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REACTIVE OXYGEN SPECIES (ROS): A BRIGHT MOON TOWARDS CANCER THERAPY- A Review

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ABSTRACT:

Most chemotherapeutics elevate intracellular levels of reactive oxygen species (ROS), and many can alter redox-homeostasis of cancer cells. It is widely accepted that the anticancer effect of these chemotherapeutics is due to the induction of oxidative stress and ROS-mediated cell injury in cancer. Reactive oxygen species (ROS) constitute a group of highly reactive molecules that have evolved as regulators of important signaling pathways. It is now well accepted that moderate levels of ROS are required for several cellular functions, including gene expression. The production of ROS is elevated in tumor cells as a consequence of increased metabolic rate, gene mutation and relative hypoxia, and excess ROS are quenched by increased antioxidant enzymatic and nonenzymatic pathways in the same cells. Our review will emphasize the molecular mechanisms useful for the development of therapeutic strategies that are based on modulating ROS levels to treat cancer. In the late 1950's free radicals (FR) and antioxidants were almost unheard of in the clinical and biological sciences but chemists had known about them for years in the context of radiation, polymer and combustion technology.

KEY WORDS: Cancer, Reactive oxygen species (ROS), Free Radicals (FR), Signaling pathways, redox-homeostasis.

1. INTRODUCTION

Reactive oxygen species (ROS) produced in eukaryotic cells through aerobic metabolism have evolved as regulators of important signaling pathways. ROS, previously considered mere byproducts of cellular respiration, are oxygen-containing molecules with high reactivity. They include hydroxyl (HO) and superoxide (O2) free radicals and nonradical molecules, such as hydrogen peroxide, which is less reactive than the majority of ROS but is able to reach any cellular compartment prior to being converted by peroxiredoxins and glutathione peroxidases into water and oxygen. In fact, hydrogen peroxide plays the role of a second messenger in some pathways that involve the transduction of extracellular signals and the control of gene expression, contributing to what is currently defined as redox signalling [1].

Oxidative stress is a state of disturbed balance between the production of reactive oxygen species (ROS) and the efficiency of antioxidants [2]. There are many biomarkers that are used for better understanding how oxidative stress is involved in cancer pathophysiology. This review focuses on 8-hidroxy-2-deoxyguanosine (8-OHdG) and antioxidative enzymes as biomarkers for measurement of oxidative stress in different types of cancer. This review also deals with the product of lipid peroxidation (LPO), malondialdehyde (MDA), across a variety of cancers. The use of detection of MDA levels, 8-OHdG, or antioxidant defense enzymes, has great diagnostic potential in

oncology. There are studies showing that low antioxidant status and increased oxidative stress levels are detected in cancer patients, even before oncology treatment starts [3].

ROS are produced in mitochondria (mainly via the electron transport chain, where ~1–2% of O2 is reduced to form superoxide anions), peroxisomes (through the β-oxidation of fatty acids) and the endoplasmic reticulum (through the oxidation of proteins). Oxidative phosphorylation in mitochondria involves four electron transporting complexes and a proton-translocating ATP synthase that direct electrons derived from the initial oxidation of NADPH and FADH2 along a multistep pathway that culminates in protons being pumped outside of mitochondria. ROS are also continuously generated by enzymatic reactions involving cyclooxygenases, NADPH oxidases, xanthine oxidases and lipoxygenases and through the iron-catalyzed Fenton reaction; indeed, it should be noted that NADPH oxidases have primarily evolved to produce ROS . Finally, ROS are generated after exposure to physical agents (ultraviolet rays and heat) and after chemotherapy and radiotherapy in cancer [4].

In recent years, the term "reactive oxygen species" (ROS) has been adopted to include molecules such as hydrogen peroxide, hypochlorous acid and singlet oxygen, which though, not radical in nature, are capable of radical transformation in the extra and intracellular environments. Whilst most ROS have extremely short half-lives, they can cause substantial tissue damage by initiating free radical chain reactions. Therefore the body contains a number of protective Anti-Oxidant mechanisms, whose specific role is to remove harmful oxidants (ROS), as soon as they form, or to repair the damage caused by ROS invivo [5].

