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PROSPECT

Reactive Oxygen Species: Current Knowledge and Applications in Cancer Research and Therapeutic

Andy T.Y. Lau, Ying Wang, and Jen-Fu Chiu*

Department of Anatomy, The University of Hong Kong, Pokfulam, Hong Kong SAR, People's Republic of China

Abstract Reactive oxygen species (ROS) are natural products inevitably generated along cellular metabolism. Due to their highly reactive nature, which can damage DNA, proteins and lipids, cells utilize antioxidative or defense systems to balance these toxic products to keep the cells in a state of redox homeostasis. However, under the situation of imbalance in redox status, depending on the magnitude of ROS encountered, high levels of ROS can induce apoptosis, whereas chronic low levels of ROS promote vascular diseases such as arteriosclerosis. Although ROS seem to be catastrophic to life, accumulating evidence points to the beneficial roles of ROS by virtue of the ability as chemotherapeutic agents to cure human diseases. Many anti-cancer drugs have been developed in this way which can generate ROS and cause oxidative stress-induced apoptosis in cancer cells. The effects of ROS are paradoxical because they can act as both disease culprits and chemotherapeutic agents. In this review, the current knowledge of ROS and the potential applications of ROS in cancer therapeutic will be discussed. J. Cell. Biochem. 104: 657–667, 2008.

Key words: ROS; redox; free radicals; antioxidant; chemotherapeutic agent

The term "Reactive Oxygen Species (ROS)" seems to have been first coined in the year of 1950's. ROS appear more likely as an evil. However, after more than five decades until now, we have gained a broad knowledge of ROS. ROS, in general, describe the varieties of oxygen-containing species that are inevitably generated along cellular metabolism [Klaunig and Kamendulis, 2004; Shi et al., 2004]. ROS, due to their highly reactive nature, possess higher reactivity than molecular oxygen that can damage DNA, proteins, and lipids [Thannickal and Fanburg, 2000]. Under normal conditions, cells utilize antioxidative or defense systems to balance these toxic products to keep the cells in a state of redox homeostasis.

Much emphasis has been put on anti-oxidant to battle against the toxic effects of ROS [Rahman and MacNee, 2000]. And numbers of review articles on anti-oxidant can be found in the literature. However, accumulating evidence points to the beneficial roles of ROS by virtue of the ability as chemotherapeutic agents to cure human diseases [Benhar et al., 2002]. Many anti-cancer drugs have been developed in this way which can generate ROS and cause oxidative stress-induced apoptosis in cancer cells.

For the vast emerging literatures published in just the few years, in this review, we intend to summarize the recent advance on the utilization of ROS in cancer research. Also, the main focus of this review will be put on the recent examples of applications of ROS rather than spending too much space on the knowledge that had already known. Nevertheless, we will recall the readers on some of the common concepts on ROS at the beginning.

ROS GENERATION

As scientists explored this field for the past decades, now we all know that ROS are formed by several different mechanisms,

Andy T.Y. Lau and Ying Wang contributed equally to this work.

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Andy T.Y. Lau's present address is Hormel Institute, University of Minnesota, Austin, MN 55912.

*Correspondence to: Prof. Jen-Fu Chiu, Department of Anatomy, The University of Hong Kong, Pokfulam, Hong Kong SAR, People's Republic of China.
E-mail: jfchiu@hkucc.hku.hk

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including (1) ionizing radiation on biological molecules; (2) as an unavoidable byproduct during cellular respiration; and (3) synthesized by enzymes (NADPH oxidase and myeloperoxidase) from phagocytic cells to battle against bacterial infection [Martindale and Holbrook, 2002; Klaunig and Kamendulis, 2004; Poli et al., 2004].

Strong ROS-producing oxidants can damage other vital cellular structures. Among the most important of these are the actions of free radicals on the fatty acid side chains of lipids in the various membranes of the cell, which caused lipid peroxidation [Girotti, 1998]. One of the most damaging things about free radicals is that they interact with other molecules to gain a stable configuration of electrons, so they convert that target molecule into a radical. Hence, a series of chain reaction begins that will propagate until two radicals meet each other and each contributes its unpaired electron to form a covalent bond linking them together.

INTRACELLULAR AND EXTRACELLULAR ROS

Every aerobic organisms need oxygen for metabolism. And ROS are generated inevitably. ROS can be produced by both endogenous and exogenous sources. The endogenous sources usually derive from oxidative phoshorylation, P450 metabolism, peroxisomes, and inflammatory cell activation [Klaunig and Kamendulis, 2004; Poli et al., 2004]. For exogenous ROS, it can be produced by environmental agents, including nongenotoxic carcinogens, chlorinated compounds, radiation, metal ions, barbiturates, phorbol esters, and some peroxisome-proliferating compounds [Klaunig and Kamendulis, 2004; Poli et al., 2004].

