Chapter from the book *Free Radicals and Diseases*

Role of Oxygen Free Radicals in Cancer Development and Treatment

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Additional information is available at the end of the chapter

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Abstract

It is well known that species derived from oxygen are cytotoxic and are involved in the etiology of cancer. Several carcinogens during metabolism exert their effect by producing reactive oxygen species (ROS). One of the consequences of oxidative damage to cellular DNA is mutated. It plays a vital role in the process of carcinogenesis (especially in the initiation and progression). The alters, including rearrangement of DNA sequence, base modification, DNA miscoding lesions, gene amplification, and the activation of oncogenes, could be implicated in the initiation stage of several cancers. Mitochondrial changes in the cancer cells are well known and as a result are respiratory injured. Mitochondrial dysfunction could lead to a low coupling efficiency of the mitochondrial electron transport chain (mETC), raising electron leakage and increased ROS formation. It has been documented that by reducing and inactivation of antioxidant system, the oxidative stress (OS) in cancer cells is higher. Cancer cells exhibit a higher oxidative stress level compared to normal cells, rendering tumor cells more vulnerable to raise ROS levels. Therefore, increasing ROS levels through redox modulation can be a strategy to selectively kill cancer cells but not normal cells. A promising anti-cancer method named "oxidation therapy" has been developed by causing cytotoxic oxidative stress for cancer therapy. In this chapter, we described the role of ROS as a double-edged sword in cancer development and treatment.

Keywords: cancer, reactive oxygen species, oxidation therapy, mitochondria

1. Introduction

Oxygen, while peremptory required for life, can also take part in the demolition of tissue and/or damage its ability to normal function. Reactive oxygen species (ROS), or low generally oxygen-free radicals (OFR), are produced by cellular metabolism in living cells. It has been appraised that the medium person has around 15,000 free radicals aggressive each cell per day. In an athlete, this number can be raised by almost 50%. In many instances, ROS are generated specifically to answer necessary biological functions, whereas in other cases, they indicate byproducts of metabolic processes. The production of ROS is an outcome of aerobic life. ROS represent a permanent source of attacks to our genetic material that can be either reduced or enhanced by environmental influences, hormonal and nutritional. Notwithstanding the antioxidant defense system of cell to neutralize oxidative injury from ROS, radical-linked damage of proteins and DNA has been suggested to play a major role in the development of diseases such as neurodegenerative disorders, arthritis, arteriosclerosis, cancer, and others diseases. All ROS have the potential to interact with cellular ingredients containing the deoxyribosyl backbone of DNA or DNA bases to generate strand breaks or damaged bases. ROS can also oxidize proteins or lipids afterward producing mediators that react with DNA by forming adducts. Many oxidative DNA damages are oxidative damage, and promutagenic is suggested to play an important role in the development of some cancers. Now, researchers do not know the correct role that injury by ROS plays a vital role in cancer development and its contributory role with other forms of genetic occurrences accelerating malignant progression and cell transformation. However, it is known that oxidative free radicals and oxidative stress (OS) could take part in the beginning of proliferation of cancerous cells. The result of OS at a given phase of carcinogenesis is immediately linked to the reactivity and the type of the radical species complicated. Nonenzymatic antioxidants accompanied by antioxidant enzymes are involved in ROS transformation. But antioxidant conservation versus ROS damages should be carried out with precaution inasmuch as the function of the antioxidant might indeed motivate the progression of cancer through the raised permanence of tumor cells. In inhibition of ROS-related cancer, the major duty looks to be decrease of exogenous and endogenous origins of ROS and the omission of carcinogens in environment. There is as well as the probability that cancer therapy could make utilization of the findings of ROS researches. Intracellular formation of ROS such as O_2^-, OH•, and H_2O_2 is associated with the suppression of cell proliferation. Similarly, production of oxidative stress in reply to several external motivations has been implicated in the activation of transcription factors and to the triggering of cell death. In this chapter, we run over how radical species induce DNA sequence changes, deletions, mutations, gene rearrangements, and alterations. These changes may lead to the beginning of apoptosis signaling leading to cell death, or to the inactivation of some tumor suppressor genes and/or the activation of several proto-oncogenes. The adjustment of gene expression by means of antioxidants, the redox state, and oxidants stays as a promising therapeutic procedure. Some anticarcinogenic agents have been demonstrated to inhibit ROS formation and oxidative DNA injury, inhibiting tumor development. As well as, new compounds vectors expressing radical-scavenging enzymes reduce apoptosis. Oxidative stress has been implicated in both the pathogenesis and apoptosis of cancer providing designed support for two concepts: free radical species may be raised in tumor cells and oxidant scavenging systems may be effective in cancer remedy. In addition, the production of ROS can be used therapeutically for cancer therapy. In this chapter, we described the role of ROS in cancer development and treatments.

