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Chapter

NQO1-Bioactivatable Therapeutics as Radiosensitizers for Cancer Treatment

Naveen Singh, Edward A. Motea, Xiumei Huang, Colton L. Starcher, Jayne Silver, I-Ju Yeh, S. Louise Pay, Xiaolin Su, Kristen A. Russ, David A. Boothman and Erik A. Bey

Abstract

Developing cancer therapeutics that radiosensitize in a tumor-selective manner remains an ideal. We developed a novel means of radiosensitization, exploiting NAD(P)H:Quinone Oxidoreductase 1 (NQO1) overexpression, and lowered catalase expression in solid human tumors using NQO1-bioactivatable drugs. Non-small cell lung (NSCLC), pancreatic (PDAC), prostate, and breast cancers overexpress NQO1. Ionizing radiation (IR) creates a spectrum of DNA lesions, including lethal DNA double-strand breaks (DSBs), and mutagenic but rarely lethal altered DNA bases and DNA single-strand breaks (SSBs). NQO1-bioactivatable drugs (e.g., β-lapachone and deoxynyboquiones) also promote abasic DNA lesions and SSBs. These hyperactivate poly (ADP-ribose) polymerase 1 (PARP1) and dramatically increase calcium release from the endoplasm reticulum (ER). Exposure of human cancer cells overexpressing NQO1 to NQO1-bioactivatable drugs immediately following IR, therefore, hyperactivates PARP1 synergistically, which in turn depletes NAD+ and ATP, inhibiting DSB repair. Ultimately, this leads to cell death. Combining IR with NQO1-bioactivatable drugs allows for a reduction in drug dose. Similarly, a lower IR dose can be used in combination with the drug, reducing the effects of IR on normal tissue. The combination treatment is effective in preclinical animal models with NSCLC, prostate, and head and neck xenografts, indicating that clinical trials are warranted.

Keywords: NQO1 expression, PARP hyperactivation, abasic site synergy, NAD+/ATP losses, DSB repair inhibition, programmed necrosis

1. Introduction

For decades, radiobiologists and physician-scientists have collaborated to develop effective combination therapies with ionizing radiation and radiosensitizing agents to reduce the overall dose of radiation required in cancer therapy. This minimizes adverse side-effects observed in normal tissues and increases the efficacy of radiation in reducing tumor burden. Here, we discuss the pros and cons of radiosensitizing

agents used in the clinic in comparison with NAD(P)H quinone oxidoreductase-1 (NQO1)-bioactivatable drugs. β-Lapachone (β-Lap) is a clinical chemotherapeutic agent discovered to be a potent DNA repair inhibitor in the late 1980s. It has since been shown to be bioactivated by NQO1, an enzyme elevated more than 20-fold in most solid human cancers, e.g., non-small cell lung, pancreas, prostate, head and neck, and breast cancers, and shows promise as a potent radiosensitizer.

2. Radiotherapy as a single agent

2.1 Initial use of ionizing radiation

The late 19th-century discovery of the X-ray by Wilhelm Roentgen led to diagnostic tools and therapies for diseases such as blood disorders and benign and malignant growths [1, 2]. Initially, radiation was delivered using unfocused beams, causing skin and blood malignancies in both patients and radiologists [1, 2]. Today, patients benefit from vast technological improvements, allowing for focused radiation beams, which markedly increased patient survival. Current approaches include conformal radiation therapy, proton beam radiation therapy, stereotactic radiation therapy (using linear accelerators or gamma knife devices), and intraoperative therapy [3]. Despite improvements in targeting tumors and reducing normal tissue damage, high doses of radiation are still required for a curative effect. Some tumors can also be resistant to radiotherapy, including hypoxic tumors and dormant cancer cells that regrow when the optimal tumor microenvironment presents itself. Thus, methods to improve the safety and efficacy of ionizing radiation were initiated, including combination with chemotherapeutics or radiosensitizers.

