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Manipulation of Cellular Redox Parameters for Improving Therapeutic Responses in B-Cell Lymphoma and Multiple Myeloma

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ABSTRACT

Developing novel combined-modality therapeutic approaches based on understanding of the involvement of redox biology in apoptosis of malignant cells is a promising approach for improving clinical responses in B-cell lymphoma and multiple myeloma. Therapeutic modalities that generate reactive oxygen species (i.e., radiation, photodynamic therapy, and specific chemotherapeutic drugs) have been shown to be selectively cytotoxic to malignant B-cells. In this review, we will discuss agents that induce apoptosis in B-cell tumors by oxidative stress. Subsequently, a novel biochemical rationale (based on fundamental differences in cancer vs. normal cell oxidative metabolism) for combining oxidative stressors with radiotherapy and chemotherapy, that may lead to designing of more effective treatment strategies for B-cell malignancies, will be discussed. Besides providing potential curative benefit, such novel therapies could also selectively target and inhibit the emergence of drug-resistance in tumor cells, which is a major determinant of treatment failure in many B-cell malignancies. J. Cell. Biochem. 113: 419–425, 2012.

KEY WORDS: OXIDATIVE STRESS; RADIOTHERAPY; CHEMOTHERAPY DRUGS; B-CELL LYMPHOMA; MULTIPLE MYELOMA

B-cell development is unique where each step along the process is influenced by the concerted action of external factors such as interaction with antigen, the microenvironment and a network of cytokines, and internal factors such as transcription factors. Fluctuations in reactive oxygen species (ROS) can potentially influence B-cell differentiation, cell cycle progression, growth arrest, and cell death. Perturbations such as aberrant chromosomal translocations can result in cells that respond abnormally to their environment, with the result being the development of B-cell lymphoma and multiple myeloma (MM) [LeBien and Tedder, 2008]. Normal B-cells undergo a rigorous selection process before they fully differentiate into plasma cells that secrete immunoglobulin. Thus, the machinery necessary to induce programmed cell death appears to be particularly important in B-cells, which may explain why B-cells tend to more sensitive to some types of treatment than other malignancies. Despite this sensitivity to therapy, indolent B-cell non-Hodgkin's lymphoma (B-cell NHL) [i.e, small lymphocytic lymphoma, follicular lymphoma (FL), mantle cell, and marginal zone lymphoma] is rarely cured. Aggressive chemotherapy potentially cures 40–50% of intermediate and high-grade lymphoma (such as B-cell lymphoblastic lymphoma/leukemia and Burkitt's lymphoma/leukemia). We are making rapid progress with new approaches to treatment of MM, but historically this disease is considered uniformly fatal with current median survival being approximately 4 years [Jemal et al., 2011].

Radiotherapy is an effective treatment modality for B-cell malignancies and is used in single and combined modality treatments [Goel et al., 2006b; Witzig, 2006] Other modalities like chemotherapy drugs (i.e., thalidomide and lenalidomide, bortezomib), monoclonal antibodies targeting tumor specific antigens, transcription-pathway modulators (targeting kinases), and dendritic cell-based vaccines are being used or evaluated in the

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treatment of B-cell lymphoma and MM [Anderson et al., 2001; Hannah, 2005; Van de Velde et al., 2008]. Cytotoxic drugs hypothesized to act via ROS-induced oxidative stress have been added to the armamentarium of conventional cytotoxic agents with some limited success in therapy of drug-resistant B-cell malignancies [Dalton, 2002]. This review will discuss few cytotoxic agents that have shown activity in B-cell lymphoma and MM by perturbing the cellular redox (reduction/oxidation) environment. Subsequently, a novel biochemical rationale (based on fundamental differences in cancer vs. normal cell oxidative metabolism) for combining oxidative stressors with radiotherapy and chemotherapy, that may lead to designing of more effective treatment strategies for B-cell malignancies, will be discussed.

