway choice is determined by end-resection, prevented by 53BP1, or promoted by BRCA1/MRN and Exo1/Dna2 exonucleases. (Left) NHEJ of unresected DSB ends initiates with Ku70/80 binding to ends and recruitment of DNA-PKcs to form the activated DNA-PK holoenzyme. Artemis is required to trim certain types of end-structures, and small gaps may be filled with polymerases *m* and *l* prior to LigIV/XRCC4/XLF-mediated ligation. NHEJ repair usually produces small indels (1-20-bp deletions, few-bp insertions). (Right) Resected 3′ single-strand ends are coated with RPA, which is then exchanged with RAD51, mediated by BRCA2, RAD52, RAD54, and RAD51 paralogs. The RAD51 nucleoprotein filament seeks and invades a homologous donor duplex (grey). RAD51 dissociates before repair synthesis; the newly synthesized strand (red dash) is released from the donor duplex and anneals with the complementary strand on the opposite side of the DSB. A second round of repair synthesis and nick sealing completes repair. DDR: DNA damage response; DSB: DNA double-strand break; NHEJ: non-homologous end-joining; HR: homologous recombination

tissue, thus the highest X-ray doses are near the skin at the entrance point. To concentrate X-ray doses within tumors, beams are intensity modulated and delivered to patients from several angles, spreading low doses to a large volume of normal tissue[3]. Protons have a small mass and a single positive charge. Proton interactions with tissue slow and eventually stop these particles at a defined depth (within a tumor), termed the Bragg peak[63]. This feature provides a clear benefit as normal tissue beyond the tumor receives essentially no dose. Carbon ions with high mass and six positive charges are high LET radiation. Because of their mass, carbon ions also stop at depth and eliminate exit dose, similar to protons. However, the high mass and high charge of carbon ions produces dense ionization tracks, especially at the end of the track as particles slow and stop[64,65].

X-rays, protons, and carbon ions induce the same number of DSBs per unit dose (~40 DSBs/Gy). Exposures to 1 Gy of X-rays or protons kills ~10%-20% of cells[66-68]. In contrast, the same dose of carbon ions kills 2-3-fold more cells, hence the relative biological effect (RBE) of carbon ions is ~2.5. Proton LET increases somewhat in the distal region of the Bragg peak, and RBE correspondingly increases to perhaps as high as 1.7[65,69]. The high RBE of carbon ions reflects the fact that these ions efficiently induce clustered DSBs, defined as two or more DSBs separated by < 200 bp[64,68,70]. Clustered DSBs are repaired inefficiently and are hence more cytotoxic than isolated DSBs. Low LET X-rays and protons induce occasional clustered DSBs - it is thought that these lesions primarily determine low LET radiation cytotoxicity, not the more prevalent isolated DSBs[64,68,71-73]. The greater cytotoxicity (RBE) of carbon ions reflects their greater efficiency at inducing clustered DSBs. NHEJ, the dominant DSB repair pathway, initiates with Ku70/Ku80 (Ku) binding to DNA ends and recruitment of DNA-PKcs [Figure 2][51]. Ku appears to efficiently bind both large and small DNA fragments, generated by isolated and clustered DSBs, respectively. However, short fragments do not activate DNA-PKcs kinase[74], which has critical roles in NHEJ, HR, DDR signaling, and checkpoint activation[75]. Thus, short DNA fragments appear to be refractory to repair by NHEJ, and this may account for both the greater cytotoxicity of clustered *vs*. isolated DSBs, and the shift from NHEJ toward HR in cells exposed to high LET radiation[64,76-79]. A greater dependence on HR was also observed with protons than X-rays[80], perhaps reflecting the higher proton LET in the Bragg peak. However, a more recent study showed minimal differences when cells were treated with X-rays vs. protons, and inhibitors of NHEJ or HR[81], suggesting additional factors determine repair pathway choices among cell types. That cells struggle to repair clustered DSBs may reflect their rarity in nature and the lack of selective pressure to evolve repair systems for this class of complex DNA lesion.