2. Role of ROS in development of cancer

2.1. Mechanisms of free radical-induced DNA base modification and mutagenesis

It has been appraised that in one human cell is exposed to nearly 10^5 oxidative hits such as hydroxyl radical and other such species in a day. Constant change of genetic material resulting from these oxidative damage incidents demonstrates the initial step of carcinogenesis involved in aging and mutagenesis. The mechanisms such as specific and nonspecific repair play an important role in the removal of DNA changes by free radicals. It has been documented that base-excision is one method of repair DNA base damage. The alterations, including base deletion and substitution, play a role of in the DNA damage (misrepairing) and carcinogenesis. Mutagenic potential is directly equal to the number of oxidative DNA changes that flee repair. It is known that repair mechanisms decline with age and so DNA damages accumulate with age. The subsequence specificity of DNA lesion locates modifies the mutation frequency. The particular mechanism by OS which helps to the expansion of carcinogenesis is mainly unknown. However, two distinct mechanisms are supposed to act an important role in the expansion of oxidative and carcinogenesis. The modulation of gene expression by oxidative damage, can affect carcinogenesis. The epigenetic effects on gene expression could lead to the stimulation of proliferation and growth signals. Chromosomal rearrangements are speculated to result from loss of heterozygosity, alterations in gene expression, contributing to genetic amplifications and strand breakage misrepair, which in turn may advance neoplastic progression. Active oxygen species have been shown to motive poly(ADP ribosylation) and protein kinase pathways, thus affecting signal transduction pathways. This can lead to modulation of the expression of necessary genes for tumor promotion and proliferation. One previous study shows that RAS signal transduction pathways play a role in the mediating free radical signaling. Second, free radicals cause genetic changes, including chromosomal rearrangements and mutations, play a vital role in the beginning of carcinogenesis process. The oxidative DNA damage leads to a wide range of chromosomal abnormalities, inducing a wide cytotoxicity and stoppage of DNA duplication. Mutations can happen a failure to arrest in G1, diminishing their capacity to repair damaged DNA. This enhancement in replication errors can begin tumor suppressor gene inactivation and additional oncogene activation, eventually contributing to malignancy. Free radical-induced cytotoxicity may also help the beginning of carcinogenesis by promoting the clonal expansion of more resistant-initiated cells depleting the normal cell population, then increasing the possibility of mutation through incorrect replication or due to misrepair, while chromosomal rearrangements can end strand breakage misrepair. The initiation potential of oxidants might help to induce carcinogenesis as a result of their ability to cause DNA base alterations in tumor suppressor genes and certain oncogenes. Researches have shown that the radicals (especially hydroxyl radicals) are able to activate some oncogenes, including C-Raf-1 and K-ras. On the one hand, the activation launches through N-terminal deletions in these genes and the induction of DNA point mutations in GC base pairs. On the other hand, the base point mutations in CpG dinucleotides are also mostly found in specific tumor suppressor genes, including retinoblastoma and p53, which leading to their inactivation. It is shown that cells containing mutant or absent p53 are attacked by hydroxyl radical, which leading to a failure to arrest in G1 stage, diminishing their ability to repair damaged DNA. This enhancement in replication errors can initiate tumor suppressor gene inactivation and additional oncogene activation, eventually contributing to malignancy. Free radical-induced cytotoxicity may also contribute to the initiation of carcinogenesis by promoting the clonal expansion of more resistant-initiated cells, depleting the normal cell population, then increasing the likelihood of mutation [1].

2.2. Role of ROS in genotoxicity and DNA damage

ROS-caused DNA lesion may be characterized both structurally and chemically and displays a typical schema of modifications. The free radicals-induced DNA lesion was detected in the various cancer tissues. Most of these alterations can be modified in the in vitro situation.

The figures of DNA lesion induced through ROS experimentally include production of base-free sites, modification of all bases, frame shifts, deletions, DNA-protein cross-links, strand breaks, and chromosomal rearrangements. The Fenton chemistry mechanism is one of the reactions involved in DNA damage through the generation of hydroxyl radical form. It is well known that hydroxyl radical responds with all ingredients of the DNA molecule: the pyrimidine bases and purine. Regarding oxidative DNA lesion, main concern has centralized on repair to bases of DNA, with over 20 yields known, but only a few have been investigated with more details. Also, hydroxyl radical is capable to aggravate to twofold bonds of DNA bases at second-order rate constants of $3–10 \times 10^9\ M^{-1}\ s^{-1}$ that grabs an H-atom from the methyl group of thymine and each of the five carbon atoms of 2′ deoxyribose with rate constants of $2 \times 10^9\ M^{-1}\ s^{-1}$. Provided OH-adduct radicals of DNA bases are produced through additional reactions, the carbon-centered sugar radicals and allyl radical of thymine are formed from abstraction reactions. Peroxyl radicals are generated in environments full of oxygen through oxygen addition to OH-adduct radicals and also to carbon-centered radicals at diffusion controlled rates. Further reactions of base and sugar radicals generate a variety of sites, modified bases and sugars, protein of DNA, strand breaks, and cross-links.