The hydroxyl radical can stimulate a classic free radical chain reaction known as lipid peroxidation. When the hydroxyl radical is generated close to membrane phospholipids, it attacks the lipid side chains to form radical intermediates called peroxyl radicals (RO_2), hydrogen peroxide and lipid hydroperoxides. Arachadonic acid is a preferential target for the hydroxyl radical. Mechanisms of preventing hydroxyl radical induced tissue damage include the binding of transition metal ions by the "preventative antioxidants" albumin, caeruloplasmin, haptoglobin, lactoferrin, and transferrin. Scavengers of the hydroxyl radical include vitamin C, uric acid and thiols, such as reduced glutathione and cysteine [6].

Hydrogen peroxide plays a key role in free radical biochemistry because, in the presence of transition metal ions, it can easily break down to produce the hydroxyl radical (OH-), one of the most reactive and damaging FR species [7]. ROS have been associated with several diseases, playing a particular role in the female reproductive tract on ovaries, and even on embryos. ROS compounds are also implicated in the reproductive functions such as ovarian steroidogenesis, oocyte maturation, corpus luteal function and luteolysis, thus being related to female fertility [8].

2. BIOLOGICAL OUTCOMES OF OXIDATION BY ROS

It has been determined that each cell is exposed to ~1.5 × 105 oxidative hits per day. If, for any reason, ROS production increases or the number of scavenged ROS decreases, then cells experience a condition known as oxidative stress. Oxidative stress has been implicated in the pathophysiology of cancer: in fact, high levels of ROS generated by ongoing aerobic glycolysis followed by pyruvate oxidation in mitochondria (the Warburg effect), increase receptor and oncogene activity, and the stimulation of growth factor-dependent pathways or oxidizing enzymes induce genetic instability [9,10]. Moreover, excessive intracellular levels of ROS may damage lipids, proteins and DNA, and this ability has been exploited in a series of anticancer strategies.

2.1 REACTIVE OXYGEN SPECIES DAMAGE TO PROTEINS, DNA, AND LIPIDS

Proteins are mainly functional biomolecules that drive cellular activity. Oxidative damage to proteins may result in protein dysfunction. ROS radical that induces most damage to DNA is hydroxyl radical. DNA damage plays significant roles in mutations, genetic instability, and epigenetic changes. Many kinds of oncogenes and tumor suppressor genes can suffer damage by oxidative stress causing mutations which are known to induce cancer. Most studied DNA lesion is 8-OHdG. It is mutagenic, and many studies showed that levels of 8-OHdG is elevated in many different types of cancer. 8-OHdG can pair with both adenine and cytosine, but if mismatch adenine and guanine (A: G) is not repaired there will be a transversion of regular pairs adenine and timidine (A: T), cytosine and guanine (C: G). This mutation is commonly found in oncogenes and tumor suppressor genes. 8-OHdG is used as a biomarker for evaluation of oxidative stress [11].

Hydroxyl and hydroperoxyl radical are the most common ROS that can affect lipids. Oxidative damage to lipids causes LPO, which mainly localize in the cellular membrane resulting in a loss of membrane property. Their reactive end products can consequently damage other molecules. When exposed to low LPO, cell defense mechanisms lead to adaption, while a higher rate of LPO induces apoptosis or necrosis. Among many different aldehydes which can be formed as secondary products during LPO, MDA has been most extensively studied [12].

3. MECHANISMS OF CARCINOGENESIS CAUSED BY REACTIVE OXYGEN SPECIES

Continuous inflammation may lead to a preneoplastic event, In chronically inflamed cells, the secretion of a large amount of ROS/reactive nitrogen species(RNS) recruits more activated immune cells, which leads to the amplification of dysregulated processes and eventually to a preneoplastic condition. If the amount of cellular ROS/RNS produced is high enough to overcome endogenous antioxidant response, irreversible oxidative damage to nucleic acids, lipids, and proteins may cause genetic and/or epigenetic alterations leading to the dysregulation of oncogenes and tumor suppressor genes. The oxidative stress and chronic inflammation processes are tightly coupled and the failure to block these processes could result in genetic/epigenetic changes that drive the initiation of carcinogenesis [13]. Several studies have shown that oxidative stress affects several signaling pathways associated with cell proliferation.