2.2 Enhancing radiation therapy with radiosensitizers

Radiosensitizing agents are molecules that enhance the dose of ionizing radiation delivered to a patient's tumor. The optimal clinical radiosensitizer (a) lowers the required dose of ionizing radiation, (b) increases its antitumor effect, and (c)

Radiosensitizer	Tumor type	Mechanism
Hyperbaric oxygen	Brain tumors	Oxygenation
Nicotinamide	Glioblastoma	Oxygenation
Metronidazole	Cervical cancer	Oxygenation
Mitomycin-C	Breast cancer	Kills hypoxic cells
5-fluorouracil (5FU)	Gastrointestinal	S-phase check points
Bromodeoxyuridine (BrDU)	Breast	Repair inhibition
Topo-inhibitors	Breast, cervical	DNA damage
NBTXR3	Solid tumors	Direct
Nimoral	Head and neck	Modifies hypoxia
Trans sodium crocetinate	Glioblastoma	Oxygenation
NVX108	Glioblastoma	Oxygenation

*List of commonly used radiosensitizing methods/agents for combination with radiotherapy in various tumor types. The last four are **emboldened** to denote their current use in ongoing clinical trials.*

Table 1.
Clinical radiosensitizers.

synergistically kills cancer cells. To date, no radiosensitizer has met these demands. Many radiosensitizers have been used clinically (**Table 1**, normal text) with limited success, or are currently in clinical trial (**Table 1**, bold text). These include suppressors of radioprotectors (e.g., thiol) [4], molecules releasing cytotoxic substances when radiolyzed [5], thymine/cytidine analogs [6], oxygen mimic sensitizers [7], and DNA repair inhibitors [8].

3. β-Lapachone, a DNA repair inhibitor

3.1 Initial discovery of β-lapachone's effect on DNA repair

In the late 1980s, our laboratory began searching for DNA repair modulators that synergize with ionizing radiation to kill cancer cells more effectively. The goal was to thwart cancer cells' ability to repair IR damage, to avoid the survival of IR-resistant malignant cells that have undergone potentially lethal damage repair (PLDR). One of those compounds was (3,4-dihydro-2,2-dimethyl-2H-naphthol[1,2-b]pyran-5,6-dione), also known as β-lapachone [9].

We found that just four micromolar β-lapachone inhibited single-strand DNA break repair in cancer cells exposed to DNA-damaging agent methyl methane sulfonate [9, 10], killing 99% of cells at an exposure time 90–120 min [11]. Additionally, we found that combining β-lapachone with ionizing radiation in Hep2 cells increased double-strand breaks and dramatically lowered the dose of radiation required for cell death, highlighting β-lapachone as a potent radiosensitizer [12].

In the 1990s and early 2000s, we conducted subtraction-hybridization screening to isolate X-ray inducible genes to investigate ionizing radiation resistance and found Xip3, also known as NQO1 [13]. Dicoumarol, an NQO1 inhibitor, specifically blocked β-lapachone's toxicity, indicating that the radiosensitizer may be bioactivated by this enzyme. As NQO1 is specifically expressed in tumor cells, this indicated a promising use of β-lapachone as a cancer therapeutic with or without ionizing radiation.

4. Mechanism of action for NQO1-bioactivatable therapies

4.1 NQO1 vs. catalase ratio and specificity

NQO1 is a Phase II detoxification enzyme that reduces ROS levels in cancer cells. NQO1 converts quinones into stable intermediate hydroquinones that are exported out of the cell by conjugation [10]. Most solid cancers, including non-small cell lung and pancreatic cancers (>85%), prostate, colon, and breast cancers (60%) and head and neck cancers (40%) overexpress NQO1 5- to 200- fold above normal tissue. Corresponding levels of catalase in these cancers were strikingly reduced, impacting the ability of cancer cells to eliminate ROS [14]. Overexpression of NQO1 appears to stabilize HIF-1alpha and promotes metastasis [15].

Though NQO1 detoxifies most quinones through two-electron oxidoreduction, a few quinones undergo a rapid futile redox cycle response, generating an unstable intermediate hydroquinone that spontaneously reverts back to its original form using two oxygenation steps and creating two superoxides. Deoxynyboquiones (DNQ), KP372 agents, and β-lapachone are three classes of NQO1-bioactivatable drugs currently known [16]. Recently, Napabucasin, an orphan drug in clinical trials for pancreatic and cervical cancer, has also been reported to be bioactivated by NQO1 [17]. Though mitomycin C and streptonigrin are metabolized by NQO1,

these agents can also be activated by other drug metabolizing enzymes [18]. Human cancer cells overexpressing NQO1 have been shown to be sensitive to NQO1-bioactivatable drugs alone and in combination with PARP inhibitors, cisplatin, radiation, and NAMPT inhibitors both in cell culture and xenograft models [14, 19].