Hydroxyl radical attacks to pyrimidines: to the C5 and C6 site of cytosine and thymine, generating C5-OH- and C6-OH-adduct radicals. Oxidative reactions of the C5-OH-adduct radicals of thymine and cytosine followed by release of proton (deprotonation) and addition of OH or water lead to the generation of glycols of cytosine and thymine. Oxygen adds to C5-OH-adduct radicals to produce 5-hydroxy-6-peroxyl radicals that may remove superoxide followed by reaction with water, giving rise to cytosine glycol and thymine glycol. Oxidation of the allyl radical of thymine generates 5-(hydroxymethyl) uracil (5-OHMeUra) and 5-formyluracil. In the lack of O_2, 6-hydroxy-5-hydropyrimidines and 5-hydroxy-6-hydroare generated by reduction of 6-OH- and 5-OH-adduct radicals of pyrimidines, respectively. Hydroxyl radical is as well as capable to attacks to purines giving rise to C4-OH-, C5-OH-, and C8-OH-adducts. One electron oxidation and one electron reduction of C8-OH-adduct radicals yield formamidopyrimidines and 8-hydroxypurines (7,8-dihydro-8-oxopurines). The most studied of these oxidized DNA products is 8-oxo-deoxyguanosine (8-oxo-dG), mainly because it is the most detectable. This base ornamentation falls out in nearly one in 10^5 guanidine residues in a healthy human cell. 8-Hydroxyguanine and 8-hydroxy-29-deoxyguanosine undergo keto-enol tautomerism, which favors the 6, 8-diketo form. Therefore, 8-OH-G is mostly named 8-oxoG or 8-oxy-7-hydroguanine. The nucleoside is thereupon named 8-oxo-7-hydro-29-deoxyguanosine or 8-oxo-dG so, 8-OH-dG and 8-oxo-dG are the identical compounds. Several methods for evaluating oxidative DNA damage exist; a favorite method engages enzymatic digestion of DNA, which releases 8-hydroxypurines for analysis by HPLC usually with electrochemical detector. Another method uses acidic hydrolysis of DNA, which releases the free base, because the glycosidic bond is cleaved by acid. Measurement is through HPLC or, transformation to volatile compounds, through GC-MS. The 8-oxoG damage is main due to it is relatively simply generated and is mutagenic, thus is a main indicator for the detection of carcinogenesis. The studies suggested mutagenic potential of 8-oxo-dG is supported by insertion of adenine opposite the lesions, or a loss of base pairing specificity, misreading of adjacent pyrimidines. Mutations that may arise due to the production of 8-oxo-dG involve GC→TA transversions. Former studies have shown that the mispairing of 8-oxo-dG with adenine appears to be feasible due to the energetically favored syn glycosidic conformation, while coupling with dG assumes the antiform. Studies demonstrated that factors such as day/night shift work, low meat intake, low BMI (<21.8), smoking, and hard physical labor significantly increased the 8-oxo-dG level, whereas medium physical exercise, such as sports, reduced its level. These data propose that the way of life might remarkably affect the level of oxidative lesion. The generation of 2-oxy-dA in the nucleotide unite is another mechanism of mutations. Studies have shown that the incorporation of 2 oxy-dA opposite G caused GC→TA transversions in the chromosomal lac I gene [2].

2.3. Lipid peroxidation and DNA damage

While major consideration has centralized on direct DNA lesion by oxygen free radicals because of the genetic outcomes of such lesion, reactive radical species may also induce damage to other cellular members. Phospholipids in the cell membrane are extremely susceptible to oxidative process and have been discovered to be repeated targets of radical-caused injury that supply them to be involved in free radical chain reactions. Several of the fatty acids are polyunsaturated, have a methylene group between two double bonds that predisposes the fatty acid more susceptible to oxidation. In addition, it is reported that polyunsaturated fatty acids (at high concentration) in phospholipids predisposes play a role of in the free radical chain reactions. Linoleic acid is the most common fatty acid in cell membranes. A set of arachidonic acid oxidation products termed isoprostanes is the best biomarker of lipid peroxidation that generally detected through GC-MS. The first products of unsaturated fatty acid oxidation are short-lived lipid hydroperoxides. When they react with metals, they produce some of products for example epoxides and aldehydes, which are themselves reactive. Malondialdehyde (MDA) is one of the important aldehyde products through lipid peroxidation. This product of lipid peroxidation is mutagenic and carcinogenic in mammalian cells and animals, respectively. MDA can react with DNA bases dA, dC, and dG, to form adducts, M_1A, M_1C, and M_1G. M_1G has been indicated in the several tissues (such as pancreas, liver, and breast). The M_1G content corresponds nearly to 6500 adducts in cell. Many researches have shown that M_1G is an electrophile in the genome. N_2-Oxo-propenyl-dG, as a yield of quantitative and rapid ring-opening of M_1G, is as well as electrophilic, but aims regions of DNA distinct from M_1G. Therefore, the conversion of M_1G and N_2-oxo-propenyl-dG may unfold varying reactive groups of DNA that could take part in the production of DNA-DNA interstrand cross-links or DNA-protein cross-links. It has been shown that hydroxypropanodeoxoguanosines (OH-PdGs) are exist in rodent and human liver DNA. It has been proposed that these propano adducts are interceded by the reaction of DNA with crotonaldehyde and acrolein, which in turn are products of lipid peroxidation. Crotonaldehyde and acrolein are mutagenic in mammalian cells and bacteria.

There is a few information associated with the repair of OH-PdGs. Studies show that PdG is a main substrate for the nucleotide cut repair complex of mammalian cells and *E. coli* and is identified and repaired through the mismatch repair system. Various exocyclic etheno DNA adducts increasing from lipid peroxidation have been found in DNA from healthy human volunteers. The most important involves etheno-dG, etheno-dC, and etheno-dA. Etheno-dC and etheno-dA are found to be strongly genotoxic but weakly mutagenic [3].