Among them, the epidermal growth factor receptor signaling pathway can be mentioned, and key signaling proteins, such as the nuclear factor erythroid 2-related factor 2, RAS/RAF, the mitogen-activated protein kinases ERK1/2, and MEK, phosphatidylinositol 3-kinase, phospholipase C, and protein kinase C are affected by oxidative stress. Moreover, ROS alter the expression of the p53 suppressor gene that is a key factor in apoptosis. Thus, oxidative stress causes changes in gene expression, cell proliferation, and apoptosis and plays a significant role in tumor initiation and progression. Furthermore, it was revealed that ROS are causing hypomethylation of long interspersed nuclear element-1 (LINE-1) in bladder cancer cells suggesting that LINE-1 and oxidative stress have the oncogenic potential to drive tumorigenesis and cancer progression. In addition, expression of LINE-A-encoded protein (ORF1p) was experimentally induced by ROS bladder cancer cells [14].

Table 1: Formation of major oxidants

Oxidant	Formula	Equation
Superoxide anion	O_2-	$NADPH + 2O_2 \leftrightarrow NADP^+ + 2O_2^- + H^+$
		$2O2- + H+ \rightarrow O2 + H2O2$
Hydrogen peroxide	H2O2	Hypoxantine + $H_2O + O_2 \leftrightarrow$ xanthine + H2O2
		Xanthine + $H_2O + O_2 \leftrightarrow$uric acid+ H2O2
Hydroxyl radical	OH	$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH^- + OH$
Hypochlorous acid	HOCl	$H_2O_2 + Cl^- \rightarrow HOCl + H_2O$
Peroxyl radicals	ROO	$R + O_2 \rightarrow ROO$
Hydroperoxyl	HOO	$O_2^- + H_2O \leftrightarrow HOO\cdot + OH^-$

4. BIOLOGICAL TARGETS FOR FR AND ROS

There are five principal targets for FR and ROS in living cells:

(1) small organic biomolecules,

(2) proteins,

(3) nucleic acids,

(4) gene activation, and

(5) unsaturated fattyacids.

4.1 SMALL ORGANIC BIOMOLECULES

Such compounds include the following: vitamins (ascorbic acid, carotenoids, cx-tocopherol, quinones), carbohydrates (glucose, ribose), amino acids (hystidine, tryptophane, cysteine, methionine), uric acid, cholesterol, and small soluble peptides like glutathione. The reaction of FR and ROS with vitamins A, C, or E, quinones, glutathione, and uric acid usually terminates the radical reaction chain. Mucopolysaccharides such as hyaluronic acid can be depolymerized by FR/ROS. Carbohydrate affected by ROS leads to receptor alterations and reduced viscosity of synovial fluid [15].

4.2 PROTEINS

The sensitivity of a protein to FR/ROS depends on its amino acid composition and on the accessibility of such species to its more susceptible amino acids. Several amino acids undergo direct oxidative modification, which may affect their physiological role, by interaction with FR and ROS. In two human diseases associated with premature aging, Werner syndrome and progeria, oxidized proteins increase at a much higher rate than is normal. The final molecular and biochemical consequences of the interaction of FR/ROS and proteins includes changes in conformation, enzymatic activity or binding as well as receptor inactivation, increased susceptibility to proteases, and changes in immunogenicity. Consequences of ROS on protein results in increased turnover, membrane damage and cell injury [15].

4.3 NUCLEIC ACIDS

ROS alteration on DNA causes mutations leads to cell injury. The hydroperoxide of linoleic acid (1 3-hydroperoxylinoleic acid) was found to cause guanine-site-specific double-stranded DNA breakage [15].

4.4 GENE ACTIVATION

FR/ROS have been capable of activating transcription and Encode transcription factors participating in the modulation of cell growth, differentiation, and development and can activate apoptosis, a "programmed" form of cell death. FR/ROS have been implicated in the regulation of mammalian transcription factors such as nuclear factor (NF)-κB and activator protein-1 (AP-1) and of so-called "heat shock" (or stress protein) transcription factors (HSTF) [15].