DEFENSE SYSTEMS TO ANTAGONIZE THE EFFECTS OF ROS

Organisms are capable of keeping the cells in a state of redox homeostasis. This kind of equilibrium can only be achieved by utilizing cellular antioxidant defense enzymes such as superoxide dismutase, glutathione peroxidase, and catalase [Klaunig and Kamendulis, 2004]. Superoxide dismutases and glutathione peroxidases, which are present in cytosol and mitochondria, reduce superoxide anion to hydrogen peroxide and water, and remove the majority of hydrogen peroxide, respectively. Meanwhile, catalase, located in peroxisomes, also remove high levels of hydrogen peroxide. Nonenzymatic antioxidants, like vitamin E, vitamin C, β-carotene, glutathione, and coenzyme Q function to quench ROS [Clarkson and Thompson, 2000].

THE CONSEQUENCES OF IMBALANCE OF REDOX STATUS—OXIDATIVE STRESS

Although a number of defense systems have evolved to combat the accumulation of ROS, unfortunately, sometimes they are not sufficient to counter the effect of ROS; oxidative stress arises when ROS are produced faster than they can be removed by the cellular defense mechanisms (Fig. 1). This imbalance in redox status, depending on the magnitude of ROS encountered, high levels of ROS can induce apoptosis, whereas chronic low levels of ROS promote a wide variety of diseases and carcinogenesis [Finkel and Holbrook, 2000].

THE ACUTE AND CHRONIC EFFECTS OF ROS

Usually, ROS can elicit a broad spectrum of responses depending on the magnitude of the level and the duration of exposure. In general, low levels of ROS are mitogenic and promote cell proliferation, while intermediate levels cause transient or permanent cell cycle arrest and induce cell differentiation. High levels of ROS are detrimental and induced cell apoptosis or necrosis [Finkel and Holbrook, 2000; Martindale and Holbrook, 2002].

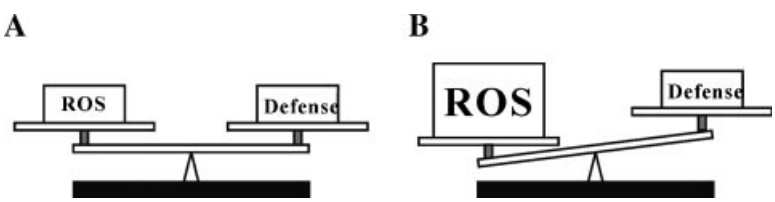

Fig. 1. Oxidative stress. **A**: A number of defense systems have evolved to combat the accumulation of ROS. **B**: Unfortunately, sometimes they are not sufficient to counter the effect of ROS, oxidative stress arises when ROS are produced faster than their removal by the cellular defense mechanisms.

ACUTE HIGH LEVELS OF ROS—INDUCE APOPTOSIS

Acute high levels of ROS damage cellular components like DNA, proteins, and lipids. Due to the strong reactivity, ROS can attack DNA and cause DNA base oxidation, DNA lesion, and DNA strand breaks [Shukla et al., 2003; Shi et al., 2004]. Protein can be damaged by oxidative modifications of amino acid as well as by ROS-mediated peptide cleavage. This results in malfunction of many enzymes including cytochrome *c* oxidase, glutathione oxidase, and catalase which in turn reduces the capacity of cell to eliminate ROS and further aggravates the cell under oxidative stress. Lipid peroxidation, as has been mentioned above, in which ROS attack lipid membrane in a chain reaction manner, produce a series of organic radicals and result in considerable demolition to the cell. Our recent proteomic study on our well-established rat lung epithelial cell line (LEC) showed that acute high levels of ROS exerted by arsenite caused oxidative stress-induced apoptosis in LEC [Lau et al., 2004]. This arsenite-induced apoptosis can be countered by free radical scavengers NAC or GSH. Acute high levels of ROS are effective in inducing cell apoptosis and the potential use of inorganic arsenical compounds in the treatment of various human cancers is being now studied intensively by scientists from all over the world [Lau and Chiu, 2003].