3. Role of ROS in treatment of cancer

3.1. Functions of ROS in the cancer cells

The findings from both *in vitro* and *in vivo* studies have shown that endogenous oxidative stress in cancer cells is higher than normal cells. ROS might function as a double-edged sword and as varied ROS levels could cause various biological responses. A low to moderate raise of ROS may help with the proliferation and survival of cells. But, at a high level, ROS may suppress the antioxidant capacity of the cell and start cell death (**Figure 1**). On the other hand, at the accumulation of ROS, these cells may be more sensitive than normal cells. The normal cells at under physiological status play an important role in maintaining redox homeostasis with a low level of basal ROS by controlling the balance between pro-oxidants and antioxidant capacity. The physiological conditions are affected by ROS inducers (such as hypoxia, metabolic defects, ER stress, and oncogenes) and ROS elimination (such as NRF2, glutathione,

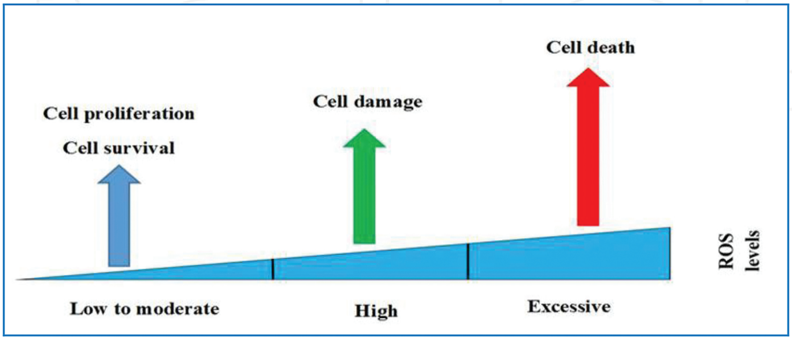

Figure 1. The interaction between different ROS levels in cancer cells. In cancer cells, ROS at low to moderate levels induces cell proliferation and cell survival, at high level induce cell damage and at an excessive level induce cell death.

NADPH, tumor suppressors, and dietary antioxidant agents) [4, 5]. The increase of ROS level of intracellular by activating signaling pathways in cancer cells represents that these cells very more vulnerable than normal cells to ROS-caused cell death. As a result, these cells in comparison to normal cells very more dependent on the capacity of the antioxidant system and more vulnerable to major oxidative stress induced through exogenous ROS-generating agents or compounds that inhibit the antioxidant system. This might constitute a biochemical basis to plan therapeutic strategies to selectively death cancer cells using ROS-moderated mechanisms [4–6].

As described above, the increase of ROS in cancer cells was induced several biological responses. These biological responses (including adaptation, increase in cellular proliferation, cell damage, and cell death) are likely to be dependent on the cellular genetic background, the types of the specific ROS involved, and the levels of ROS at the duration of the oxidative stress [7].

3.2. Antioxidant and oxidation pathways regulate ROS generation

Oxidative stress plays an important role in cell signaling as a sensor and regulator. It was reported that a lot of regulator agents have a considerable effect on up-expression and down-expression of antioxidant genes. In the following, we explain some of the major factors that act directly in the expression of antioxidant genes (**Figure 2**). On the other hand, good understanding of the particular pathways that are affected by these regulators is important before designing therapeutic approaches to the adjustment of ROS levels [4].

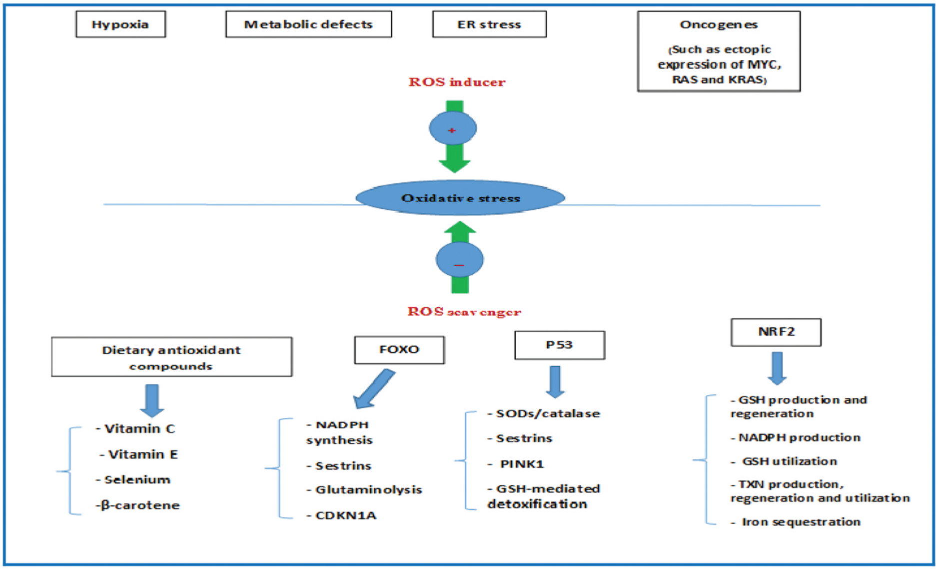

Figure 2. The regulation of ROS level by ROS inducers and ROS scavengers. The oxidative stress/ROS generation increase by agents such as, oncogenes, mitochondrial mutations, hypoxia, ER stress and decrease expression levels of antioxidant proteins that prevent increasing ROS level.