Low and high LET radiation are distinguished in two other ways. Low LET X-rays and protons induce ROS most efficiently in well-oxygenated tissue. At low oxygen levels, the cytotoxic effects of X-rays and protons is reduced ~3-fold, the so-called oxygen enhancement ratio (OER)[82]. Importantly, high LET carbon ions show far less reliance on oxygen (lower OER), owing to the greater ionization potential of these high mass/high charge ions[82,83]. Radiosensitivity varies during the cell cycle. Low LET X-rays and protons show highest cytotoxicity during G1 and M phases, and ~2-fold less cytotoxicity during S-phase, termed S-phase radioresistance[84]. Interestingly, high LET carbon ions show the opposite effect: ~2-3-fold S-phase radiosensitivity relative to G1 cells (Kato, unpublished results). This suggests one mechanism by which mixed high and low LET exposures might yield synergistic cell killing[85-87].

The highly damaging effects of high LET radiation initially raised concerns about the safety of carbon ions in radiotherapy[88], but serious side effects occur no more often than with X-rays or protons[89-91]. This safety profile probably reflects the fact that high LET ions behave similarly to low LET X-rays and protons while traveling through (normal) tissue at high speed, gaining their high LET properties only when slowing and stopping at the end of their tracks (in tumors)[63,92]. Thus, carbon ion LET and RBE are relatively low in the entrance region and increase dramatically in the Bragg peak, and the most damaging effects are confined to the tumor volume[63,93,94].

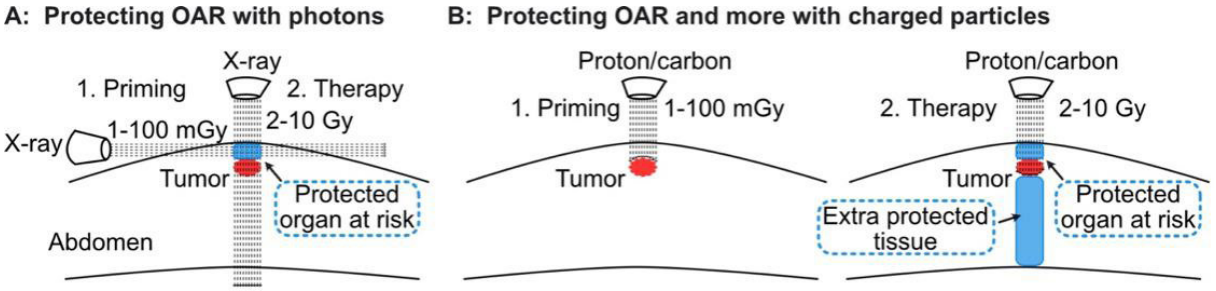

Figure 4. Proposed approach to protect normal tissue by stimulating radioadaptive responses. Horizontal X-ray beam delivers a priming dose to protect OAR (blue), but not the tumor (red) from subsequent, high doses delivered with a vertical beam(A); low priming dose of charged particles (left) protect OAR (blue) from subsequent high doses (right) (B). With charged particles, priming and therapeutic doses can be delivered along the same beamline since particles stop at predetermined depths. Charged particles also protect normal tissue distal to the tumor (larger blue section). OAR: organs at risk

being augmented, the types of agents administered, tumor location, and the organs at risk. Therapeutic efficacy can be increased, and side effects decreased, by employing multi-targeted approaches[236]. For example, the Li lab combined physical (radiation) targeting with two other targeting approaches. The first was an oncolytic adenovirus to deliver hTERT promoter-driven E1a gene for conditional replication in hTERT-positive (tumor) cells, and the second was a replication-defective adenovirus expressing shRNA to repress DNA-PKcs[237]. This downregulated NHEJ specifically in tumor cells within the (physically-targeted) radiation beam. Another tumor-specific targeting approach is illustrated by recent studies targeting triple-negative breast cancer. Here, CRISPR/Cas9 designed to knock out the Lcn2 oncogene was delivered to breast cancer cells using a tumor-tropic, ICAM1 antibody-linked nanomaterial[238,239]. These and other targeting strategies can be combined to enhance a wide variety of therapeutic interventions.

The adaptive response raised concerns about improved tumor cell survival when tumors are "primed" with 5-10 mGy diagnostic CT scans to localize tumors before treatment with a 2-10 Gy "challenge" (therapeutic) dose[134]. It may be possible to invert this paradigm and exploit the adaptive response to protect normal tissue and increase therapeutic gain. This might be done, for example, by using a transverse photon (X-ray) beam to expose normal tissue above the tumor to low (mGy) doses. This could induce a transient adaptive response in at-risk normal tissue [specifically, organs at risk (OAR)], protecting this tissue from high dose radiotherapy delivered with a perpendicular beam [Figure 4A]. Such a strategy might be optimized with particle radiation, as priming doses can be delivered to just the normal tissue region that will be subsequently exposed to therapeutic doses in the entrance region, and particles also spare distal tissue [Figure 4B].