CHRONIC LOW LEVELS OF ROS—CAUSE CARCINOGENESIS

Recent studies show that chronic low levels of ROS induce DNA or protein damages which affects the cells' genome stability and redox homeostasis [Klaunig and Kamendulis, 2004]. It has also been shown that chronic low levels of ROS caused oxidative modifications of DNA bases and resulted in gene mutation, and since low levels of ROS may serve as messengers in cellular proliferation, in the presence of DNA mutation and enhanced cell proliferation, in conjunction with compromised cellular anti-oxidative stress defense mechanisms, largely promoted genotoxic events to occur and this may play a role in the process of carcinogenesis. Our recent proteomic study on LEC showed that chronic low levels of ROS exerted by arsenite caused malignant cell transformation [Lau and Chiu, 2006]. Chronic low levels of ROS also promote a wide variety of diseases such as vascular diseases like arteriosclerosis. This is largely caused by perturbation of endothelial cell signaling. This signaling requires oxidation of regulatory enzymes and activates pathways leading to increased nuclear translocation of NF-κB. Increased binding of proteins to genomic κB sites may induce a mitogenic or inflammatory response in the endothelial cells. This suggests that low levels of ROS promote vascular diseases by activating the endothelium rather than causing vascular cell death [Barchowsky et al., 1996].

SIGNAL TRANSDUCTION OF CELLS IN RESPONSE TO ROS

From the past two decades, research on the signal transduction pathways mediated by ROS has been studied intensively. ROS can activate diverse signaling pathways and affect a spectrum of various gene expressions.

ROS PERTURB MULTIPLE SIGNAL TRANSDUCTION PATHWAYS AND INDUCE GENE EXPRESSIONS

The signaling pathways generally activated by ROS and their ultimate cellular outcomes have recently been reviewed by several research groups [Benhar et al., 2002; Martindale and Holbrook, 2002; Leonard et al., 2004; Poli et al., 2004]. In brief, ROS can activate such as, members of the mitogen-activated protein kinases (MAPKs), phosphatidylinositol-3-kinase (PI3K)/Akt pathway, phospholipase C-γ1 (PLC-γ1) signaling, protein kinase C, p53 signaling, ataxia-telangiectasia-mutated (ATM) kinase, nuclear factor-kappaB (NF-κB) signaling, and Jak/Stat pathway. Certain pathways tend to enhance survival, while others promote cell death. Hence, the battle between survivals from cell death was really determined by the relative balance among the activities regulated. Through distinct signal transduction cascades, ROS can induce families of heat shock protein expression, immediate early genes of the bZip family members like c-Jun and c-Fos, hypoxia inducible factor, and antioxidative enzymes expression which help to regulate redox homeostasis and the expression of transforming oncoproteins and growth factors.

BIOMEDICAL APPLICATIONS

The above sections mentioned seem that ROS are disease culprits. However, this is not always

the case. As mentioned at the beginning of this article, in our human body, a kind of defense mechanism against pathogens is actually beneficial to us. Activated macrophages, neutrophils, and eosinophils produce ROS during phagocytic respiratory burst which can generate free radicals to kill the pathogens. Indeed, the utilization of ROS-generating agents in cancer research is now gradually unveiled and applied to biomedical fields, the results are promising and the potentiality is currently under-estimated, indicating that further extensive research is warranted.

MECHANISM OF ACTIONS OF ROS-GENERATING DRUGS

In light of the natural role of ROS as defense mechanism in human body, it is not surprising to see increased applications of ROS as a chemotherapeutic agent in human diseases. The fact that the more intrinsic oxidative stress in cancer cells and thereby relatively susceptible to oxidative stress-induced apoptosis than normal cells. This makes the basis for development of ROS-generating drugs in cancer research [Benhar et al., 2002]. The actions of these ROS-generating drugs can act directly and/or indirectly. The whole idea is to cause an imbalance in ROS status to the cell causing oxidative catastrophe and this can be achieved by several means including direct generation of ROS by the agent itself, depletion of intracellular GSH level, cause decrease of mitochondrial membrane potential, enhance the activity of ROS producing enzymes, inhibition of antioxidative proteins, and proteasome inhibition. Moreover, due to the more intrinsic oxidative stress in cancer cells than normal cells, the selective use of modulator (e.g., kinase inhibitor/activator) [Blagosklonny, 2004] which targets certain signaling pathways (reactivate desensitized apoptotic pathways or inhibit survival pathways) in cancer cells and then perturb the cellular redox-status by ROS-generating agent can possibly gain more selective killing of cancer cells in patients while causing less cytotoxicity to normal cells as well as providing therapeutic advantage in the treatment of cancers that do not respond to ROS-generating agent alone (Fig. 2).