CELLULAR REDOX HOMEOSTASIS

Metabolic redox reactions are fundamental to producing energy and the biosynthetic capacity that is necessary for the normal function of all living systems. These reactions lead to production of ROS such as superoxide anion ($O_2^{\bullet-}$), hydrogen peroxide (H_2O_2), organic hydroperoxides (ROOH), hydroxyl radical ($OH^{\bullet}$), peroxyl radical ($ROO^{\bullet}$), and alkoxyl radical ($RO^{\bullet}$) as well as reactive nitrogen species (RNS) such as nitric oxide ($NO^{\bullet}$) and peroxynitrite ($ONOO^-$). ROS can be produced metabolically as by-products of mitochondrial-catalyzed electron transport reactions during respiration, products of metal-catalyzed reactions, by neutrophils and macrophages during inflammation, and by enzymatic reactions [Trachootham et al., 2008]. Steady-state levels of ROS production are balanced by cellular antioxidant systems that include detoxifying enzymes such as superoxide dismutases (SODs), catalase (CAT), glutathione peroxidases (GPxs) and small molecule antioxidants such as glutathione (GSH), peroxiredoxins, vitamin C and E, and thioredoxin [Finkel and Holbrook, 2000; Trachootham et al., 2008]. A dysregulation in the intracellular redox homeostasis can occur by enhanced production and/or attenuated degradation of ROS resulting in increased steady-state levels of prooxidants referred to as a condition of "oxidative stress" which if it persists, can lead to pathological accumulation of oxidative damage to critical biomolecules [Finkel and Holbrook, 2000; Spitz et al., 2004].

Under physiological conditions a cellular redox homeostasis is robustly maintained in a primarily reducing state whereby modest fluctuations in ROS can function as signaling molecules regulating cellular processes such as differentiation, cell cycle progression, growth arrest, apoptosis, and alteration of the immune response [Spitz et al., 2004; Trachootham et al., 2008]. When ROS serve as effectors of intracellular signaling pathways they exert their effects through direct effects on kinases and phosphatases as well as regulating redox sensitive transcription factors such as nuclear factor-κB (NF-κB) family, activator protein 1 (AP-1), hypoxia-inducing factor-1α (HIF-1α), p53, nuclear factor-erythroid 2-related factor 2 (Nrf2), controlling gene expression [Gius and Spitz, 2006]. However, persistent high levels of oxidative stress can lead to adverse events such as oncogene activation, genomic instability, de-differentiation, and mitogenesis, culminating in carcinogenesis as well as cancer progression to metastatic disease [Gius and Spitz, 2006]. The anti-cancer activity of a variety of therapies is partially based on the concept of selectively inducing an "oxidative catastrophe" in cancer cells to eliminate the malignant cells by apoptosis or mitotic-linked cell death [Renschler, 2004]. Studies have shown that compared to normal cells, cancer cells can be rendered susceptible to ROS-mediated cytotoxicity as they are intrinsically under oxidative stress due to increased steady-state levels of $O_2^{\bullet-}$ and H_2O_2 from mitochondrial metabolism [Szatrowski and Nathan, 1991; Aykin-Burns et al., 2009], they demonstrate altered expression of antioxidant enzymes (such as SODs, CAT, GPxs) [Oberley and Buettner, 1979], and/or they respond aberrantly to low molecular weight antioxidants [Schafer and Buettner, 2001]. Utilizing this concept for attaining selective cancer cell apoptosis, oxidative stressors are being combined with agents that deplete intracellular antioxidants, inhibit antioxidant enzyme activity, and/or disrupt mitochondrial membrane potential to release cytochrome c. [Spitz et al., 2000; Renschler, 2004].

B-cells are intrinsically associated with genomic instability and their malignant transformation to B-cell lymphoma and MM may be a result of several inappropriate tumor-associated mechanisms, including the formation of ROS. Chronic lymphocytic leukemia (CLL) patients show increased leukocytic H_2O_2, serum malondialdehyde concentration, lower GSH levels, with reduced activity of CAT, SODs, and glucose-6-phosphate dehydrogenase [Al-Gayyar et al., 2007]. In diffuse large B-cell lymphoma (DLBCL), the most common type of aggressive lymphoma, a low-level expression of CAT, GPx1, SOD2, and thioredoxin-binding protein-2 has been associated with poor prognosis and drug resistance [Andreadis et al., 2007]. Polymorphisms in genes affecting NADPH oxidase (NOX) system [Tome et al., 2005] and SOD2 [Wang et al., 2006] were reported to correlate with increased risk of DLBCL. Similarly, genetic polymorphisms (and related oxidative stress) in SOD2, GPx1, and CAT have been suggested to play a role in etiology of marginal zone lymphoma, B-cell NHL, and FL [Lightfoot et al., 2006]. Also, pediatric patients with acute lymphoblastic leukemia (ALL) show increased oxidative stress [Mazor et al., 2008] indicated by high plasma thiobarbituric acid reactive substances and serum protein carbonylation, with decreased antioxidant activity (as measured by whole blood CAT and SOD activities, plasma and erythrocyte thiol levels, and serum vitamin E concentration) [Battisti et al., 2008]. MM patients show higher levels of lipid peroxidation with reduced activities of SODs and GPxs suggesting a role of free radicals in progression of myeloma [Zima et al., 1996]. In a clinical study, myeloma patients treated on vincristine–adriamycin–dexamethasone (dex) therapy show an inhibition in antioxidant enzymes including SODs, GPxs, and CAT [Kuku et al., 2005]. Myeloma patients show higher serum levels of interleukin-1β (IL-1β), soluble interleukin-2 receptor, interleukin-6 (IL-6), interleukin-8, tumor necrosis factor-α (TNF-α), and C-reactive protein compared to healthy controls and a correlation between excessive production of proinflammatory cytokines and increased ROS production and tumorigenesis has been shown [Kundu and Surh, 2008]. Expression of the inducible isoform of nitric oxide synthase (iNOS or NOS2) has also been detected in MM and in B-cell NHL patients [Mendes et al., 2001]; so the relevance of NOS expression to the steady-state