In conclusion, multi-targeted strategies that combine DNA repair and DDR-modulated tumor-specific radiosensitization, advanced photon and particle beam focusing, and radioprotection of normal tissues are a rational path to tumor cures with minimal side effects.

DECLARATIONS

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Authors' contributions

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161. Balbous A, Cortes U, Guilloteau K, et al. A radiosensitizing effect of RAD51 inhibition in glioblastoma stem-like cells. *BMC Cancer* 2016;16:604.
162. King HO, Brend T, Payne HL, et al. RAD51 Is a selective DNA repair target to radiosensitize glioma stem cells. *Stem Cell Reports* 2017;8:125-39.
163. Pastushok L, Fu Y, Lin L, et al. A novel cell-penetrating antibody fragment inhibits the DNA repair protein RAD51. *Sci Rep* 2019;9:11227.
164. Turchick A, Liu Y, Zhao W, Cohen I, Glazer PM. Synthetic lethality of a cell-penetrating anti-RAD51 antibody in PTEN-deficient melanoma and glioma cells. *Oncotarget* 2019;10:1272-83.
165. Turchick A, Hegan DC, Jensen RB, Glazer PM. A cell-penetrating antibody inhibits human RAD51 via direct binding. *Nucleic Acids Res* 2017;45:11782-99.
166. Cyteir Therapeutics I. A phase 1/2 study of CYT-0851, an oral RAD51 inhibitor, in B-cell malignancies and advanced solid tumors. In; 2019.
167. Yu D, Sekine E, Fujimori A, Ochiya T, Okayasu R. Down regulation of BRCA2 causes radio-sensitization of human tumor cells in vitro and in vivo. *Cancer Sci* 2008;99:810-5.
168. Hirai T, Shirai H, Fujimori H, et al. Radiosensitization effect of poly(ADP-ribose) polymerase inhibition in cells exposed to low and high linear energy transfer radiation. *Cancer Sci* 2012;103:1045-50.
169. Hirai T, Saito S, Fujimori H, et al. Radiosensitization by PARP inhibition to proton beam irradiation in cancer cells. *Biochem Biophys Res Commun* 2016;478:234-40.
170. Jannetti SA, Zeglis BM, Zalutsky MR, Reiner T. Poly(ADP-ribose)polymerase (PARP) inhibitors and radiation therapy. *Front Pharmacol* 2020;11:170.
171. Chang L, Graham PH, Hao J, et al. PI3K/Akt/mTOR pathway inhibitors enhance radiosensitivity in radioresistant prostate cancer cells through inducing apoptosis, reducing autophagy, suppressing NHEJ and HR repair pathways. *Cell Death Dis* 2014;5:e1437.
172. Schotz U, Balzer V, Brandt FW, et al. Dual PI3K/mTOR inhibitor NVP-BEZ235 enhances radiosensitivity of head and neck squamous cell carcinoma (HNSCC) cell lines due to suppressed double-strand break (DSB) repair by non-homologous end joining. *Cancers* 2020;12:467.
173. Gil del Alcazar CR, Hardebeck MC, Mukherjee B, et al. Inhibition of DNA double-strand break repair by the dual PI3K/mTOR inhibitor NVP-BEZ235 as a strategy for radiosensitization of glioblastoma. *Clin Cancer Res* 2014;20:1235-48.
174. Hirakawa H, Fujisawa H, Masaoka A, et al. The combination of Hsp90 inhibitor 17AAG and heavy-ion irradiation provides effective tumor control in human lung cancer cells. *Cancer Med* 2015;4:426-36.