5. THE ROS LANDSCAPE DURING CANCER DEVELOPMENT

Normal somatic cells require ROS for a number of cellular processes, such as immune defense mechanisms and obligate secondary signalling. In cancer cells, ROS levels are increased due to both environmental and internal mechanisms (Fig. 1)

The overall balance of ROS and the combined positive and deleterious effects of ROS all contribute to the final impact on cancer biology. This topic has been studied extensively in the literature and has been summarized in a number of excellent reviews. Firstly, environmental toxins linked to cancer have been shown to increase the amount of ROS species, for example smoking and UV. Also, as ROS are an inevitable by-product of metabolism, the increased metabolism sustaining increased proliferation in cancer cells results in increased ROS production. ROS are generated as a result of activation of a number of well-known oncogenes, for example Cmyc, Kras and BRCA1. ROS are also increased due to hypoxia induced in tumors when the vasculature can no longer adequately supply the growing lesion. Finally, alterations in signaling associated with tumorigenic transformation, such as altered integrin activation during cancer metastasis are also linked to increased ROS species production. All of these mechanisms combined result in a significant increase of cancer cell ROS levels around which there remains much controversy regarding the impact of ROS in the tumor.

In cancer cells ROS are usually considered oncogenic because they have been implicated in initiation, progression and metastasis of cancers however this is not clear cut, as ROS may also be crucial for tumor clearance. A clear mechanism by which ROS impact tumor development is by direct DNA damage during carcinogenic transformation such as catalyzing the modified DNA base 8-OHdG resulting in mutation. ROS catalysis of disulfide bond formation can impact a wide range of cellular proteins and lipid modifications which result in unstable, short lived lipids that ultimately propagate reactive species by secondary messenger breakdown

products. Finally, anoikis is the process by which normal cells induce apoptosis after the loss of cell matrix attachment. ROS have been shown to promote anoikis resistance and uncouple attachment and programmed cell death in cancer cells, thereby enabling metastasis. While a plethora of information support ROS mediate tumor development, data also supports that ROS removal is correlated with increased tumorigenesis. Antioxidant therapy, which should remove the cancer promoting ROS, paradoxically correlates with decreased survival in clinical trials. This may occur due to antioxidants decreasing ROS to a level supporting tumor proliferation and migration while minimizing some of the negative impacts of ROS in cancer cells, such as DNA damage. The obvious contradiction is a continuing area for resolution, and it is becoming more likely that ROS has both positive and negative roles in tumors. [16-18].