prooxidant levels in these malignancies may also be very significant.

Studies have shown that malignant B-cells undergo apoptosis following exposure to therapeutic agents such as ionizing radiation [Bera et al., 2010; b, 2007] and certain cytotoxic drugs that increase oxidative stress [Dalton, 2002; Villamor et al., 2004]. Monoclonal antibodies directed against tumor specific antigens represent a major advance in the therapy of B-cell NHL and exert cytotoxicity by various mechanisms, perhaps including the generation of ROS within the targeted B-cell. Rituximab, an anti-CD20 monoclonal antibody, has demonstrated efficacy in patients with indolent and aggressive forms of B-cell NHL and CLL. In primary B-cell lymphoproliferative disorders, rituximab induced complement-mediated cell death by increased production of $O_2^{\bullet -}$ and loss of mitochondrial transmembrane potential [Bellosillo et al., 2001]. Anti-HLA-DR antibodies have also shown ROS-mediated cytotoxicity in malignant B-cells [Mone et al., 2004]. Signaling events downstream to B-cell receptor such as B-cell linker protein (BLNK) and spleen tyrosine kinases (Syk) are partially governed by oxidative stress [Tohyama et al., 2004]. In B-cells, millimolar concentrations of H_2O_2 were found to induce necrosis by tyrosine phosphorylation of focal adhesion kinases downstream of Lyn and Syk [Tohyama et al., 2004]. BLNK is required for coupling Syk to phospholipase C-γ2, thereby accelerating apoptosis in B-cells exposed to H_2O_2 [Han et al., 2001].

In a Phase I clinical trial, unmethylated CG dinucleotides (CpG ODN) has been safely administered to refractory B-cell NHL patients with immuno-modulatory effects [Link et al., 2006]. A role of increased pro-oxidant environment by increase in ROS production and alterations in the GSH and glutathione disulfide [GSSG] ratio has been shown to potentiate CpG ODN-induced IL-6 and TNF-α secretion by macrophages [Kirsch et al., 2002]. The formation of ROS in phagocytic cells (such as macrophages) involves NOX enzymes and CpG ODN was found to induce interleukin-12 secretion in macrophages via ROS generated by NOX enzymes [Aramaki et al., 2002]. Recently, the CpG ODN receptor TLR9-mediated activation of c-Jun N-terminal kinases pathway, phosphorylation and upregulation of cytosolic phospholipase A_2 enzyme, and increased ROS production by the NOX1 has been shown to induce the secretion of monocyte chemoattractant protein-1 from macrophages [Lee et al., 2008].

In an ex vivo study myeloma cells (and not normal hematopoietic stem cells) could be selectively eliminated by photodynamic therapy (PDT), which generates singlet O_2 and induces lipid peroxidation [Brasseur et al., 2000]. Malignant B-cells are also highly sensitive to killing by radiotherapy and cytotoxic drugs that modify cellular redox systems such as anthracycline-derivatives, motexafin gadolinium, arsenic trioxide (ATO), non-steroidal anti-inflammatory drugs, dex, and imexon. Naturally occurring compounds such as catechin (green tea), resveratrol, chaetocin, curcumin (from turmeric), and parthenolide have also been hypothesized to induce ROS-mediated anti-B-cell activity.