175. Lee Y, Li HK, Masaoka A, et al. The purine scaffold Hsp90 inhibitor PU-H71 sensitizes cancer cells to heavy ion radiation by inhibiting DNA repair by homologous recombination and non-homologous end joining. *Radiother Oncol* 2016;121:162-8.
176. Noguchi M, Yu D, Hirayama R, et al. Inhibition of homologous recombination repair in irradiated tumor cells pretreated with Hsp90 inhibitor 17-allylamino-17-demethoxygeldanamycin. *Biochem Biophys Res Commun* 2006;351:658-63.
177. Segawa T, Fujii Y, Tanaka A, et al. Radiosensitization of human lung cancer cells by the novel purine-scaffold Hsp90 inhibitor, PU-H71. *Int J Mol Med* 2014;33:559-64.
178. Lee Y, Sunada S, Hirakawa H, et al. TAS-116, a novel Hsp90 inhibitor, selectively enhances radiosensitivity of human cancer cells to X-rays and carbon ion radiation. *Mol Cancer Ther* 2017;16:16-24.
179. Fujii Y, Kato T, Kubota N, et al. p53 independent radio-sensitization of human lymphoblastoid cell lines by Hsp90 inhibitor 17-allylamino-17-demethoxygeldanamycin. *Oncol Rep* 2010;23:199-203.
180. Shimomura A, Yamamoto N, Kondo S, et al. First-in-human Phase I study of an oral HSP90 inhibitor, TAS-116, in patients with advanced solid tumors. *Mol Cancer Ther* 2019;18:531-40.
181. Vormoor B, Schlosser YT, Blair H, et al. Sensitizing Ewing sarcoma to chemo- and radiotherapy by inhibition of the DNA-repair enzymes DNA protein kinase (DNA-PK) and poly-ADP-ribose polymerase (PARP) 1/2. *Oncotarget* 2017;8:113418-30.
182. Fok JHL, Ramos-Montoya A, Vazquez-Chantada M, et al. AZD7648 is a potent and selective DNA-PK inhibitor that enhances radiation, chemotherapy and olaparib activity. *Nat Commun* 2019;10:5065.
183. Zhang Q, Green MD, Lang X, et al. Inhibition of ATM increases interferon signaling and sensitizes pancreatic cancer to immune checkpoint blockade therapy. *Cancer Res* 2019;79:3940-51.
184. Chen BP, Uematsu N, Kobayashi J, et al. Ataxia telangiectasia mutated (ATM) is essential for DNA-PKcs phosphorylations at the Thr-2609 cluster upon DNA double strand break. *J Biol Chem* 2007;282:6582-7.
185. Cornell L, Munck JM, Alsinet C, et al. DNA-PK-A candidate driver of hepatocarcinogenesis and tissue biomarker that predicts response to treatment and survival. *Clin Cancer Res* 2015;21:925-33.
186. Abdel-Fatah TM, Arora A, Moseley P, et al. ATM, ATR and DNA-PKcs expressions correlate to adverse clinical outcomes in epithelial ovarian cancers. *BBA Clin* 2014;2:10-7.
187. Toulany M, Maier J, Iida M, et al. Akt1 and Akt3 but not Akt2 through interaction with DNA-PKcs stimulate proliferation and post-irradiation cell survival of K-RAS-mutated cancer cells. *Cell Death Discov* 2017;3:17072.
188. Baptistella AR, Landemberger MC, Dias MVS, et al. Rab5C enhances resistance to ionizing radiation in rectal cancer. *J Mol Med* 2019;97:855-69.
189. Zou M, Li Y, Xia S, et al. Knockdown of CAVEOLIN-1 sensitizes human basal-like triple-negative breast cancer cells to radiation. *Cell Physiol Biochem* 2017;44:778-91.
190. Saki M, Makino H, Javvadi P, et al. EGFR mutations compromise hypoxia-associated radiation resistance through impaired replication