ROS-GENERATING DRUGS AS CHEMOTHERAPEUTICS IN HUMAN DISEASES

Various ROS-generating drugs from natural sources or chemically synthesized have been proven to be good means of curing human diseases. In this article, we are particularly interested in the (1) natural occurring compounds; (2) arsenical compounds; (3) proteasome inhibitors; (4) agents that enhance or inhibit the activity of ROS producing enzymes or antioxidative (detoxificative) proteins, respectively; (5) drugs that target DNA or DNA topoisomerase; and (6) the metalloporphyrins, and the amazing effects of their combined actions with other modulators in cancer research.

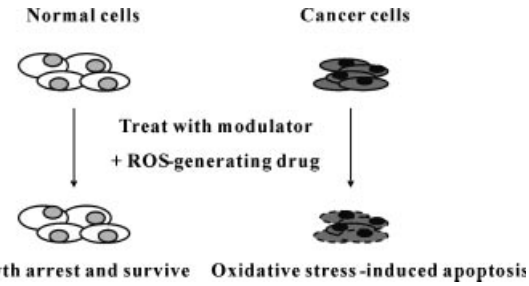

Fig. 2. Combined treatment of modulator and ROS-generating agent. By the selective use of modulator (e.g., kinase inhibitor/activator or histone deacetylase inhibitor) which targets certain signaling pathways in cancer cells and then perturb the cellular redox-status by ROS-generating agent can possibly gain more selective killing of cancer cells while causing less cytotoxicity to normal cells as well as providing therapeutic advantage in the treatment of cancers that do not respond to ROS-generating agent alone.

NATURAL OCCURRING COMPOUNDS AS EFFECTIVE CHEMOTHERAPEUTIC AGENTS

Various active compounds isolated from natural sources and traditional Chinese medicine revealed promising results against tumor. Non-pungent capsaicinoids, isolated from sweet pepper, was demonstrated to possess anti-cancer potential. The induction of apoptosis in Jurkat cells was preceded by an increase in the production of ROS and by a subsequent loss of mitochondria transmembrane potential [Macho et al., 2003]. C-phycocyanin, which is a major billiprotein of the blue-green algae, has been shown to induce the generation of ROS in AK-5 tumor cells, which in turn induced apoptosis accompanied with downregulation of Bcl-2, which is critical in the apoptotic death processes [Pardhasaradhi et al., 2003].

Vitamins D and E and their respective analogs induced apoptosis in breast cancer and non-small cell lung cancer cell lines [Ravid et al., 1999; Weitsman et al., 2003; Kang et al.,

2004; Weitsman et al., 2005]. Calcitriol (1,25-dihydroxyvitamin D_3), the hormonal form of vitamin D_3, potentiated the action of H_2O_2 and activity of some common anti-cancer drugs and agents of the anti-cancer immune system, including tumor necrosis factor α (TNF α) and doxorubicin. It has been shown to generate ROS stress, enhance caspase-dependent and -independent pathway and induced MCF-7 breast cancer cell death by augmentation in the drop of mitochondrial membrane potential and release of cytochrome *c* from mitochondria [Ravid et al., 1999; Weitsman et al., 2003; Weitsman et al., 2005]. α-Tocopheryl succinate (TOS), a vitamin E analog, has been shown to induce the generation of ROS in caspase-independent apoptosis in human lung cancer A549 and H460 cell lines [Kang et al., 2004].

The anti-cancer roles of some active compounds sesquiterpene lactones purified from Chinese medicine like cynaropicrin (from *Saussurea lappa*) and parthenolide (from *Tanacetum parthenium*) have been investigated recently. Cynaropicrin potently inhibited the proliferation of leukocyte cancer cells, such as U937, Eol-1, and Jurkat T cells, the cytotoxic effect of cynaropicrin was due to inducing apoptosis and cell cycle arrest at G1/S phase, and that cynaropicrin-induced proteolytic cleavage of PKCδ suggest that ROS and PKCδ may play an important role in mediating its pro-apoptotic activity [Cho et al., 2004]. Parthenolide effectively inhibits hepatoma cell growth in a tumor cell-specific manner and triggers apoptosis of hepatoma cells as well as invasive sarcomatoid hepatocellular hepatoma SH-J1 cells. This parthenolide-induced apoptosis, accompanied with depletion of glutathione, generation of ROS, reduction of mitochondrial transmembrane potential, activation of caspases (caspase-7, -8, and -9), overexpression of GADD153 (an oxidative stress or anti-cancer agent inducible gene), and subsequent apoptotic cell death [Wen et al., 2002].