4.2 NQO1-dependent ROS formation and PARP hyperactivation

Cancer cells overexpressing NQO1 and exposed to NQO1-bioactivatable drugs, such as β-lapachone, DNQ or IB-DNQ, acquire extensive DNA lesions as evidenced by alkaline comet assays [11]. The unstable hydroquinone form of these NQO1 bioactivatable drugs reacts with two oxygen molecules spontaneously to regenerate the original compound [20]. This futile redox cycle consumes ~60 moles of NADPH to generate ~120 moles of ROS in ~2 min for β-lapachone, leading to the generation of permeable hydrogen peroxide (H_2O_2). This diffuses into the nucleus and causes massive oxidative stress and SSBs [16]. Initial DNA damage is mainly through the formation of altered bases, SSBs, and apurinic/apyrimidinic (AP) sites generated through incorporation of 8-oxo-deoxyguanine [21]. Ultimately, damage caused by H_2O_2 results in extensive SSBs and DSBs. These lesions lead to PARP hyperactivation that can be prevented by BAPTA-AM (chelates Ca^{2+}), PARP inhibitors, or the NQO1 inhibitor dicoumarol, in NQO1+ cells. In contrast, cells deficient in NQO1 due to NQO1 polymorphisms, *2[C609T] or *3[C465T], are unaffected by exposure to NQO1-bioactivatable compounds [14], lacking the enzyme activity for redox cycling Hyperactivation of PARP rapidly degrades the increased NAD+ pools generated as a result of the oxidation of NADH in the futile cycle [11, 20, 22]. NAD+ loss is not seen in cells treated with PARP1 inhibitors; instead, cells exposed to PARP inhibitors in combination with NQO1-bioactivatable drugs undergo a synergistic apoptotic cell death response [14].

4.3 Calcium release, DNA damage and μ-Calpain-dependent programmed necrosis

One of the key components in the cell death response by NQO1-bioactivatable drugs is the release of calcium from the core endoplasmic reticulum (ER) stores, which is otherwise inert [11, 23]. This results in specific programmed necrosis referred to as NAD+ -Keresis. Pre-treatment, with the calcium chelator, BAPTA-AM, suppresses PARP hyperactivation and results in specific inhibition of NQO1-dependent cell death by NQO1-bioactivatable drugs. Extensive DNA damage along with Ca^{2+} release from the ER results in the hyperactivation of PARP1 in NQO1+ cancer cells. PARP1 hyperactivation rapidly degrades the NAD+ and causes concomitant ATP losses within 30–40 min of drug treatment. μ-Calpain activation is observed upon treatment with NQO1-bioactivatable drugs within 8–24 h [16, 24]. The multitude of damage caused by treatment with these drugs overwhelms DNA repair machinery and depletes the cells of the energy resources, culminating in cell death [10, 11, 16, 20, 24–27].

4.4 NQO1-bioactivatable drugs lead to perturbations in metabolic pathways

Treatment with NQO1-bioactivatable drugs causes wide-scale metabolic changes in the cell, which can be attributed to cell death overwhelming the cellular machinery. Altering key enzymes in NAD metabolism results in synergy with NQO1-bioactivatable drugs. NAMPT is an important source of reducing equivalents for redox balance in cancer cells. Pretreatment with FK866, a NAMPT inhibitor, leads to accelerated cell death due to decrease in NAD+/NADH levels and reduced doses

of NQO1-bioactivatable drugs [28]. NAMPT knockdown has also been shown to sensitize cancer cells to ROS induction through ionizing radiation [29, 30].