Thus, redox parameters have a significant impact on the sensitivity of B-cell malignancies to a broad range of treatments including ionizing radiation, traditional cytotoxic chemotherapy, targeted therapy, and immunotherapy.

B-CELL THERAPY UTILIZING OXIDATIVE STRESS-INDUCING AGENTS

The following section summarizes few therapeutic agents that are proposed to be inducers of oxidative stress in malignant B-cells and may have clinical utility in the treatment of B-cell lymphoma and MM. The proposed mechanism of action of radiotherapy and few anti-cancer agents, and combination strategies that could improve B-cell therapy are discussed.

RADIOTHERAPY

Radiotherapy results in the transfer of energy to cellular biomolecules as well as water molecules forming ion pairs resulting in the formation of highly reactive intermediates (ROS; such as $ROO^{\bullet}$ and $OH^{\bullet}$) causing cytotoxicity by covalent alterations in DNA, proteins, and lipids [Spitz et al., 2004]. Radioimmunotherapy (RIT) with CD20 radioimmunoconjugates yttrium-90 ibritumomab tiuxetan (zevalin) and iodine-131 tositumomab (bexxar) target high-energy, short path length radiation to the disseminated lymphoma tumor deposits and are approved in the United States for use in relapsed or refractory indolent, or transformed B-cell lymphoma [Schaefer-Cutillo et al., 2007]. In a recently conducted phase I clinical trial, we have combined zevalin with CpG ODN to achieve a further improvement in B-cell NHL responses [Kapoor et al., 2008]. As CpG ODN, rituximab, and radiotherapy are potent inducers of ROS, it is conceivable that the impressive overall response rate achieved in this trial could be due to increased ROS production and apoptosis of B-cells. This study also opens up new avenues of combining other ROS producing agents or inhibitors of antioxidant pathways with RIT to improve response rates in other B-cell malignancies.

In MM, radiotherapy is used as a definitive treatment for patients with solitary plasmacytoma and for palliation of pain from focal disease. Recent studies combining targeted radiotherapy with bortezomib [Berenson et al., 2009; Berges et al., 2008; Goel et al., 2006a], dex [Bera et al., 2010], or thalidomide [Marchand et al., 2008] suggest the effectiveness of radiation in the context of MM therapy. However, ROS act as secondary messengers in signal transduction pathways that involve redox-sensitive transcription factors such as NF-κB that is believed to be a pro-survival transcription factor in MM [Gilmore, 2007] and activates antioxidant enzymes such as SODs, CAT, and GPxs leading to drug resistance [Klaunig and Kamendulis, 2004]. We have combined radiotherapy with the proteasome-inhibiting drug PS-341/Bortezomib (BTZ) to inhibit constitutive and radiation-induced activation of NF-κB resulting in synergistic killing of myeloma cells in vitro and in vivo [Goel et al., 2006a; Goel et al., 2005]. Further studies that combine radiotherapy with NF-κB inhibitors may prove useful in ROS-induced cytotoxicity of myeloma cells with the added benefit of inhibiting the emergence of chemo-radioresistance in myeloma cells in the bone marrow microenvironment.

ARSENIC TRIOXIDE (ATO)

ATO has an established clinical activity in acute promyelocytic leukemia and preclinical data shows ATO activity in B-cell lymphoma and MM. In acute myeloid leukemia (AML), ATO was also found to inhibit NF-κB activity via depletion of GSH levels and accumulation of H_2O_2 with down regulation of cyclo-oxygenase-2

expression [Han et al., 2005]. Studies have shown that the sulfhydryl oxidizing action of ATO exerts cytotoxic effects by elevating oxidative stress and by inhibiting the proper function of the GSH/GPx system [Dalton, 2002; Hussein, 2003]. In support of this mechanism, myeloma cell lines with lower antioxidant capacity, as measured by GSH, GST, GPx, CAT, and SOD levels, were found to be sensitive to ATO-induced apoptosis [Zhu et al., 2000]. Several agents that deplete cellular GSH, such as green tea, ascorbic acid, PI3K/Akt inhibitor, and buthionine sulfoximide, have been shown to enhance ATO-induced apoptosis. Furthermore, ATO has been combined with trolox (an analogue of α-tocopherol) or polyunsaturated fatty acid docosahexaenoic acid (forming toxic lipid peroxidation products) with increased oxidative cell death of B-cell malignancies [Diaz et al., 2007]. Recently, ATO and 2-methoxyestradiol (2-ME2) have been combined with BTZ to enhance BTZ-induced toxicity in myeloma cell lines via inhibition of β-catenin protein accumulation [Zhou et al., 2008].