3.3. NRF2 (nuclear factor, erythroid-derived 2, like 2)

NRF2 is an important regulator of the antioxidant system and cellular stress responses in the several cancers. From the support on the function of Nrf2 target genes, one can easily conclude that activation of Nrf2 may protect cells from several stresses imposed through toxic exposure. Actually, it is recognized that NRF2 regulate various anti-oxidative stress responses and for detoxification reactions, its expression in the tissues increases [8, 9].

NRF2 adjust the common various different antioxidant pathways such as GSH production and regeneration, GSH utilization, NADPH production, thioredoxin (TXN) production, regeneration and utilization, Quinone detoxification and Iron sequestration (**Figure 2**). It is directly (through GSH metabolism) and indirectly (controlling free Fe(II) homeostasis) involved in ROS detoxification. NRF2 decreases the generation of harmful hydroxyl radicals from ROS by increasing the release of Fe(II) from haem molecules [4].

It was suggested that phytochemical compounds such as dietary and medicinal plants through the effect on NRF2 pathway played a key role in cancer therapy [8, 9].

3.4. FOXO (Forkhead box O) and p53

FOXO, as a transcription factors, are involved in different signaling pathways and play key roles in some physiological and pathological processes such as cancer. It could play and act as a self-regulatory mechanism, which protects cells from an oxidative damages, via keep in good condition a balance of ROS and antioxidant productions.

FOXO and p53 (as a tumor suppressor) have a key role in inhibiting oxidative stress process through inducing antioxidant gene expression [4]. It was reported that an increase of ROS level leads to up-regulation of anti-oxidative proteins, such as MnSOD and catalase through FOXO3a- and FOXO1 [10]. The p53, as a final transcription factor, has an important role in regulating antioxidant gene expression is p53 and a double-edged (as a pro- and antioxidant) role in ROS controlling. The p53 and FOXO play a role of the regulate antioxidant pathways such as SODs/catalase, PTEN-induced putative kinase 1 (PINK1), NADPH synthesis, and sestrins [4].

3.5. Hypoxia and hypoglycemia

Hypoxic conditions caused by the imbalance between intake and oxygen consumption [4]. The production of ROS through the mitochondrial complex I and III, xanthine oxidase, and NADPH oxidase related to hypoxia is recognized as one of the most harmful causes of oxidative process. Some studies have shown that hypoxia condition-caused superoxide generation occurs through the activation of NADPH oxidase placed in the cell membrane and under moderate condition, NO is generated in mitochondria. Studies suggested that hypoxia-induced loss in mitochondria membrane potential and this and this event is related to raising ROS [11].

Studies have shown that the mitochondria complex III (at the Qo site) at during the transfer of electrons from ubisemiquinone to molecular oxygen is the main source for ROS generation under hypoxia. In addition, it has shown that activation the transcription factor hypoxia-inducible factor (HIF) dependent on ROS level. HIFs regulate physiological responses to hypoxia, such as pathophysiological processes (especially in cancer) [4].

It was reported that, there is a relation between an increase in the level of H_2O_2 generation of mitochondria and an increase in susceptible cancer cells to apoptosis. This susceptibility caused cytotoxicity and also to oxidative stress-induced apoptosis when compared to normal cells [12].

3.6. Oncogenes

The oncogenes such as RAS, c-MYC, and Bcr/Abl, as a ROS-generators, are common agents that induce increase ROS levels in cancer cells by alteration in balance between pro-oxidant and anti-oxidant systems [11]. In the cancer cells, oxidative stress (due to increase ROS level and decrease antioxidant level) is higher than normal cells. At a time when equivalent levels of oxidative stress are added by the administration of exogenous ROS-inducing agents, oxidative stress levels in cancer cells but not normal cells can readily over the threshold of cell death. Hence, cancer cells in compared normal cells are expected to be more vulnerable to cell damage caused by ROS-inducing agents and this vulnerability can be exploited to selectively kill these cells [11].

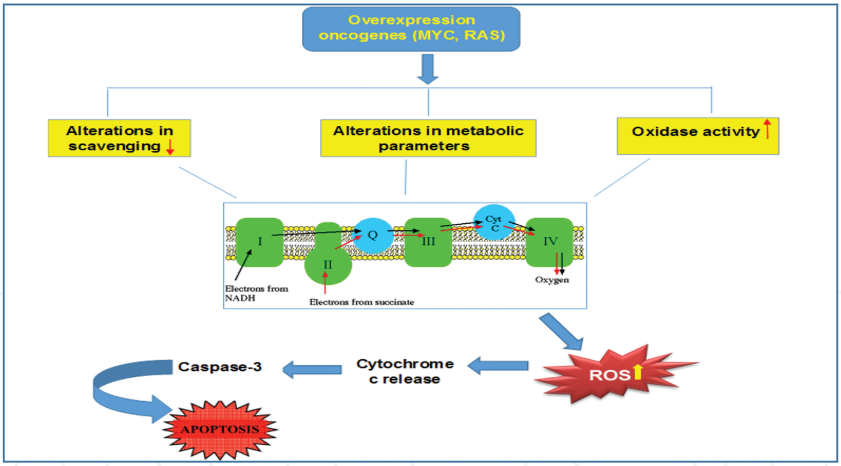

Figure 3. A model for increase ROS and apoptosis signaling by oncogenes such as c-MYC.

