

Free radicals and antioxidants in normal versus cancerous cells — An overview

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Oxygen is vital for aerobic processes of metabolism and respiration- It has been also implicated in many diseases and degenerative conditions. Free radicals formed from reactive oxygen and nitrogen species act as key players in the initiation and progression of tumor cells and enhance their metastatic potential. The imbalance in the formation and use of free radicals in the tissue creates oxidative stress. Inadequacy in normal cells antioxidant defense system or excessive free radical formation or even both can cause the cell to experience the oxidative stress. This review outlines the involvement of free radicals in different aspects of cancer, from prevention to initiation, progression, treatment and to reduce morbidity and mortality.

Keywords: Cancer therapy, Reactive oxygen species (ROS), Superoxides, Xenobiotic

Introduction

Human life on earth is possible due to the presence of oxygen which is essential for cellular activities. Out of the total inhaled oxygen around 5% is converted to Reactive Oxygen Species (ROS) by the univalent reduction of oxygen. This poisonous property of O₂ is disclosed by the Gershan's free radical theory of oxygen toxicity in 1954. According to this theory the oxygen toxicity is due to incomplete reduction of oxygen. It reveals the dual role of ROS and RNS (Reactive Nitrogen Species). Further, it clarifies that ROS and RNS have both beneficial and harmful effects in accordance with their concentration in the body^{1,2}.

At the low and moderate concentrations, ROS shows beneficial effect by participating in the physiological mechanisms, Such as cellular defense against infectious agents, cellular signaling, induction of mutagenic response, electron transport chain, *etc.* On the other hand, the free radicals at high concentration, show deleterious effect due to imbalance between the production of free radicals and cellular antioxidants which creates oxidative stress³. In the biological systems, oxidative stress is due to the excess production of ROS/RNS as well as deficiency

of enzymatic and non-enzymatic antioxidant. The redox regulation mechanism of the living organisms creates the equilibrium between the beneficial and deleterious effect of the free radicals which is essential for the survival. This redox regulation mechanism forms a redox homeostasis, protects living organisms from various oxidative stress by managing the redox status *in vivo*⁴. This review deals with (i) The mechanism of formation of free radicals in respiring cells under aerobic conditions; (ii) The antioxidant systems involved in the scavenging process; (iii) Free radicals mediated damage to the cellular macromolecules (Lipids, Proteins, and DNA); (iv) The involvement of free radicals in different aspects of Cancer, from prevention to initiation, progression, treatment, and recovery; and (v) Controversial views of antioxidants in cancer therapy.

Free radicals

Free radicals are highly unstable, and reactive with short half-lives. Their hyper reactivity is due to the presence of one or more unpaired electrons in its outermost shell of their atoms. These unpaired electrons try to attain balance by binding with electrons of neighbouring atoms, giving rise to chain reactions⁵. Free radicals are produced from oxygen during various cellular metabolisms and for a variety

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of tasks such as signaling, metabolizing xenobiotic, initiating apoptosis, stimulation of antioxidants, repair process. Hence, its production in an animal cell is inevitable⁶. Increase in free radical level leads to oxidative stress. Increased number of free radicals, in turn steals number of electrons from neighbouring, DNA, enzymes and cell membranes which affect their structure and composition, leads to cell damage. Free radicals are not only the byproducts of cellular processes but also introduced into our bodies exogenously by cigarette smoking, radiation, drinking alcohol, air and water pollution, certain gases and even sunlight. The major forms of free radicals present in our body are hydroxyl radicals and superoxides.

Hydroxyl free radicals are necessary for hydroxylation of lysine and proline amino acids to hydroxylysine and hydroxyproline, respectively, it is also necessary for collagen biosynthesis⁷. Defense mechanism against bacteria and virus, *via* macrophages and leucocytes, also contribute to the formation of free radicals. For normal metabolism of cell free radicals are needed, However, the presence of free radicals poses a risk of damage for large molecules such as nucleic acids, proteins, polysaccharides and lipids⁷⁻¹⁰.