Plumbagin (5-hydroxy-2-methyl-1,4-naphthoquinone), occurs in the plants belonging to *Plumbagineae* and *Droseraceae* families, has potential as a chemotherapeutic agent. Treatment of human cervical cancer ME-180 cells with plumbagin caused loss of mitochondrial membrane potential, and morphological changes characteristic of apoptosis, including the translocation of phosphatidyl serine, nuclear condensation, and DNA fragmentation. Interestingly, plumbagin-induced apoptosis involved release of cytochrome *c* and apoptosis-inducing factor (AIF), thereby activating both the caspase-dependent and -independent pathways, respectively [Srinivas et al., 2004]. Cinnamaldehyde, an active compound isolated from the stem bark of *Cinnamomum cassia*, a traditional oriental medicinal herb, has been shown to inhibit tumor cell proliferation. Cinnamaldehyde is a potent inducer of apoptosis in human promyelocytic leukemia HL-60 cells and that it transduces the apoptotic signal via ROS generation, thereby inducing mitochondrial permeability transition (MPT) and cytochrome *c* release to the cytosol [Ka et al., 2003]. To sum up, the identification of active compounds from natural sources and traditional Chinese medicine that can generate ROS-stress is definitely worth to be further studied as means of curing various types of cancers.

PROMISING ANTI-CANCER ACTIVITY OF ARSENIC TRIOXIDE (As_2O_3) AS A SINGLE AGENT AND INDUCES APOPTOSIS IN VARIOUS CELL LINES AND MALIGNANCIES

Arsenical compounds, like As_2O_3, are potent therapeutics for the treatment of acute promyelocytic leukemia (APL) and also other human cancers. It has been shown that As_2O_3 induces apoptosis via the generation of ROS in cancer cells. Due to the promising results of As_2O_3 in act against cancers, extensive research has been undertaken intensively. In the past 5 years, the possible actions of As_2O_3 were uncovered. It can be seen that the actions of As_2O_3 in inducing apoptosis are diverse and complicated. As a single agent, As_2O_3 inhibits cell growth and induces apoptosis in several types of cancer cells including APL, prostate, and ovarian carcinomas [Bode and Dong, 2002; Dong, 2002; Lau and Chiu, 2003]. These actions of arsenic may result in the induction of apoptosis, the inhibition of growth and angiogenesis and the promotion of differentiation. Such effects have been observed in cultured cell lines and animal models as well as clinical studies. Because arsenic affects so many cellular and physiological pathways, a wide variety of malignancies, including hematologic cancer, pancreatic cancer, and solid tumors derived from several tissue types, may be suitable to As_2O_3 therapy [Miller et al., 2002]. However, drawbacks do occur despite its potent

apoptotic mechanism, it is not equally effective in all leukemic cells which has prompted a search for agents enhancing As_2O_3 efficacy.

COMBINED TREATMENT WITH As_2O_3 ENHANCES APOPTOSIS IN VARIOUS CANCERS

Recent research data show that the use of mild ROS generators in conjunction with As_2O_3 as novel regimens to sensitize tumor cell apoptosis. The use of L-buthionine-sulfoximine (BSO), which inhibited the critical step in glutathione synthesis, effectively enhanced in vitro growth inhibition effect of As_2O_3 on all 11 investigated cell lines arising from prostate, breast, lung, colon, cervix, bladder, and kidney cancers, compared with As_2O_3 treatment alone [Maeda et al., 2001, 2004]. The combination therapy of As_2O_3 with BSO is a valid means of blockade of H_2O_2-scavenging system, and that the combination of a ROS-generating agent with an inhibitor of major scavenging system is effective in terms of both efficacy and selectivity.

It has been shown that Docosahexaenoic acid (DHA), a kind of polyunsaturated fatty acid, sensitized various cancer cells to ROS-inducing anti-cancer agents. Recently, DHA also enhances the apoptotic effect of clinically achievable concentration (1–2 μM) of As_2O_3 in several As_2O_3-resistant human leukemic cell lines via a ROS-dependent mechanism as well as 12 different solid tumor cell lines. The cytotoxic effect of As_2O_3 and DHA was not observed in normal cells and only selectively toxic for malignant cells associated with the induction of apoptosis and a concomitant increase of intracellular lipid peroxidation products [Sturlan et al., 2003; Baumgartner et al., 2004].