The activation of MYC in cancer cells leads to an elevating in intracellular ROS through mechanisms such as changes in scavenging process, metabolic rate, and eventual activation of intracellular oxidases (**Figure 3**). The previous investigations show that the increased expression of c-MYC and E2F1 induces accumulation of ROS and increases ROS by c-MYC and E2F1 sensitizes host cells to apoptosis.

Hypoxia, on the one hand, as a ROS inducer, can also directly induce the raised expression of oncogenes such as MYC and RAS through HIF-2α. On the other hand, MYC increases mitochondrial biogenesis, which adds to the raised ROS generation under hypoxic conditions and the raised mitochondrial ROS generation elevate the oxidative stress process [12].

It has been suggested that RAS, p53, and c-MYC through the mitochondria to regulate ROS generation, thereby affecting apoptosis. RAS in K-Ras-transformed fibroblast cell under the condition that glucose deprivation induces apoptosis through changes in mitochondrial complex gene expression. It has also been reported that RAS in p66SHC overexpression condition in the transformed cells increase ROS generation through mitochondria and p53, MPTP opening and mitochondrial swelling could induce apoptosis (**Figure 3**) [12].

3.7. Mitochondria

In recent decades, several studies have shown that mitochondria organelle plays an important role in human health and disease. These studies led to the emergence of a new field of study named "mitochondrial medicine." In the mitochondria, the molecules that are located on or inside have been considered as an initial pharmacological target and a wide range of efforts are in progress to use these targets to develop targeted treatments for cancer.

It has been confirmed that these organelles are the principal intracellular source of ROS production in most tissues. The under physiological status approximately 2% the O_2 consumed is changed to ROS molecules. In the mitochondria, ROS generation (such as O_2^-) often occurs by complexes I and III respiratory chain. Studies have shown that under physiological condition, complex II respiratory chain could also be a main regulator of ROS generation from mitochondria [13].

In eukaryotic cells, the mitochondrion is an important organelle that plays a main role in several critical processes. The important role of mitochondria is mentioned in the physiology of cancer, such as in energy metabolism and cell cycle regulation. There is powerful documentary evidence to support the rationale for the expansion of anticancer strategies based on mitochondrial targets. This organelle is recognizing to play an important role in the apoptosis mechanism and initiate cell death through various mechanisms that comprise disrupting electron transport and energy metabolism in the respiratory chain, releasing agents or proteins (such as cytochrome c) that mediate apoptosis signaling, and changing the cellular redox status by ROS generation.

The therapeutic targeting of cancer cells based on mitochondria have often depended on the intrinsic various differences between mitochondria in normal and cancer cells, which allow for better options, manipulation different pathways, and destruction of these cancer cells. These differences are including bioenergetics change, disturbance of the mitochondrial DNA (mtDNA), and morphological and physiological changes in the cancer cell (**Table 1**). The mtDNA is one main target of ROS and the lack of sufficient protective histones surrounding the mtDNA in cancer cells makes the mtDNA more easily tending to ROS-caused DNA damage. In addition, various researches have shown that the mitochondria were different in cancer cells than in normal cells, such as grow faster, fewer and smaller, and also had the morphological transformed [13].

	Cancer cells	**Normal cell**
Bioenergetics process	Aerobic glycolysis condition "Warburg Effect"	Aerobic condition
Mitochondrial DNA (mtDNA)	mtDNA is mutated	mtDNA is normal
Morphological and physiological differences (shape and count)	Size and shape: smaller	Size and shape: larger
MMP level	Higher (~60 mV)	Lower
ROS level	Higher	Lower
Intracellular pH	Acidic	No acidic
Metabolic rates	Higher	Lower

Table 1. Some differences between cancer and normal cells such as bioenergetics process, MMP and ROS level, and morphological and physiological differences.

Today, several mitochondria targeted strategies for cancer therapy have been focused on the development of agents that manage increased the ROS generation in mitochondria from the cancer cells without effect on the normal cells. It has been shown that ROS generation in the mitochondria is evaluated through the rates of both mitochondria ROS (mtROS) disposal and production, and ROS levels in mitochondria are regulated by several agents, including mitochondria O_2 levels, mitochondrial membrane potential (MMP or $\Delta\psi$m), the metabolic condition of mitochondria, and other factors (**Figure 4**). A number of recent researches reveal the fact that mtROS at low to high levels act as several functions. That is it at low levels, involved in the hypoxia adaptation process, at moderate levels, involved in controlling inflammatory response, and at high level involved in regulating apoptosis signaling.