4.5 Exploiting NQO1-bioactivatable drugs as radiosensitizers

Cancer cells, tissues, and organs subjected to ionizing radiation experience a wide spectrum of DNA lesions including SSBs, DSBs, AP sites and DNA-protein cross-links. One unrepaired DSB is lethal to the cell [21, 31]. Hence, NQO1-bioactivatable drugs, when combined with IR (**Figure 1**), synergistically kill cancer cells due to the combined effect of DNA damage and PARP1 hyperactivation [21, 32]. Sublethal doses of NQO1 drugs and IR combine to release massive amounts of ROS due to

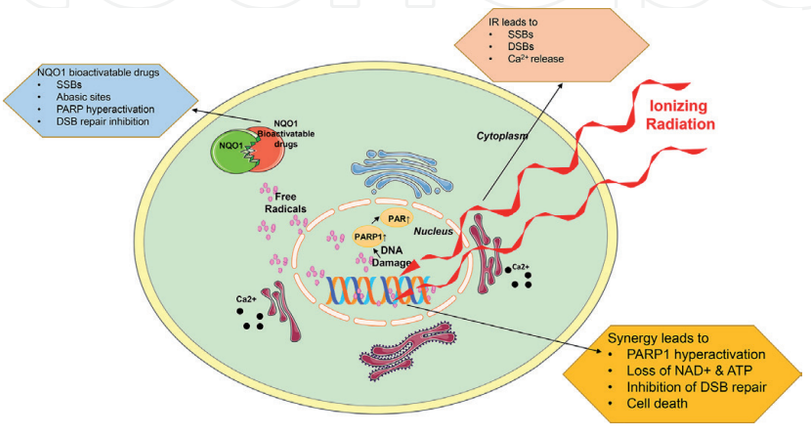

Figure 1.
Radiation sensitization by NQO1 bioactivatable drugs: sublethal doses of β-lapachone when bioactivated by NQO1 release massive amounts of ROS, resulting in synergy with IR and increased programmed necrosis. NQO1 bioactivatable drugs in combination with IR show tremendous synergy even at low doses. The combined effect of DNA damage and PARP hyperactivation provides more lethality to a cancer cell whereas NQO1 provides the specificity. This leads to increased ROS, gH2AX formation, hyperactivation of PARP, massive NAD and ATP losses, prevention of DSB repair, perturbations in the metabolic pathways, and μ-Calpain-mediated programmed necrosis known as NAD + -Keresis.

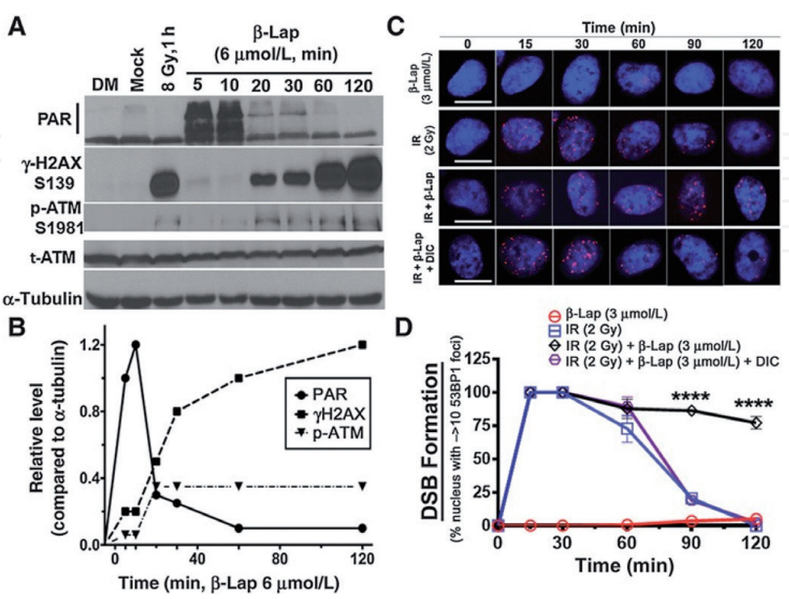

Figure 2.
*Sublethal doses of IR and β-lap in NQO1+ LNCaP cells cause PARP-1 hyper-activation and dramatic ATP loss: A, LNCaP cells expressing or lacking NQO1 were treated with IR + β-lap and monitored for PAR formation—UT, untreated control for IR; V, vehicle; DMSO only. B, Synergistic ATP loss was noted after IR + β-lap compared to single treatments alone. Results are means ± SE for experiments performed three times in duplicate. Student's t-tests compared single to combined treatments. ***$p < 0.001$, **$p < 0.01$.*