The use of ATO in B-cell lymphoma and MM clinical trials has however resulted in modest success. In MM patients that are refractory to conventional salvage therapy, ATO produced responses in 3/14 patients and prolonged stable disease in a fourth patient [Munshi et al., 2002]. ATO has also been combined with BTZ in patients with relapsed/refractory MM with objective responses and ATO with DVd (Doxil, vincristine, and dex) in newly diagnosed myeloma patients failed to improve the response rate compared to DVd alone [Hofmeister et al., 2008]. Overall, ATO has shown promising preclinical and clinical responses in malignant B-cells and an increased understanding of how ATO can be combined with other cytotoxic agents to enhance cancer cell oxidative stress may lead to more effective therapeutic regimens for B-cell lymphoma and MM.

PROTEASOME INHIBITORS

BTZ (also known as Velcade/PS-341) is boronic acid inhibitor of the catalytic site of the 20S proteasome and is first in the class to be approved by FDA for clinical use. BTZ is highly active in MM and is being used in upfront and second line therapeutic regimen [Armand et al., 2007] and has shown single agent activity in mantle cell lymphoma, FL, and Waldenstrom macroglobulinemia [Leonard et al., 2006]. BTZ induces myeloma cell apoptosis in its supportive bone marrow microenvironment by disrupting multiple signaling pathways and recent studies have shown that BTZ induces apoptosis in cancer cells by increasing ROS generation in mitochondria [Nerini-Molteni et al., 2008] and endoplasmic reticulum [Fribley et al., 2004]. In MM, combined treatment of BTZ with the BCl-2 inhibitor [Pei et al., 2003] or histone deacetylases inhibitor [Feng et al., 2007] have shown synergistic myeloma cell killing by oxidative injury. Our group has shown that BTZ when combined with γ-irradiation [Goel et al., 2005] or 153-Sm-EDTMP [Goel et al., 2006a] selectively and synergistically increases radiation-mediated killing of myeloma cells. In MM, SOD2 gene is transcriptionally silenced by DNA methylation [Hodge et al., 2005]; it is therefore feasible that BTZ treatment in myeloma may result in reactivation of the SOD2 gene (by de-methylation) and may thus attenuate its therapeutic efficacy. Recently, BTZ was shown to induce Nrf-2-mediated antioxidant responses [Nerini-Molteni et al., 2008]. Thus, a more detailed understanding of how best to combine proteasome inhibitors with radiotherapy and/or ROS-producing therapeutic agents are warranted that would improve clinical benefits of proteasome inhibitors in B-cell lymphoma and MM.

DEXAMETHASONE

Dex is a synthetic steroidal glucocorticoid that is widely used in the treatment of MM in single and combination chemotherapy regimens [Rajkumar et al., 2002]. Dex has been combined with ifosfamide, cisplatin, and etoposide as salvage chemotherapy for patients with relapsed and refractory lymphoma [Lazar et al., 2009]. Newly diagnosed myeloma patients treated with dex exhibit gene expression changes indicative of oxidative stress [Burington et al., 2008] and we have shown that dex, in combination with radiotherapy enhances the killing of myeloma cells while protecting normal bone marrow hematopoiesis through a mechanism that involves selective increases in oxidative stress [Bera et al., 2010]. Since glucocorticoids constitute one of the most active class of agents that has been used historically to treat MM, a more detailed understanding of how best to incorporate these in combination treatment approaches and impact remission rates in myeloma patients.

ROLE OF ROS-PRODUCING AGENTS IN OVERCOMING THERAPEUTIC RESISTANCE IN B-CELL LYMPHOMA AND MM

In B-cell lymphoma and myeloma patients, therapy protocols combining conventional cytotoxic drugs and novel agents provide some transient benefit, followed by relapse usually caused by drug-resistant disease [Harousseau et al., 2004]. To improve patient outcome, it is therefore important to develop a biochemical rationale for combined modality therapies that enhance cytotoxicity and avoid drug resistance in malignant B-cells in vivo.