The mitochondrion is the major site of the superoxide free radical ($O_2^{\bullet-}$) by the action of NADPH (Nicotinamide Adenine Dinucleotide Phosphate) oxidase enzyme¹¹. In dismutation, the reactive hydrogen peroxide (H_2O_2) is formed by the action of superoxide dismutase (SOD). It happens when the superoxide radical reacts with itself and forms oxygen and hydrogen peroxide⁶. Continuous reduction process transforms the hydrogen peroxide *via* the Fenton reaction into the hydroxyl free radical ($\bullet OH$) which is highly reactive, and finally, water is formed, mediated by the action of catalase (CAT) or glutathione peroxidase (GPx)^{6,12}. In some cases, oxygen molecule binds to a proton, another free radical is formed known as the hydro peroxide radical ($HO_2 \rightarrow \bullet$)¹³. Figure 1 depicts different reactive forms of oxygen^{6,14,15}

The free radicals in Reactive Nitrogen Species (RNS) are nitric oxide ($NO\bullet$), peroxyntirite ($ONOO^-$), nitrogendioxide ($NO_2\bullet$) and nitrite (NO_2^-). $NO\bullet$ is synthesized from a guanidine group of L-arginine by an enzyme of the nitric oxide synthetase (NOS) family. The formation of $ONOO^-$ takes place by the reaction of $NO\bullet$ with a molecule of $O_2\bullet$, which forms nitrogen dioxide ($NO_2\bullet$) as an

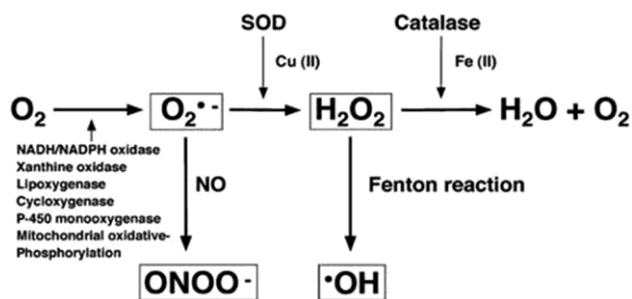


Fig. 1 — The different reactive forms of oxygen and antioxidants

intermediary. This intermediary reacts with $NO\bullet$ to finally generate anhydride nitrous (N_2O_3)^{13,15,16}.

The isoforms of NOS are neuronal (nNOS or NOSI), inducible (iNOS or NOSII), endothelial (eNOS or NOSIII) and mitochondrial (mtNOS). All these forms dependent on NADPH and calmodulin¹⁷. eNOS has a remarkable role in tumor development, as it modulates various tumor-related processes, such as apoptosis, angiogenesis, cell cycle, invasion, and metastasis¹⁸. Different biological actions of $NO\bullet$ are mediated by guanylcyclase (sGC) and cyclic guanosine monophosphate (cGMP). $NO\bullet$ spreads to nearby cells and readily enters the cytosol, where it activates sGC by binding to the “hem” component of iron on the porphyrin ring. As the concentration increases, its cytotoxic effects get activated which leads to inhibition of mitochondrial enzymes, including succinate, ubiquinone oxidoreductase, and aconitase, which are important in cell metabolism^{13,19}.

Antioxidants

Antioxidants are molecules which protect the body from damage caused by excess free radicals. Antioxidants neutralize these free radicals by sharing their extra electrons- so that they don't steal extra electrons from other vital organs of the cell. Free radical exposure of any organism leads to the development of defense mechanism in their body²⁰. As per the nature of an antioxidant molecule, it is mainly divided into two categories, Enzymatic and Non enzymatic antioxidants. Enzymatic antioxidant defense includes superoxide dismutase (SOD), glutathione peroxidase (GPx), catalase (CAT). Non-enzymatic antioxidants are mainly vitamin C (ascorbic acid), vitamin E (α -tocopherol), vitamin A (β carotene), and glutathione (GSH), flavonoids. There is a balance between both free radicals and the level of the antioxidants in the body under normal conditions. For the survival of the organism and its health, this balance is essential.

Glutathione disulphide (GSSG) is the oxidized form of glutathione. Glutathione is the major soluble antioxidant in cell compartments²¹. It is highly abundant in the cytosol (1-11 mM), nuclei (3-15 mM), and mitochondria (5-11 mM). GSH is synthesized in the cytosol by the sequential action of glutamate–cysteine ligase and glutathione synthetase. Its mitochondrial presence requires inner membrane transport. The dicarboxylate carrier protein and the 2-oxoglutarate carrier protein are the two mitochondrial electro neutral antiport carrier proteins shows the capacity to transport GSH.

Studies points that, externally added GSH is readily taken up against the concentration gradient by mitochondria, despite the ~8 mM GSH presents in the mitochondrial matrix²². The redox state of critical protein sulfhydryl's which are necessary for DNA repair and expression is maintained by GSH in the nucleus. Studies concluded that oxidized glutathione is accumulated inside the cells and the ratio of GSH/GSSG is a good measure of oxidative stress of an organism^{23,24}. A high concentration of GSSG may damage many enzymes oxidatively.