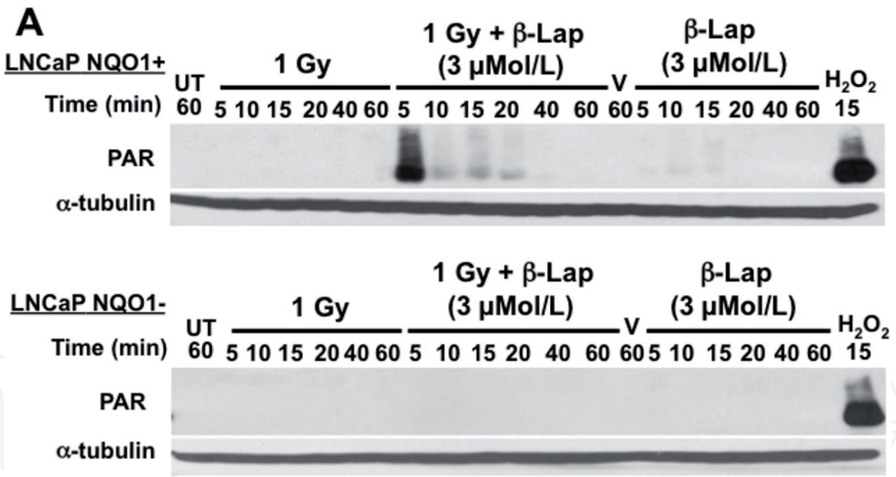

Figure 3.
β-Lap inhibits DNA double strand break repair: A. log-phase A549 NSCLC cells were treated with or without β-lap (6 μM) and cell extracts prepared at various times during treatment to detect PAR-PARP formation, γ-H2AX (pS139), pS1981 ATM, total ATM (t-ATM) and α-tubulin steady-state levels by Western blot. A549 cells were also exposed or not to IR (8 Gy) and analyzed 1 h later. Mock, non-irradiated cells. DM, media alone. B. Graphical representation of data shown in **Figure 2A**. *C. Representative images of A549 cells exposed or not to IR (2 Gy) alone, β-lap (3 μM, 2 h) alone, the combination [IR (2 Gy) + β-lap (3 μM, 2 h)], or the combination with DIC (50 μM, NQO1 inhibitor) and assessed for DSB breaks over time (0–120 min) using 53BP1 as the surrogate marker (in red). Cells were also stained for nuclear DNA using DAPI (in blue). Scale bar = 10 μm. D. Graphical representation of data presented in* **Figure 2C**; *****p < 0.0001.*