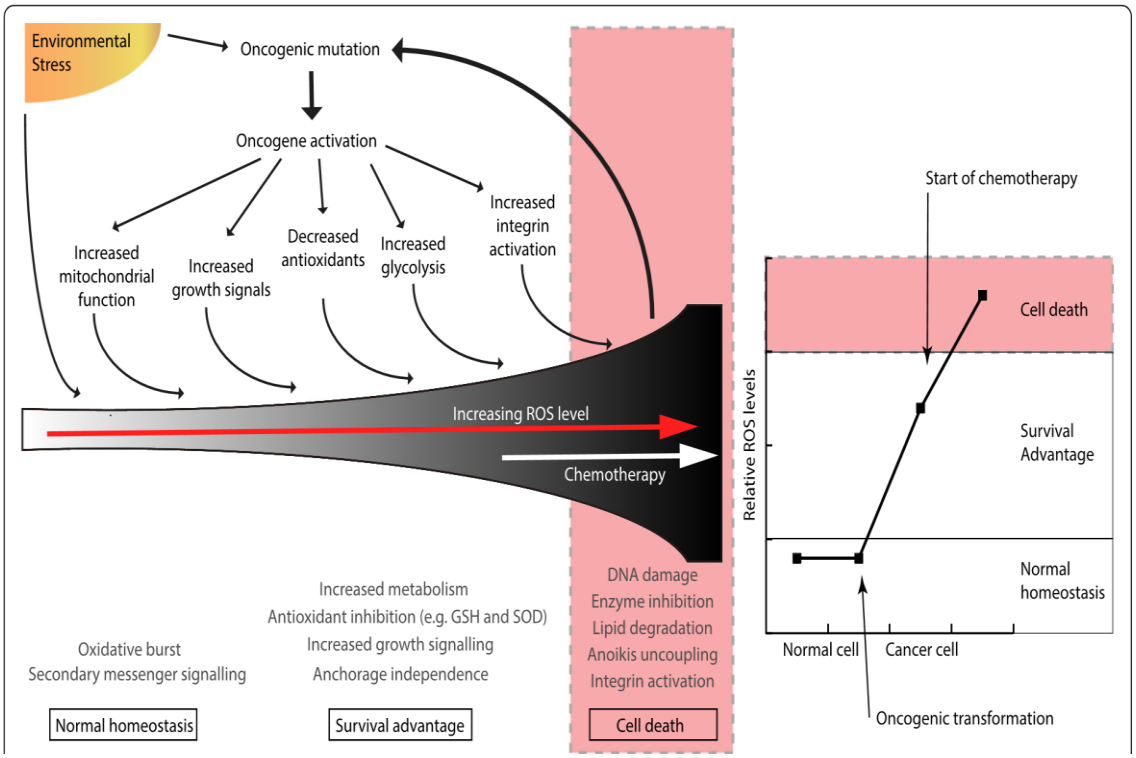

Fig. 1 Many factors contribute to increasing ROS levels in cancer, which in turn lead to a number of biological consequences. Overall, current theories suggest the culmination of increased ROS during cancer development confers a survival advantage, which is increased further during chemotherapy. Chemotherapy pushes ROS levels over a critical threshold proposed to induce biological processes leading to cell death, mostly via apoptosis.

6. EFFECTS OF ROS

Free radicals and reactive oxygen species (ROS) are essential to many normal biologic processes. At low concentrations, these free radicals stimulate the growth of fibroblasts and epithelial cells in culture, but at higher concentrations it may result in tissue injury [19].

A variety of biological processes, eg., antimicrobial defense, inflammation, carcinogenesis, radiation damage, photobiological effects, and aging, involve reactive oxygen species. Reactive oxygen species (ROS) play crucial roles in normal physiological processes including response to growth factors, the immune response, and apoptotic elimination of damaged cells but are also highly toxic and destructive when generated during the respiratory burst as it represents an important pathogenic mechanism for tissue damage and diseases associated with phagocytic insiltration. Excessive production of ROS resulting in oxidative stress has been implicated in the pathogenesis of many human diseases including periodontitis [20].

7. OXIDATIVE STRESS PROMOTES CANCER AND REVEALS ITS ACHILLES HEEL

Cancer is the second cause of death worldwide and is characterized by several hallmarks; cell transformation, genome instability, hyperproliferation, immortalization, angiogenesis, epithelial-mesenchymal transition (EMT) and metastasis, which are all influenced in several ways by intracellular ROS [21].

ROS as double-edged swords in cancer

Several noncancer cells associate with tumors: among these, cancer-associated fibroblasts (CAFs), particularly represented in the tumor microenvironment (TME), actively contribute to the regulation of tumor homeostasis, promoting tumor progression and the invasion of cancer cells. CAFs and ROS engage in two-way cross-talk: on the one hand, fibroblasts are targeted by ROS, which is able to convert them into active CAFs through the upregulation of HIF1α; on the other hand, CAFs are critical for the increase in ROS levels observed in cancer [22]. CAFs can also promote cancer growth and invasiveness, and both CAFS and ROS are linked through the increases in ROS-generated CAFs to which most cancers respond by increasing the expression of antioxidant genes [22]. (Fig-2)

However, a growing body of evidence supports the view that antioxidant activities are essential for tumorigenesis. It has been recently reported that targets of the Nrf2 gene, such as HMOX1, facilitate cancer development because they counteract the effect of oxidative stress in transformed cells. Moreover, established oncogenes such as K-RAS and c-MYC, which had been previously demonstrated to induce intracellular ROS56, have been recently shown to stabilize Nrf258. In this regard, mutations to NRF2 and its regulator KEAP1 have been found in cancer cells, supporting the supposition that antioxidant genes are pivotal in tumor progression. In fact, it has been found that the breast cancer susceptibility 1 (BRCA1) gene interacts with and induces Nrf2 expression with positive outcomes on cancer cell survival63. Interestingly, estrogen stimulation of breast cancer cells that do not express BRCA1 and, as a result, suffer from high intracellular ROS levels rescues NRF2 transcription, enhancing the survival of these cancer cells.