The different protective roles of glutathione against oxidative stress are:

- (i) It acts as a cofactor for many detoxifying enzymes against oxidative stress, *e.g.* Glutathione transferase, Glutathione peroxidase and others.
- (ii) Through plasma membrane, GSH participates in amino acid transport.
- (iii) It directly scavenges hydroxyl radicals and singlet oxygen while catalytic actions of glutathione peroxidase detoxify hydrogen peroxide and lipid peroxidases.
- (iv) GSH has the ability to regenerate antioxidants and return to their active forms, like reduction of tocopherol radical of vitamin E, reduction of semi dehydroascorbate to ascorbate²⁵.

The ability of glutathione to regenerate the antioxidant is associated with the redox state of glutathione disulphide-glutathione couple (GSSG/2GSH). Multiple roles of enzymatic and non-enzymatic antioxidants in rescue against oxidative stress are described in different studies²⁶⁻⁴².

Free radicals mediated damage to the cellular macromolecules

The essential cell components like lipids, proteins and nucleic acids are under the threat of high

concentrations of free radicals². These free radicals can alter intrinsic membrane properties like fluidity, ion transport, loss of enzyme activity, protein cross-linking, and inhibition of protein synthesis. All components of the DNA molecule are under the threat of hydroxyl radicals, damaging both the purine and pyrimidine bases and also the deoxy-ribose backbone, ultimately resulting in cell death⁵.

Lipid peroxidation

The oxidative modifications of lipids are catalase by reactive oxygen species⁴³. The hydrogen becomes more prone to abstraction in the presence of a double bond adjacent to a methylene group makes the C-H bond of polysaturated fatty acid (PUFA) weaker. Lipid peroxidation is initiated by OH•, alkoxy radicals (RO•), and peroxy radicals (ROO•), not by the O₂⁻ and H₂O₂⁴⁴. Peroxy radicals act as both reaction initiators as well as the products of lipid peroxidation, leads to a self-perpetuating process. Oxidation of the substrate is possible by the transfer of electrons when lipid peroxy radicals react with other lipids, proteins, and nucleic acids. Cell membranes, undergoes changes in membrane fluidity, permeability, and cellular metabolic functions during oxidative attack due to the presence of large amount of PUFA. The crucial aldehyde products of lipid peroxidation are malondialdehyde (MDA) and 4-hydroxy-2-nonenal (HNE). In bacterial and mammalian cells MDA is mutagenic and in rats it is carcinogenic. The major toxic product of lipid peroxidation is Hydroxynonenal, it is weakly mutagenic⁴⁵⁻⁴⁹.

DNA damages

Reactive Oxygen Species may induce oxidative damage to DNA, both nuclear and mitochondrial. The major types of damages observed are base modifications, deoxyribose oxidation, strand breakage, and DNA-protein cross-links. Out of all the ROS, hydroxyl radicals produce many products from the DNA base which mainly include C-8 hydroxylation of guanine to form 8-oxo-7, 8 dehydro-2 β -deoxyguanosine, a ring-opened product; 2, 6-diamino-4-hydroxy-5-formamimidopyrimidine, 8-OH-adenine, 2-OH-adenine, thymine glycol, cytosine glycol, *etc*⁵⁰. ROS also cause different mutagenic alterations in DNA. The most widely studied DNA lesion is the formation of 8-OH-G. Permanent modification of genetic material resulting from these “oxidative damage” incidents represents the first step involved in mutagenesis, carcinogenesis and ageing. Mutations

originating from selective modification of G:C sites indicates an oxidative attack on DNA by ROS. The action of 8-oxodeoxy-guanosine as a promutagen in altering the binding of methylase to the oligomer so as to inhibit methylation of adjacent cytosine has been reported in cases of cancer development^{51,52}.

ROS has shown activate mutations in human C-Ha-ras-1 proto-oncogene and induce mutation in the p53 tumor-suppressor gene⁵³. It also interferes with normal cell signaling, resulting in alteration of the gene expression and development of cancer by redox regulation of transcriptional factors/activator and/or by oxidatively modulating the protein kinase cascades. Early response or stress-response genes like *c-fos*, *c-jun*, *jun-B*, *jun-D*, *cmyc*, *erg-1*, and *hemoxygenase-1* are also produced by various ROS. A vital role is played by the early response proto-oncogenes in signal transduction, leading to cell proliferation and transformation⁵⁴. Abnormal components of the electron transport chain are formed by the oxidative damage of mitochondrial DNA which involves base modifications and strand break. This leads to the formation of more ROS through increased leakage of electrons and further cell damage, which promotes cancer and ageing⁵⁵.