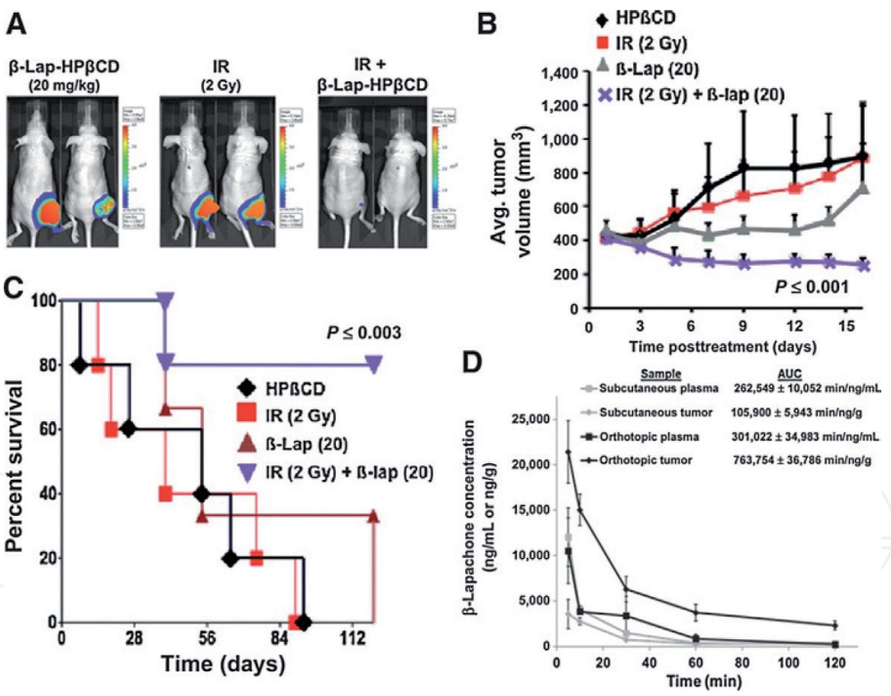

Figure 4.
β-Lap radiosensitizes subcutaneous A549-luc xenografts in athymic nude mice: A. subcutaneous A549-luc xenografts (400 mm³) were generated in athymic nude mice and then treated with or without IR (2 Gy) then immediately with or without β-lap (20 mg/kg) for 5 treatments every other day. Representative antitumor responses (at day 20 post-treatment) are demonstrated for β-lap alone, IR alone, and the IR + β-lap combination. B. Antitumor responses (tumor volumes, mm³) over time are shown for the treatments described in **Figure 3A**. *C. Overall survival of animals treated as described in* **Figure 3A**. *D. PK values for plasma and subcutaneous vs. orthotopic A549-luc tumors in athymic nude mice. Note the significantly high levels of β-lap in orthotopic vs. subcutaneous A549 tumor tissue, whereas plasma levels were identical in both sets of mice.*

their synergy, resulting in PARP hyperactivation, loss of nucleotides and increased programmed necrosis (**Figures 2** and **3**), beyond the capabilities of the single agents (IR or NQO1-bioactivatable drug) alone. Head and neck cancers, PDA and NSCLC

have been shown to be sensitive to nontoxic doses of β-lapachone when combined with IR [21, 32]. Using NQO1-bioactivatable drugs as radiosensitizers leads to increases in ROS, γH2AX formation, hyperactivation of PARP1, massive NAD+ and ATP losses, inhibition of DSB repair, perturbation in carbon flux pathways and μ-Calpain mediated programmed necrosis known as NAD + -Keresis. The cell death responses observed are independent of any oncogenic drivers [21, 31–33]. This lethal combination between radiation therapy and NQO1-bioactivatable drugs prolongs long-term survival and promotes enhanced tumor shrinkage at non-toxic doses of each agent (IR and Drug, **Figure 4**). Thus, combining NQO1-bioactivatable drugs with radiation therapy, should be a long-standing treatment modality for tumors overexpressing NQO1.

5. Discussion

5.1 Advantages of NQO1-bioactivatable drugs vs. other radiosensitizers

The major advantage of using NQO1-bioactivatable drugs as radiosensitizers is the tumor selectivity afforded by the drugs themselves. Synergy is afforded by a number of tumor-selective responses to the drugs. First, the dependence of the drugs on NQO1 levels is perfect for the specific treatment of various difficult-to-treat human cancers, including non-small cell lung, pancreatic, breast, prostate, and head and neck cancers. Tumor selectivity requires approximately 100 units of enzyme activity, whereas lower levels of NQO1 results in mild metabolomic alterations used for the treatment of metabolic syndromes [34]. Second, the minimum time of exposure of 30–120 min fits the pharmacokinetics of the drug. It should be noted that all studies thus far indicate that the drugs have to be available immediately after or at the same time as exposure with IR. Pre-treatment prior to IR is ineffective. Third, synergy between NQO1-bioactivatable drugs and IR occurs due to PARP1 hyperactivation causing massive NAD+ and ATP loss, preventing repair of the DNA damage created by IR. NQO1-bioactivatable drugs are highly specific to tumors, causing little normal tissue toxicity, which is unaffected by IR treatment [14, 16, 20, 25, 31]. Preclinical in vivo data suggest that radiosensitization trials with NQO1-bioactivatable drugs are warranted for non-small cell lung, pancreatic, breast prostate, and head and neck cancers.