Additionally, FOXO transcription factors have recently been implicated in tumorigenesis: in fact, rhabdomyosarcomas present FOXO genes with a high percentage of mutations that render them insensitive to inhibition by AKT signaling. Moreover, increased intracellular levels of GSH are required for the initiation and progression of various types of cancer, and inhibitors of GR behave as anticancer drugs66, while high levels of NADPH boost the metastatic ability of melanoma cells, and protocols based on depletion of GSH (isothiocyanates and aziridine derivatives that bind GSH) or based on blocking the uptake of a rate limiting precursor of its synthesis (inhibitors of the cysteine/glutamate antiporter, XCT) greatly impact cancer cell survival. Specifically, sulfasalazine, an XCT inhibitor, appears useful in the treatment of pancreatic and small-cell lung cancer cells, while NOV-002, a glutathione disulfide mimetic that alters the GSSG/GSH ratio and induces oxidative stress, has been favorably used in patients with HER2-negative breast cancer. In addition, inhibitors of the enzyme glutaminase (GLS) that converts glutamine to glutamate, which is subsequently transformed to GSH via the glutamate–cysteine ligase complex, efficiently induce cancer cell death through dysregulation of their antioxidant system. As mentioned above, another central player in these redox systems is thioredoxin, which is reduced by NADPH to induce the transfer of electrons for use in DNA synthesis, signal transduction and redox regulation. Interestingly, auranofin, which functions as a thioredoxin inhibitor, has been used with beneficial effects in the treatment of head and neck carcinoma cell lines; prevention of this effect by the ROS scavenger N-acetylcysteine (NAC) confirms t he role of ROS in these cancers [23].