Oxidative damage of proteins

In the mitochondrial electron transport chain, free radicals are produced which can initiate protein degradation. Oxidative protein damage may be brought about by metabolic processes which degrade a damaged protein to promote synthesis of a new protein. On the basis of further studies on aging processes, it has been found out that, catalytically inactive or less active, more thermo labile forms of enzyme accumulate in cells during aging and show a noticeable increase in the level of protein carbonyl content which is an index of metal-catalyzed oxidation of proteins⁵⁶⁻⁵⁸. Studies in which amino acids, simple peptides, and proteins were exposed to ionizing radiations under conditions where hydroxyl radicals or a mixture of hydroxyl/superoxide radicals are formed describes the mechanisms involved in the oxidation of proteins by ROS⁵⁹. By the action of ROS/RNS, the side chains of all amino acid residues particularly cysteine and methionine residues of proteins are susceptible to oxidation⁵⁹. Oxidation of cysteine residues may lead to the reversible formation of mixed disulphides between protein thiol groups (-SH) and low molecular weight thiols, in particular GSH (S-glutathionylation).

The amount of carbonyl groups, generated by many different mechanisms is a good measure of ROS-mediated protein oxidation. Numerous highly sensitive methods have been developed for the assay of protein carbonyl groups^{60,61}.

Levels of glyceraldehyde-3-P-dehydrogenase, aspartate aminotransferase, and phosphoglycerate kinase decline with age together with an increase in protein carbonyl content in human erythrocytes⁶². The carbonyl content of protein in rat hepatocytes also elevated with age along with a decline in the activities of glutamine synthetase and glucose-6-P-dehydrogenase, without any loss in the total enzyme protein⁶³. An age-related oxidative modification of human ceruloplasmin, a copper-containing protein in human plasma has also been reported⁶⁴. The mechanism of oxidative damage of proteins by ROS has been studied *in vitro* by different ways, generating these reactive species in solution which is non-specific (global) damage, or 'site-specifically' within the protein (localized) damage⁶⁵. Non-specific damage can be formed by generating activated oxygen species *in situ*, using either a radiation source (Co⁶⁰)- or using pulse radiolysis techniques; which lead to aggregation and fragmentation of the protein and modification of almost all the amino acid residues⁶⁶. On the other hand, localized or 'site-specific' damage, (which was extensively studied using glutamine synthetase as the model enzyme⁶⁵⁻⁶⁸) can occur when the ROS, such as •OH, are formed at putative metal-binding sites in proteins. When these sites are occupied by iron or copper, they can (in the presence of suitable reductants, O₂ – or ascorbate) react with H₂O₂ to generate highly reactive •OH which reacts preferably with specific amino acids present in the vicinity of the metal-binding site^{58,69}, thereby inducing specific damage (site-specific) that shows no gross structural modification. The concept of 'site-specificity' has been studied in detail by Halliwell, Gutteridge, and Stadtman^{65,68}. It means that (i) catalytic metal ions, such as iron or copper would be bound to the target molecule (protein, DNA, or cell membrane), and the •OH produced by O₂⁻ (or ascorbate) and H₂O₂ produced at the iron or copper binding site would then react preferentially with the target molecule; (ii) the damaging effect of •OH is observed at a specific site where catalytic ions are bound; and (iii) the defensive action of the free radical scavengers to remove •OH from the specific site decreases dramatically, since they are unable to access the microenvironment. Although tryptophan,

phenylalanine, and tyrosine residues of proteins are not the major sites of oxidation (as in the case of global damage) by the site-specific oxidative system; arginine, lysine, histidine, cysteine and proline are particularly sensitive to this oxidation, resulting in the formation of carbonyl derivatives^{67,70}.

Involvement of free radicals in different aspects of cancer

The studies reveal that free radicals induced oxidative stress is closely related to all aspects of cancer, from prevention, initiation, progression, treatment, and recovery. The human body is constantly under oxidative stress arising from both endogenous and exogenous origin. When such oxidative stress exceeds the capacity of the body system to maintain oxidation-reduction homeostasis, gene mutation may result, affecting the intracellular signal transduction and transcription factors directly or *via* antioxidants, leading to carcinogenesis.