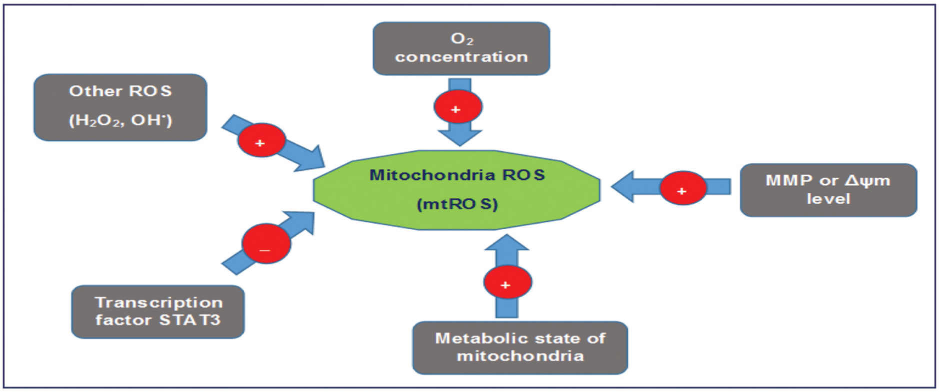

Figure 4. Adjustment of mitochondria ROS (mtROS) generation. Several agents such as MMP, the metabolic state of mitochondrial, O_2 level and STAT3 adjust the generation of mtROS.

As mentioned in the previous section, the increase of ROS level of intracellular by sever agents through activating signaling pathways in cancer cells represents that these cells are more vulnerable than normal cells to ROS-caused cell death. The results of the studies suggest that ROS (such as H_2O_2) could affect the extrinsic apoptosis pathway through changing the intracellular space. The up-regulation of receptor shows in various systems with increase ROS by exogenous ROS and ROS causing agents. It has also been found to sensitize cancer cells, but not normal cells to TRAIL-caused apoptosis.

Adenine nucleotide translocator (ANT), as an inner mitochondrial protein, is also a target of ROS regulation by integrity of its redox-sensitive cysteines, supplying an extra mechanism by which drug-caused ROS generation may activate mitochondrial apoptosis signaling. In addition, it has shown that $O_2\bullet^-$ plays a key role, on the one hand, to regulate the function of voltage-dependent anion channel (VDAC) to promote cytochrome c explosion. On the other hand, ROS also could regulate protein complexes inner place the mitochondrial electron transport chain (mETC), activate caspases-3 and initiate apoptosis signaling.

Today, agents that are used in the treatment of cancer through the mechanism of ROS generation are known as an important drug class. Some studies have shown that several mitochondria-targeted drugs have potency useful in selective cancer cell killing and no effect on normal cells in pre-clinical and clinical testing, such as ROS regulators. It has been shown that cancer cells in comparison with normal cells are more vulnerable to irreversible damage induced by stress oxidative and subsequent apoptosis. Researchers in previous studies have been used of the differences between the mitochondria between cancer and normal cells as a means to kill cancer cells by anti-cancer drugs. The phrase "mitocan" has been suggested to categorize mitochondria-targeted anticancer drugs, particularly those that caused increase ROS generation in mitochondria.

A promising anti-cancer strategy named "oxidation therapy" has been expanded by inducing cytotoxic oxidative stress in cancer cells and no effect on normal cells through several mechanisms for cancer treatment. Developing cancer therapies based on increasing further the high ROS level in cancer cells to a toxic level by the several mechanisms such as triggering ROS accumulation directly and inhibiting the antioxidant systems display powerful phenomenon of selectively killing cancer cells [6]. A number of drugs class have been recognized as increasing ROS production. It is well documented that some of chemotherapeutic agents can induce ROS generation through mitochondrial respiratory chain complexes in patients during cancer therapy. These compounds can be separated into various categories such as alkylating agents, anthracycline antibiotics, platinum compounds, mitotic inhibitors, antimetabolites, biological response modifiers, and hormone therapies.

3.8. Targeting mitochondrial respiratory chain

Arsenic trioxide (ATO) is used in the treatment of acute promyelocytic leukemia (APL). It was reported that ATO induces apoptosis signaling in several cancer cells such as lung, leukemia, and myeloma cancer through the induction of ROS. The mechanism by which ATO cause increased ROS generation is not completely well known. The most recent investigations indicated that ATO can impair the function of respiratory chain in the mitochondria, leading to increased generation of superoxide, likely by causing leakage of electrons from the mitochondrial respiratory chain complexes (**Figure 5**). On the other hand, ATO could be used in mixture with several anticancer drugs, which play a role through increasing ROS production.

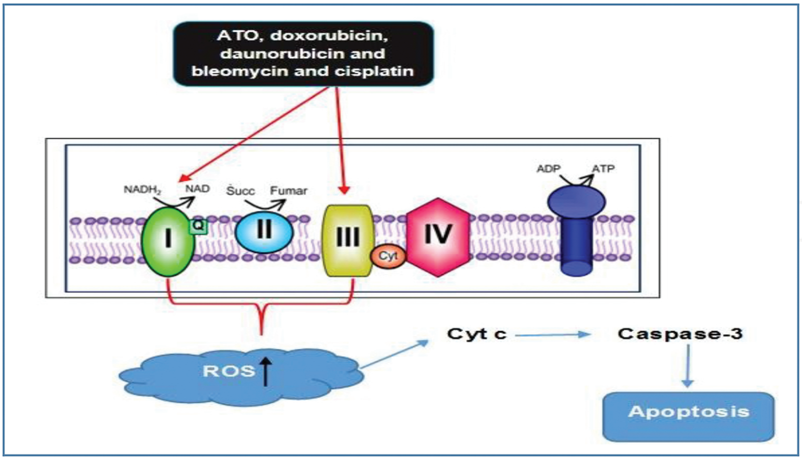

Figure 5. Mechanism of ROS generation and apoptosis induction by the ATO, doxorubicin, daunorubicin and bleomycin (anthracycline antibiotics) and cisplatin. These drugs, leading to increased generation of ROS, likely by causing leakage of electrons from the mitochondrial respiratory chain complexes.