5.2 Future directions for NQO1-bioactivatable drugs

A clinical trial of radiation sensitization effects of the new drug, isobutyldeoxynyboquione (IB-DNQ), against non-small cell lung (NSCLC) and/or pancreatic adenocarcinomas (PDAC) is warranted. These cancers are almost uniformly NOQ1 over-expressive and they have routinely low levels of catalase [14]. We have developed CLIA assessments of NQO1 status and enzymatic levels for these studies. The pharmacokinetics of IB-DNQ in these cancers, particularly in NSCLC and PDAC cancers, is relatively short at about 6 h, but long enough for sensitization of tumors to the NQO1-bioactivatable drug + IR. Biomarker and DSB repair kinetics are ongoing in our laboratory in preparation for these radiosensitization studies.

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References

[1] Brady LW. The changing role of radiation oncology in cancer management. Cancer. 1983;**51**:2506-2514

[2] Lederman M. The early history of radiotherapy: 1895-1939. International Journal of Radiation Oncology, Biology, Physics. 1981;**7**:639-648

[3] Ahmad SS, Duke S, Jena R, Williams MV, Burnet NG. Advances in radiotherapy. BMJ. 2012;**345**:e7765

[4] Durand RE. Roles of thiols in cellular radiosensitivity. International Journal of Radiation Oncology, Biology, Physics. 1984;**10**:1235-1238

[5] Wardman P. Chemical radiosensitizers for use in radiotherapy. Clinical Oncology (Royal College of Radiologists). 2007;**19**:397-417

[6] Lee MW, Parker WB, Xu B. New insights into the synergism of nucleoside analogs with radiotherapy. Radiation Oncology. 2013;**8**:223

[7] Revesz L, Palcic B. Radiation dose dependence of the sensitization by oxygen and oxygen mimic sensitizers. Acta Radiologica. Oncology. 1985;**24**:209-217

[8] Raleigh DR, Haas-Kogan DA. Molecular targets and mechanisms of radiosensitization using DNA damage response pathways. Future Oncology. 2013;**9**:219-233

[9] 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-naphtho[1,2-b] pyran-5,6-dione), a novel DNA repair inhibitor. Cancer Research. 1987;**47**:5361-5366

[10] Pink JJ, Planchon SM, Tagliarino C, Varnes ME, Siegel D, Boothman DA. NAD(P)H:Quinone oxidoreductase activity is the principal determinant of beta-lapachone cytotoxicity. The Journal of Biological Chemistry. 2000;**275**:5416-5424

[11] Bentle MS, Reinicke KE, Bey EA, Spitz DR, Boothman DA. Calcium-dependent modulation of poly(ADP-ribose) polymerase-1 alters cellular metabolism and DNA repair. The Journal of Biological Chemistry. 2006;**281**:33684-33696

[12] Boothman DA, Pardee AB. Inhibition of radiation-induced neoplastic transformation by beta-lapachone. Proceedings of the National Academy of Sciences of the United States of America. 1989;**86**:4963-4967

[13] Boothman DA, Meyers M, Fukunaga N, Lee SW. Isolation of x-ray-inducible transcripts from radioresistant human melanoma cells. Proceedings of the National Academy of Sciences of the United States of America. 1993;**90**:7200-7204

[14] Huang X, Motea EA, Moore ZR, et al. Leveraging an NQO1 bioactivatable drug for tumor-selective use of poly(ADP-ribose) polymerase inhibitors. Cancer Cell. 2016;**30**:940-952

[15] Oh ET, Kim JW, Kim JM, et al. NQO1 inhibits proteasome-mediated degradation of HIF-1alpha. Nature Communications. 2016;**7**:13593

[16] Huang X, Dong Y, Bey EA, et al. An NQO1 substrate with potent antitumor activity that selectively kills by PARP1-induced programmed necrosis. Cancer Research. 2012;**72**:3038-3047

[17] Froeling FEM, Mosur Swamynathan M, Deschenes A, et al. Bioactivation of napabucasin triggers reactive oxygen species-mediated cancer

cell death. Clinical Cancer Research. 2019

[18] Siegel D, Yan C, Ross D. NAD(P)H:Quinone oxidoreductase 1 (NQO1) in the sensitivity and resistance to antitumor quinones. Biochemical Pharmacology. 2012;**83**:1033-1040