The adherence of myeloma cells to bone marrow stromal cells leads to the overproduction of several proinflammatory cytokines that enhance the survival and growth of myeloma cells through paracrine and autocrine loops [Anderson and Carrasco, 2011]. IL-6 is a pro-proliferative cytokine that is robustly secreted by MM and bone marrow resident cells and is associated with resistance to dex [Richardson et al., 2005]. We recently showed that combining dex with radiation selectively increased the oxidative stress induced killing of myeloma cells and inhibited the release of IL-6 from irradiated bone marrow stromal cells [Bera et al., 2010]. The levels of manganese superoxide dismutase (MnSOD), a superoxide scavenging enzyme located in mitochondria, has been shown to be low in cancer cells and a tumor-suppressor role of MnSOD has been suggested [Oberley and Buettner, 1979]. In a human hepatocellular carcinoma cell line, TNF-α, IL-1, and IL-6 were found to increase in MnSOD protein levels [Ono et al., 1992] similar to IL-1- and IL-6-mediated induction of MnSOD mRNA levels in normal hepatocytes [Dougall and Nick, 1991]. It is therefore reasonable to hypothesize that proinflammatory cytokine(s) mediated upregulation of MnSOD and possibly other cellular antioxidants could significantly contribute to the acquisition of cellular resistance to oxidative stress-inducing agents in the treatment of MM. A phase II clinical trial with 2-ME2, a proposed inhibitor of MnSOD, showed minor responses and prolonged stable disease in relapsed myeloma

patients [Rajkumar et al., 2007] and may show improved responses when combined with a ROS-generating therapeutic agents such as radiotherapy and/or chemotherapy. Indeed, improved myeloma cell killing has also been reported when 2-ME2 was combined with ROS-generating drugs BTZ [Chauhan et al., 2004] and ATO [Zhou et al., 2008]; however, the role of ROS in these combination therapies still warrants investigation.

The GSH redox system represents one of the most important cellular defense systems against oxidative stress [Schafer and Buettner, 2001]. Increased cellular concentration of GSH has been associated with resistance to chemotherapeutic agents like platinum drugs. Therapeutic approaches that enhance the production of ROS within mitochondria with simultaneous inhibition of GSH production may lead to oxidative stress and the induction of apoptosis of myeloma cells. Buthionine sulfoximide is a chemical inhibitor of γ-glutamylcysteine synthetase, the rate-limiting enzyme for GSH synthesis. In addition, buthionine sulfoximide is clinically relevant, well tolerated in humans, and a relatively specific pharmacological agent that inhibits GSH synthesis and may be used to inhibit the emergence of chemo-radioresistance in myeloma cells. ATO has been combined with ascorbic acid in patients with relapsed and refractory B-cell NHL [Chang et al., 2008] and in patients with relapsed/refractory myeloma [Hofmeister et al., 2008].

BTZ has been hypothesized to induce apoptosis in malignant cells by ROS-mediated mechanisms as well as possessing anti-myeloma and anti-lymphoma activity via inhibiting the activation of NF-κB. In addition, SOD2 is regulated by NF-κB as well as being epigenetically silenced by promoter hypermethylation in myeloma cell lines [Hodge et al., 2005]. Myeloma cell lines and primary myeloma cells have been shown to have a lower endogenous MnSOD levels with increased susceptibility to 2-ME2-mediated oxidative cell death via apoptosis [Hurt et al., 2007]. Also, 2-ME2 has also been combined with PDT with increased myeloma cell killing in vitro and in vivo [Golab et al., 2003].

CONCLUSION

Emerging data indicates malignant B-cells are intrinsically under metabolic oxidative stress due to increased steady-state levels of $O_2^{\bullet-}$ and H_2O_2 from mitochondrial metabolism and/or altered expression of antioxidant enzymes. Preclinical and clinical studies in B-cell lymphoma and MM have shown selective cytotoxicity of agents (such as radiation, PDT, and specific chemotherapeutic drugs) that influence ROS. These ROS-inducing modalities are being combined with agents that deplete intracellular antioxidants (such as buthionine sulfoximide, ascorbic acid), inhibit antioxidant enzyme activity (such as 2ME2), inhibit the secretion of pro-proliferative cytokines (such as dex), and/or other cytotoxic drugs that disrupt mitochondrial membrane potential to release cytochrome c (such as BTZ and farnesyl transferase inhibitors) such that a selective ROS catastrophe can be induced in malignant cells with minimal cytotoxicity to the normal cells. Designing complementary therapeutic approaches based on fundamental differences in oxidative metabolism between malignant versus normal cells offers a promising strategy to developing more effective treatment strategies that may also inhibit the emergence of in vivo resistance in B-cell lymphoma and myeloma cells in their supportive microenvironment leading to improved clinical responses.

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