The doxorubicin, daunorubicin, and bleomycin are an anthracycline antibiotics, cisplatin is a platinum compound, and amitriptyline as a tricyclic antidepressant are used in the treatment of several types of cancer. The mechanism of doxorubicin bleomycin, cisplatin, and amitriptyline in the ROS production is the same of ATO. These drugs with impair the function of respiratory chain in the mitochondria, leading to increase generation of superoxide. These compounds due to this mechanism (ROS generation) are used for the treatment of several types of cancers (**Figure 5**).

Studies have shown that other drugs such as dequalinium chloride (preclinical) and metformin (preclinical and clinical, Phase I) through inhibition of mitochondrial complex 1 have the ability to produce ROS.

3.9. Targeting VDACs

VDACs, also known as mitochondrial porins, show high similarity between some animals (especially mice) and humans. VDAC play an important role in the cell, such as regulating mitochondrial shape and structural changes, regulating apoptosis signaling, regulating ATP and calcium transport. Several studies have demonstrated the role of VDAC in the regulation of apoptosis signaling and VDAC is being studied as a cancer-specific target.

Erastin (Phase I/II) and Lanperasone (FDA-approved), as a modified form of tolperisone, down-regulate mitochondrial VDACs and alter the mitochondrial membrane permeability induce increase ROS generation. As has been mentioned, these drugs promote ROS production through the disturbance of VDAC and cause non-apoptotic form of cell death in KRAS.

3.10. Targeting NOX

Several studies have shown that some drugs and agents including, paclitaxel (taxol), ionizing radiation, niclosamide, AGX-891, AG-221 with effect on NOX induces ROS generation. Taxol is a mitotic inhibitory drug and it has been shown that induced ROS production/accumulation in the cell. The recent results from both *in vitro* and *in vivo* studies have shown that this drug causes the translocation of Rac1, which favorably regulates the activity of NOX, thereby furthering ROS (H_2O_2) production. It was reported that taxol can raise the levels of ROS in the extracellular and subsequently induced cancer cell death resulting in the release of cytochrome *c* from the mitochondria.

3.11. Targeting p53

P53 act as a transcription factor to regulate the expression of many pro-oxidant genes. The 5-fluorouracil (5-FU) is an antimetabolites and pyrimidine analog. It is used for therapies for several types of cancers, such as gastrointestinal, colon, rectal, and head and neck cancer, through inducing intracellular increase in superoxide. The mechanism by which 5-FU cause increased ROS generation from mitochondria is through a p53-dependent pathway [4]. The level of ROS production is different among these compounds and thus that anthracyclines (such as, doxorubicin), alkylating agents (such as, cyclophosphamide), platinum complexes are considered as the highest generation of ROS and taxanes, vinca alkaloids, and nucleotide/nucleoside analogs such as 5-FU as the lowest generation of ROS.

3.12. Targeting antioxidant system

It has been confirmed that GSH, catalase, and thioredoxin (TXN), as an antioxidant system, play a main role in equivalent pro-oxidant/antioxidant system through the scavenging various types of ROS. The two pathways GSH through enzymes such as GPX and GST and catalase can act directly on scavenging ROS in cells. For that reason, oxidative stress can be promoted with methods based on the loss of the reduced GSH storage and other antioxidant sources. A number of drugs class have been recognized as increasing oxidative stress process and ROS generation through targeting antioxidant system [4, 5].

These drugs are, including buthionine sulfoximine (BSO), imexon, phenylethyl isothiocyanate, mangafodipir, 2-methoxyestradiol, tetrathiomolybdate (ATN-224), and auranofin, used in the treatment of various types of cancer. For example, BSO through inhibition of the antioxidant system (especially GSH) in cancer cells (such as ovarian and breast cancers) can induce an accumulation of ROS due to the high basal ROS output in ovarian and breast cancers, and initiate cell death [4, 5]. Other studies have shown that imexon through decrease GSH pool and subsequently increase the production of ROS and decrease mitochondria function was induced apoptosis. Other studies have shown that some other drugs, including ascorbic acid and diethylmaleate are able effects on GSH (GSH depletion). One the other hand, mercaptosuccinic acid, aminotriazol, and 2-Methoxyoestradiol were able to inhibit of GPx, catalase, and SOD, respectively, and thereby increase ROS production [7].

4. Conclusion

Cancer is a multistage disease including initiation, promotion, and progression. The increased ROS causes DNA damage, which may lead to DNA damage or gene mutation, resulting in the progression of cancer. Increased generation of ROS and an altered redox status have observed in cancer cells, and investigations suggest that this biochemical property of cancer cells can be exploited for cancer therapy. For treatment of cancer, since high levels of ROS can induce cell death, treatment of radiation, chemotherapy, and molecule compounds all can increase the level of intracellular ROS to induce cancer cell death and apoptosis. The increased intracellular ROS levels could make cancer cells more vulnerable than normal cells to oxidative stress-induced cell death.

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