[19] Terai K, Dong GZ, Oh ET, et al. Cisplatin enhances the anticancer effect of beta-lapachone by upregulating NQO1. Anti-Cancer Drugs. 2009;**20**:901-909

[20] Bey EA, Bentle MS, Reinicke KE, et al. An NQO1- and PARP-1-mediated cell death pathway induced in non-small-cell lung cancer cells by beta-lapachone. Proceedings of the National Academy of Sciences of the United States of America. 2007;**104**:11832-11837

[21] Li LS, Reddy S, Lin ZH, et al. NQO1-mediated tumor-selective lethality and radiosensitization for head and neck Cancer. Molecular Cancer Therapeutics. 2016;**15**:1757-1767

[22] Bentle MS, Reinicke KE, Dong Y, Bey EA, Boothman DA. Nonhomologous end joining is essential for cellular resistance to the novel antitumor agent, beta-lapachone. Cancer Research. 2007;**67**:6936-6945

[23] Tagliarino C, Pink JJ, Dubyak GR, Nieminen AL, Boothman DA. Calcium is a key signaling molecule in beta-lapachone-mediated cell death. The Journal of Biological Chemistry. 2001;**276**:19150-19159

[24] Tagliarino C, Pink JJ, Reinicke KE, Simmers SM, Wuerzberger-Davis SM, Boothman DA. Mu-calpain activation in beta-lapachone-mediated apoptosis. Cancer Biology & Therapy. 2003;**2**:141-152

[25] Bey EA, Reinicke KE, Srougi MC, et al. Catalase abrogates beta-lapachone-induced PARP1 hyperactivation-directed programmed necrosis in NQO1-positive breast cancers. Molecular Cancer Therapeutics. 2013;**12**:2110-2120

[26] Chakrabarti G, Silvers MA, Ilcheva M, et al. Tumor-selective use of DNA base excision repair inhibition in pancreatic cancer using the NQO1 bioactivatable drug, beta-lapachone. Scientific Reports. 2015;**5**:17066

[27] Silvers MA, Deja S, Singh N, et al. The NQO1 bioactivatable drug, beta-lapachone, alters the redox state of NQO1+ pancreatic cancer cells, causing perturbation in central carbon metabolism. The Journal of Biological Chemistry. 2017;**292**:18203-18216

[28] Moore Z, Chakrabarti G, Luo X, et al. NAMPT inhibition sensitizes pancreatic adenocarcinoma cells to tumor-selective, PAR-independent metabolic catastrophe and cell death induced by beta-lapachone. Cell Death & Disease. 2015;**6**:e1599

[29] Chakrabarti G, Gerber DE, Boothman DA. Expanding antitumor therapeutic windows by targeting cancer-specific nicotinamide adenine dinucleotide phosphate-biogenesis pathways. Clinical pharmacology: Advances and Applications. 2015;**7**:57-68

[30] Chakrabarti G, Moore ZR, Luo X, et al. Targeting glutamine metabolism sensitizes pancreatic cancer to PARP-driven metabolic catastrophe induced by ss-lapachone. Cancer & Metabolism. 2015;**3**:12

[31] Dong Y, Bey EA, Li LS, et al. Prostate cancer radiosensitization through poly(ADP-ribose) polymerase-1 hyperactivation. Cancer Research. 2010;**70**:8088-8096

[32] Motea EA, Huang X, Singh N, et al. NQO1-dependent, tumor-selective radiosensitization of non-small cell

lung cancers. Clinical Cancer Research. 2019;**25**:2601-2609

[33] Planchon SM, Pink JJ, Tagliarino C, Bornmann WG, Varnes ME, Boothman DA. Beta-lapachone-induced apoptosis in human prostate cancer cells: Involvement of NQO1/xip3. Experimental Cell Research. 2001;**267**:95-106

[34] Li LS, Bey EA, Dong Y, et al. Modulating endogenous NQO1 levels identifies key regulatory mechanisms of action of beta-lapachone for pancreatic cancer therapy. Clinical Cancer Research. 2011;**17**:275-285