Anthraquinones, as well as its natural occurring derivatives like emodin are potent generators of ROS due to their semiquinone structure, were elevated whether they can facilitate As_2O_3-induced apoptosis in tumor cells. Results showed that anthraquinones could produce ROS and sensitize tumor cells to arsenic both in vivo and in vitro [Yang et al., 2004]. The combination of emodin and arsenic promoted the major apoptotic signaling events in esophageal-derived carcinoma cells. Emodin also sensitized HeLa cells to As_2O_3 via generation of ROS and ROS-mediated inhibition on two major prosurvival transcription factors NF-κB and AP-1 [Yi et al., 2004]. This indicated that the use of mild ROS generators is able to facilitate the induction of apoptosis-inducing drugs.

PROTEASOME INHIBITORS ON ROS GENERATION AND MITOCHONDRIA DYSFUNCTION

The ubiquitin-proteasome pathway is vital for the degradation of misfolded or unwanted proteins inside the cell. The proteasome inhibitor, Bortezomb (PS-341), shows substantial anti-tumor activity in a variety of tumor cell lines. Recently, its action mechanisms become clear in which it appears to effect on the components of the mitochondrial apoptotic pathway that caused generation of ROS, alteration in the mitochondrial membrane potential, and release of cytochrome *c* in human H460 lung cancer cells [Ling et al., 2003]. Besides, it has been shown that bortezomib induced apoptosis through induction of endoplasmic reticulum (ER) ROS-stress in head and neck squamous cell carcinoma (HNSCC) [Fribley et al., 2004]. Recently, synergistic anti-cancer activity has been demonstrated. By the combined use of histone deacetylase inhibitor FK228 or CBHA and proteasome inhibitor PSI or bortezomb, synergistically induced apoptosis in human gastric MKN45 and colorectal DLD-1 adenocarcinoma cells. The fact that HDAC inhibitors can synergize the action of proteasome inhibitors is that histone hyperacetylation activates transcription of Bim and Bim-mediated mitochondrial damage augmented cell death [Adachi et al., 2004]. Interestingly, the nonsteroidal anti-inflammatory drug sulindac, which is a promising chemopreventive agent against colon cancer, synergistically augments the anti- cancer effects of bortezomb. The combined effects primarily through cooperative ROS generation and oxidative DNA damage, thereby representing a novel combination therapy against colon cancer [Minami et al., 2005]. These results indicated that by combination of proteasome inhibitors with traditional inhibitors or drugs against certain types of cancer, could lead to an unexpectedly promising outcome and these works shed lights in the future regimens for cancer therapy.

ENHANCEMENT OF THE ACTIVITY OF ROS PRODUCING ENZYMES OR INHIBITION OF ANTIOXIDATIVE (DETOXIFICATIVE) PROTEINS

Apart from the above, agents like bryostatin 1 that can enhance the activity of ROS producing

enzyme NADPH oxidase has been shown to play a prominent cooperative role in arsenic-induced ROS formation and cytotoxicity in myeloid leukemia cells [Chou et al., 2004]. Conversely, the use of inhibitors of antioxidative (detoxificative) proteins like glutathione *S*-transferase (GST) inhibitors [non-GSH-peptidomimetic derivatives of 7-nitro-2,1-3-benzoxadiazole (NBD)] has been shown to trigger apoptosis in human leukemic K562 and CCRF-CEM (human T-lymphoblastic leukemia) cell lines by the activation of the JNK/c-Jun-mediated pathway that resulted in a typical process of apoptosis [Turella et al., 2005]. 2-methoxyestradiol (2-ME), a natural occurring metabolic of estradiol, has been demonstrated to inhibit superoxide dismutase (SOD) and induce apoptosis in leukemia cells [Hileman et al., 2004]. This is due to malignant cells in general are more active than normal cells in the production of O_2^-, are under intrinsic oxidative stress, the increased oxidative stress in cancer cells forces these cells to rely more on antioxidative enzymes like SOD for O_2^- elimination, thus making the malignant cells more vulnerable to SOD inhibition than normal cells. Therefore, using exogenous ROS-producing agents such as arsenic trioxide in combination with 2-ME to enhance the antileukemia activity is of potential clinical significance [Zhou et al., 2003].

DRUGS TARGETING DNA TOPOISOMERASE IN CANCER THERAPY

The effects of topoisomerase inhibitors induced-ROS in apoptosis were also under investigation. Inhibitors of DNA topoisomerase I have been widely used to induce apoptosis under experimental conditions and in phase III clinical trials for colon cancer [Cunningham and Glimelius, 1999]. The DNA topoisomerase I inhibitor camptothecin (CPT) caused an increase in ROS level inside leishmanial cells, which was responsible for the collapse of mitochondrial membrane potential [Sen et al., 2004a]. Recent results from Sen et al. demonstrated that CPT-induced ROS caused an increase in cytosolic Ca^{2+} due to opening of nonselective and L-type voltage-gated calcium channels as well as caused dysregulation of sarcoplasmic Ca^{2+} ATPase channels. The excessive free cytosolic Ca^{2+} led to uncoupling of mitochondrial oxidative phosphorylation and directed cells to follow the executing part of apoptosis [Sen et al., 2004b]. These results, together with other reports [Shiah et al., 1999; Sordet et al., 2004] suggested that ROS played an essential and important role in topoisomerase I inhibitor-induced apoptosis.