fork-associated DNA damage repair. *Mol Cancer Res* 2017;15:1503-16.
191. Amunugama R, Fishel R. Homologous recombination in eukaryotes. *Prog Mol Biol Transl Sci* 2012;110:155-206.
192. Shrivastav M, Miller CA, De Haro LP, et al. DNA-PKcs and ATM co-regulate DNA double-strand break repair. *DNA Repair* 2009;8:920-9.
193. Nickoloff JA, Brenneman MA. Analysis of recombinational repair of DNA double-strand breaks in mammalian cells with I-SceI nuclease. *Methods Mol Biol* 2004;262:35-52.
194. Yoshino Y, Endo S, Chen Z, et al. Evaluation of site-specific homologous recombination activity of BRCA1 by direct quantitation of gene editing efficiency. *Sci Rep* 2019;9:1644.
195. Price BD, D'Andrea AD. Chromatin remodeling at DNA double-strand breaks. *Cell* 2013;152:1344-54.
196. Kaushal S, Freudenreich CH. The role of fork stalling and DNA structures in causing chromosome fragility. *Genes Chromosomes Cancer* 2019;58:270-83.
197. Wray J, Liu J, Nickoloff JA, Shen Z. Distinct RAD51 associations with RAD52 and BCCIP in response to DNA damage and replication stress. *Cancer Res* 2008;68:2699-707.
198. Groth P, Orta ML, Elvers I, et al. Homologous recombination repairs secondary replication induced DNA double-strand breaks after ionizing radiation. *Nucleic Acids Res* 2012;40:6585-94.
199. Murnane JP. Telomere dysfunction and chromosome instability. *Mutat Res* 2012;730:28-36.
200. Budke B, Logan HL, Kalin JH, et al. RI-1: a chemical inhibitor of RAD51 that disrupts homologous recombination in human cells. *Nucleic Acids Res* 2012;40:7347-57.
201. Budke B, Lv W, Kozikowski AP, Connell PP. Recent developments using small molecules to target RAD51: How to best modulate RAD51 for anticancer therapy? *ChemMedChem* 2016;11:2468-73.
202. Lv W, Budke B, Pawlowski M, Connell PP, Kozikowski AP. Development of small molecules that specifically inhibit the D-loop activity of RAD51. *J Med Chem* 2016;59:4511-25.
203. Mersch J, Jackson MA, Park M, et al. Cancers associated with BRCA1 and BRCA2 mutations other than breast and ovarian. *Cancer* 2015;121:269-75.
204. Sekhar D, Pooja S, Kumar S, Rajender S. RAD51 135G > C substitution increases breast cancer risk in an ethnic-specific manner: a meta-analysis on 21,236 cases and 19,407 controls. *Sci Rep* 2015;5:11588.
205. Evans MK, Longo DL. PALB2 mutations and breast-cancer risk. *N Engl J Med* 2014;371:566-8.
206. Jette NR, Kumar M, Radhamani S, et al. ATM-deficient cancers provide new opportunities for precision oncology. *Cancers* 2020;12:687.
207. Byrum AK, Vindigni A, Mosammaparast N. Defining and modulating 'BRCAness'. *Trends Cell Biol* 2019;29:740-51.
208. Pommier Y, O'Connor MJ, de Bono J. Laying a trap to kill cancer cells: PARP inhibitors and their mechanisms of action. *Sci Transl Med* 2016;8:362ps17.
209. Yi M, Dong B, Qin S, et al. Advances and perspectives of PARP inhibitors. *Exp Hematol Oncol* 2019;8:29.
210. del Rivero J, Kohn EC. PARP inhibitors: the cornerstone of DNA repair-targeted therapies. *Oncology* 2017;31:265-73.
211. Gil Del Alcazar CR, Todorova PK, Habib AA, Mukherjee B, Burma S. Augmented HR repair mediates acquired temozolomide resistance in glioblastoma. *Mol Cancer Res* 2016;14:928-40.
212. Zhang X, Ma N, Yao W, Li S, Ren Z. RAD51 is a potential marker for prognosis and regulates cell proliferation in pancreatic cancer. *Cancer Cell Int* 2019;19:356.
213. Liu X, Han EK, Anderson M, et al. Acquired resistance to combination treatment with temozolomide and ABT-888 is mediated by both base excision repair and homologous recombination DNA repair pathways. *Mol Cancer Res* 2009;7:1686-92.
214. Noordermeer SM, van Attikum H. PARP inhibitor resistance: a tug-of-war in BRCA-mutated cells. *Trends Cell Biol* 2019;29:820-34.
215. D'Andrea AD. Mechanisms of PARP inhibitor sensitivity and resistance. *DNA Repair* 2018;71:172-6.
216. Tian H, Gao Z, Li H, et al. DNA damage response--a double-edged sword in cancer prevention and cancer therapy. *Cancer Lett* 2015;358:8-16.
217. Bakr A, Oing C, Kocher S, et al. Involvement of ATM in homologous recombination after end resection and RAD51 nucleofilament formation. *Nucleic Acids Res* 2015;43:3154-66.
218. Ahlskog JK, Larsen BD, Achanta K, Sorensen CS. ATM/ATR-mediated phosphorylation of PALB2 promotes RAD51 function. *EMBO Rep* 2016;17:671-81.
219. Jackson SP, Helleday T. Drugging DNA repair. *Science* 2016;352:1178-9.
220. Glorieux M, Dok R, Nuyts S. Novel DNA targeted therapies for head and neck cancers: clinical potential and biomarkers. *Oncotarget* 2017;8:81662-78.
221. Riches LC, Trinidad AG, Hughes G, et al. Pharmacology of the ATM inhibitor AZD0156: potentiation of irradiation and olaparib responses preclinically. *Mol Cancer Ther* 2020;19:13-25.
222. Zhou C, Parsons JL. The radiobiology of HPV-positive and HPV-negative head and neck squamous cell carcinoma. *Expert Rev Mol Med* 2020;22:e3.
223. Ferri A, Stagni V, Barila D. Targeting the DNA damage response to overcome cancer drug resistance in glioblastoma. *Int J Mol Sci* 2020;21:4910.
224. Philip CA, Laskov I, Beauchamp MC, et al. Inhibition of PI3K-AKT-mTOR pathway sensitizes endometrial cancer cell lines to PARP inhibitors. *BMC Cancer* 2017;17:638.
225. Wang D, Li C, Zhang Y, et al. Combined inhibition of PI3K and PARP is effective in the treatment of ovarian cancer cells with wild-type PIK3CA genes. *Gynecol Oncol* 2016;142:548-56.
226. Cossar LH, Schache AG, Risk JM, et al. Modulating the DNA damage response to improve treatment response in cervical cancer. *Clin*