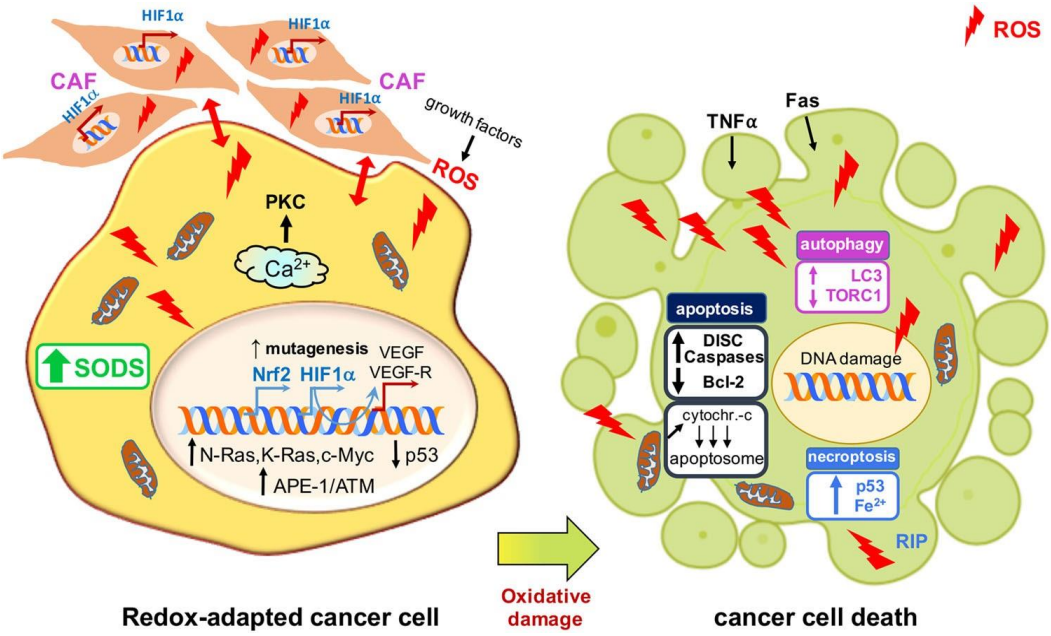

Fig. 2 The three types of programmed cell death induced by elevated ROS levels in cancer cells. ROS, in response to death-inducing ligands (TNFα and Fas), enhance the assembly of DISCs and the activation of effector caspases and reduce Bcl-2 activity or, as a consequence of increased permeability of mitochondrial PTPs, stimulate the intracytoplasmic release of cytochrome c, which interacts with Apaf-1 and procaspases and forms the apoptosome (apoptosis). ROS can also inhibit the negative regulators of autophagy (TORC1) and increase the formation of LC3-dependent autophagosomes (autophagy). Finally, high levels of ROS, induced by several receptor-interacting protein kinases (RIPs), increase p53 expression, which increases ROS levels via a mechanism that depends on intracellular iron (ferroptosis).

8. RESPONSES OF CANCER CELLS TO CHEMOTHERAPY-INDUCED ROS

Many questions regarding the role of ROS in chemotherapy remain, largely focusing on whether the ROS are a major reason for the induction of cell death, or just a side effect induced by the chemotherapy-induced mechanism of cell death. The role of ROS in cellular outcome during chemotherapy is more diverse than anticipated. The cell death triggered by most chemotherapeutics, such as cisplatin, doxorubicin and arsenic agents, involve both ROS-dependent and ROS-independent pathways. For example, the cytotoxic effect of cisplatin, one of the most effective and widely used anticancer chemotherapeutics, is thought to be mediated primarily by the generation of nuclear DNA adducts, which, if not repaired, interfere with DNA replication and cause DNA damage, which can induce cellular ROS generation [24].

However, the ability of cisplatin to induce nuclear DNA damage per se is not sufficient to explain its high degree of effectiveness for the treatment of a number of cancers. Recent work shows that exposure to cisplatin induces a mitochondrial-dependent ROS response that significantly enhances the cytotoxic effect caused by nuclear DNA damage in cancer cells [25].

ROS generation is independent of the amount of cisplatin-induced nuclear DNA damage and occurs in mitochondria as a consequence of protein synthesis impairment. Cellular responses to chemotherapy-induced ROS reflect the complex integration of ROS type, location, duration, and levels. For example, doxorubicin-induced mitochondrial ROS, particularly H2O2, are reportedly central to contribute to apoptosis and autophagy in cancer cells, while arsenic-induced NOX-generated ROS at the membrane are more often described as contributing cell death via necrosis and ferroptosis [26].

The dynamic sequence of some chemotherapy for cell readjustments may eventually promote the evolution of resilient and drug-resistant cells, which can repopulate the tumor and contribute to the emergence of a new heterogenic, more metastatic and drug-resistant tumor. Although it is questionable if mitochondrial ROS are

important contributors to drug resistance, its role and modulation of metabolic events may be central to the process and results [27].

9. CONCLUSION

Excess of everything is harmful such as no oxygen supply will prevent aerobes to live and similarly 100% oxygen can also lead to death to aerobes. Excessive ROS can cause chronic disease including cancer but inflammation cannot be controlled without ROS. Also excess or decrease of ROS can also affect fertility. ROS do not serve as simple biochemical entities, but as topological and temporal secondary messengers in cancer cells. Although most chemotherapeutics globally increase ROS to cytotoxic levels in targeting cancer cells, such ROS exposure may also inevitably reduce the efficacy of chemotherapy in the long term. To leverage cellular redox changes towards the development of a safe and effective therapeutic strategy necessitates experimental delineation of specific redox signaling pathways that are uniquely required by cancer cells to grow, survive or die. In this regard, our understanding of the complicated redox biology in cancer is still in its infancy. We anticipate that new delivery strategies, such as nanoparticle delivery systems, will be developed and applied in the clinic to further increase cellular ROS levels in cancer and reverse drug resistance. New chemotherapeutics can be engineered to target to specific cellular compartments for ROS generation and maintenance for a certain period of time.

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