DNA-DAMAGING DRUGS INDUCE APOPTOSIS IN CANCER CELLS

Growing interest has been focused on metal-based anti-cancer drugs, especially some DNA-damaging agents, including platinum [Zamble et al., 1998] and chromium [Rajaram et al., 1995] containing compounds. It is generally believed that this kind of chemotherapeutic agent induces oxidative stress and DNA damage by oxygen radicals through oxidative nucleic acid modification and scission of DNA strands [Higuchi, 2003]. The involvement of oxidative stress in the induction of apoptosis by chemotherapeutic agent's treatment has been suggested in several cell models [Ye et al., 1999; Biroccio et al., 2001; Huang et al., 2003].

The role of oxidative stress in cisplatin-induced apoptosis has been demonstrated in a number of studies. Zupi et al. reported that ROS generation did not constitute the primary event in cisplatin-induced apoptosis but depended on caspase 1-like protease activation [Biroccio et al., 2001]. Others reported that cisplatin promoted ROS production, which in turn contributed to Fas receptor aggregation and cell death [Huang et al., 2003]. The novel coupling between ROS and Fas clustering likely played a significant role in apoptosis triggered by cisplatin in Fas-expressing leukemia cells. A possible explanation for the role of oxidative stress in cisplatin-induced apoptosis is that Pt could potentiate the reactivity of superoxide [Theron et al., 2004]. However, the role of oxidative stress in another B class metal, chromium, induced apoptosis was not likely the same as that in platinum-containing compounds. It was demonstrated that ROS generated through chromium reduction was responsible for the early stage of apoptosis, whereas p53 contributed to the late stage of apoptosis and was responsible for the enhancement of chromium-induced apoptosis at that stage [Ye et al., 1999]. Reports from other groups demonstrated that chromium induced apoptosis by mediating through production of ROS, which in turn activated the Src-family tyrosine kinases [Balamurugan et al., 2002; Vasant et al., 2003]. The essential role of oxidative stress in

chromium-induced apoptosis was because chromium acted as a direct ROS-promoting agent that caused mitochondria damage and induced apoptosis [Ye et al., 1999; Hayashi et al., 2004].

METALLOPORPHYRINS IN CANCER THERAPY

Metalloporphyrins are a newly emerging class of stable catalytic antioxidants possessing a broad range of antioxidant capacities, including dismutation of superoxide, hydrogen peroxide, and scavenging of peroxynitrite [Szabo et al., 1996; Day et al., 1997]. The antioxidant properties of metalloporphyrins were previously reviewed by Patel and Day [1999]. Several metalloporphyrin compounds are under investigation as antioxidants at present, including pegylated zinc protoporphyrin [Fang et al., 2004], manganese porphyrins [Day et al., 1999], and chlorophyllin [Kamat et al., 2000].

Heme oxygenase (HO) catalyzes the initial and rate-limiting step of heme degradation in which oxidative cleavage of the porphyrin ring leads to the formation of biliverdin, carbon monoxide (CO), and free iron [Maines, 1988; Shibahara, 1988]. Among the three isozymes of HO, HO-1 was recently found to protect cells from apoptosis induced by cisplatin [Fang et al., 2004] and TNF-α [Chen et al., 2004]. It is conceivable that upregulated expression of HO-1 will lead resistance to anti-cancer agents that induced oxidative stress in the process of apoptosis. In view of that, it is reasonable to use HO-1 inhibitors to enhance antitumor effect of chemotherapeutic agents. Many groups have reported metalloporphyrins contained various other metals such as cobalt, zinc, manganese, chromium, or tin [Drummond, 1987] rather than iron of heme as HO-1 inhibitors. These metalloporphyrins can compete for HO reaction because of their inefficient binding to molecular oxygen, which prevents HO from degrading the metalloporphyrins [Drummond, 1987]. Among these metalloporphyrins, ZnPP and a water-soluble derivative of ZnPP, poly(ethylene glycol)-conjugated ZnPP (PEG-ZnPP), have been reported as HO-1 inhibitor both in vitro and in vivo [Fang et al., 2003, 2004]. Further studies of the effects of PEG-ZnPP and conventional anti-cancer drugs that generate ROS, such as cisplatin and CPT, are warranted.