Oncol 2017;29:626-34.
227. Weitzman MD, Fradet-Turcotte A. Virus DNA replication and the host DNA damage response. *Annu Rev Virol* 2018;5:141-64.
228. Jafari A, Rezaei-Tavirani M, Farhadihosseinabadi B, Taranejoo S, Zali H. HSP90 and co-chaperones: impact on tumor progression and prospects for molecular-targeted cancer therapy. *Cancer Invest* 2020;38:310-28.
229. Garcia-Carbonero R, Carnero A, Paz-Ares L. Inhibition of HSP90 molecular chaperones: moving into the clinic. *Lancet Oncol* 2013;14:e358-69.
230. Jhaveri K, Modi S. HSP90 inhibitors for cancer therapy and overcoming drug resistance. *Adv Pharmacol* 2012;65:471-517.
231. Kamal A, Thao L, Sensintaffar J, et al. A high-affinity conformation of Hsp90 confers tumour selectivity on Hsp90 inhibitors. *Nature* 2003;425:407-10.
232. Moulick K, Ahn JH, Zong H, et al. Affinity-based proteomics reveal cancer-specific networks coordinated by Hsp90. *Nat Chem Biol* 2011;7:818-26.
233. Sessa C, Shapiro GI, Bhalla KN, et al. First-in-human phase I dose-escalation study of the HSP90 inhibitor AUY922 in patients with advanced solid tumors. *Clin Cancer Res* 2013;19:3671-80.
234. Renouf DJ, Velazquez-Martin JP, Simpson R, Siu LL, Bedard PL. Ocular toxicity of targeted therapies. *J Clin Oncol* 2012;30:3277-86.
235. Balmus G, Pilger D, Coates J, et al. ATM orchestrates the DNA-damage response to counter toxic non-homologous end-joining at broken replication forks. *Nat Commun* 2019;10:87.
236. Nickoloff JA. Improving cancer therapy by combining cell biological, physical, and molecular targeting strategies. *Chin J Cancer Res* 2013;25:7-9.
237. Kon T, Zhang X, Huang Q, et al. Oncolytic virus-mediated tumor radiosensitization in mice through DNA-PKcs-specific shRNA. *Transl Cancer Res* 2012;1:4-14.
238. Guo P, Yang J, Jia D, Moses MA, Auguste DT. ICAM-1-targeted, Lcn2 siRNA-encapsulating liposomes are potent anti-angiogenic agents for triple negative breast cancer. *Theranostics* 2016;6:1-13.
239. Guo P, Yang J, Huang J, Auguste DT, Moses MA. Therapeutic genome editing of triple-negative breast tumors using a noncationic and deformable nanolipogel. *Proc Natl Acad Sci USA* 2019;116:18295-303.