It is anticipated that the development of novel anti-cancer drugs with different modes of action is needed for the treatment of cancer patients, particularly for those refractory to standard treatment (e.g., cisplatin-resistant patients). The potential applications of gold(III) complexes as a new class of anti-cancer drugs with higher cytotoxicity and fewer side effects than existing metal anti-cancer drugs have been explored recently. We have been attempting to develop gold(III) *meso*-tetraarylporphyrins as potential chemotherapeutic leads by elucidating their action mechanisms [Che et al., 2003]. Our recent promising results demonstrated that, gold(III) porphyrin 1a, as a single agent alone, exhibited anti-cancer activities in a number of human cancer cell lines [Che et al., 2003; Wang et al., 2006] and induced apoptosis by mitochondrial death pathways related to ROS (Fig. 3) [Wang et al., 2005]. These results support the findings that gold(III) porphyrin 1a is a promising anti-cancer drug lead and possibly a novel therapeutic agent directed towards the mitochondria.

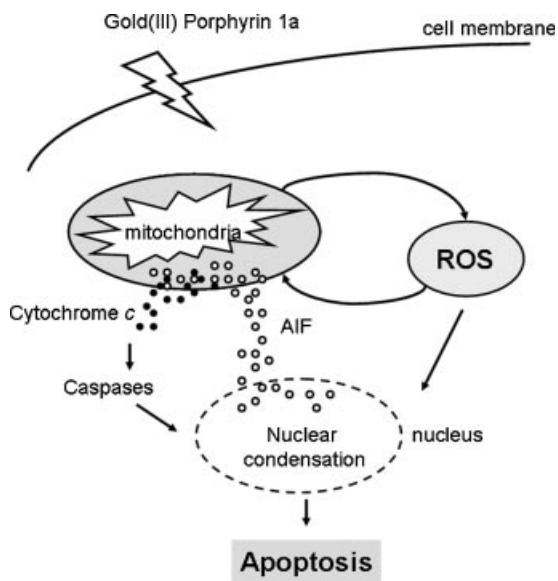

Fig. 3. Proposed model for the cellular mechanisms of gold(III) porphyrin 1a-induced apoptosis in nasopharyngeal carcinoma cells (HONE1). Gold(III) porphyrin 1a directly caused depletion of $\Delta\Psi_m$, leading to the alteration of Bcl-2 family proteins, AIF nuclear translocation, and cytochrome *c* release, this further activated caspase-9 and caspase-3, and subsequently caused PARP-1 cleavage, in which ROS were generated. The altered cellular oxidative state affected cytotoxicity of gold(III) porphyrin 1a by regulating mitochondrial permeabilization [Wang et al., 2005].

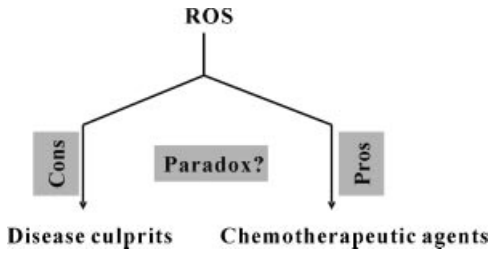

Fig. 4. The paradoxical effects of ROS.

SUMMARY AND FUTURE PROSPECTS OF ROS IN CANCER RESEARCH—DEVELOPMENT OF MORE EFFECTIVE ANTI-CANCER DRUGS

Chronic exposure to ROS is associated with increased risk of cancers. ROS undoubtedly induces apoptosis and specifically targets certain tumor cells. Recent research shows that ROS influences distinct signaling pathways involved in mediating proliferation or apoptosis, including MAPKs, p53, AP-1, or NF-κB. From the therapeutic point of view, ROS is definitely worth further studying to explore its potentiality in the future. However, it should be noted the tumor promotion threat of ROS. In conclusion, ROS can have adverse or beneficial effects depending on cellular homeostasis, concentration, and duration of ROS exposed (Fig. 4), and we should utilize these properties as beneficial chemotherapeutic agents rather than as disease culprits. We forecast that future anti-cancer therapy will focus on the selective combination of modulators with ROS-generating drugs, that is, achievement of maximum synergism to reach the drug target and enhance the therapeutic efficacy. We are now actively engaged ourselves in cancer research by utilizing novel ROS-generating drugs against cancer cells and will hopefully provide more insights to this area in the future.

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