In cancer prevention

The cellular mechanism of defense against the consequences of increased concentrations of reactive species (mainly ROS) is by antioxidants⁷¹. Proofs for prevention of carcinogenesis and increased life span by the antioxidant supplementation is documented by Kovacic and Jacintho in 2001⁷². For a correct cellular response, the concentration of antioxidant supplementation is very important, as a low concentration may have no effect while a high concentration causes a negative effect and act as pro-oxidant or the free radical⁷³. Oxidative stress leads to the over production of free radicals in the body which increases the chances for tumor initiation. Maintaining a proper equilibrium between free radicals and antioxidants prevent the body from all types of carcinomas. By donating their extra electrons antioxidant enzymes like SOD, Catalase and Glutathione peroxidase prevent oxidation and reduce the rate of chain initiation. They can also prevent oxidation by stabilizing transition metal radicals such as copper and iron^{74,75}. The intake of dietary antioxidants like vitamins E, C, and β -carotene is useful in preventing carcinogenesis⁷⁶. Different studies revealed that antioxidants can be used in the inhibition of inflammation in relation to the risk of carcinogenesis⁷⁷. The risk of formation of pro-oxidant is always associated with excess supplementation of antioxidants, for example, vitamin C⁷⁸. The type of antioxidant and its amount to be ingested for

obtaining a preventive effect in cancer remains under investigation.

In cancer initiation

Due to the presence of free radicals, cancer cells compared with normal cells forms a cellular redox imbalance, this redox imbalance thus related to oncogenic stimulation. The first step involved in mutagenesis, carcinogenesis, and ageing is "Oxidative damage" from free radicals resulting from the permanent modification of genetic material. In various tumors DNA mutation is a crucial step and elevated levels of oxidative DNA lesions have been noticed, which strongly implicating such damage in the etiology of cancer. More than 100 oxidized DNA products have been identified. Free radical based DNA damage includes single or double-stranded DNA breaks, purine, pyrimidine, or deoxyribose modifications, and DNA crosslinks. DNA damage may end up with arrest or induction of transcription, transduction pathways, replication errors and genomic instability, all of which are associated with carcinogenesis^{2,79}, 8-OH-Gformation is the most extensively studied DNA lesion. It is a potential biomarker of carcinogenesis as it is relatively easily formed and is mutagenic. DNA damage, mutations, and altered gene expressions, all are having a crucial role in the process of carcinogenesis. The participation of oxidants seems to be the common factor to all these events^{2,80,81}. It is clearly documented that inflammation and carcinogenesis are inter-related, numerous reports of cancer originating at sites of previous chronic inflammation are there in the literature⁸². Studies have been published on changes in morphology and in gene expression of mouse mammary epithelial cells after prolonged exposure to H₂O₂, which simulates chronic inflammation. In such oxidation conditions, a phenotypic cell conversion with similarities to malignant transformation was observed, including a fibroblastic morphology with intercellular spaces, implying a decrease in intercellular connections⁸³. In the mechanisms of carcinogenesis and ageing along with ROS, various redox metals, due to their ability to generate free radicals, or non-redox metals, due to their ability to bind to critical thiols have been participated⁸⁴⁻⁹⁰. Iron-mediated oxidative stress is considered to be a principal cause of human colorectal cancer⁸⁰. The second most important cause of lung cancer is occupational exposure to asbestos-containing about 30% (weight) of iron is associated with an increased risk of asbestosis⁸⁸.

Chances for oxidative stress and cancer increased in occupational exposure to cadmium⁸⁷. Directly cadmium is not capable of producing free radicals, but through indirect methods, it can cause free radical-induced damage the gene expression. Cadmium can cause activation of cellular protein kinases (protein kinase C), which results in enhanced phosphorylation of transcription factors and consequently lead to the transcriptional activation of target gene expression⁹⁰. It has been suggested that cadmium can also be implicated in the pathogenesis of human pancreatic cancer and renal carcinomas.

One of the potential lung carcinogen is Hexavalent chromium; Cr (VI)-induced cytotoxicity is associated with mitochondrial/lysosomal toxicity substantiated by the enhanced formation of free radicals⁸⁵.

Arsenic compounds are well established human carcinogens, which bind with SH groups and inhibiting various enzymes, including glutathione reductase⁸⁶. Researchers agree with the hypothesis that arsenic may act as a co-carcinogen not by causing cancer directly, but by allowing other factors, such as cigarette smoke or UV radiation, to cause DNA mutations more effectively^{89,90}. The effect of arsenic on p53 is not completely understood. The experimental results show both p53 dependent and p53 independent induction of apoptosis and also both, increased and decreased expression of the protein⁹⁰.

The oxidative DNA damage rate increased 35–50% by tobacco smoke, as estimated from the urinary excretion of 8-OH-G, or by 20–50%, estimated from the level of 8-OH-G in leukocytes⁹¹. The oxygen consumption, which is a main endogenous source of ROS, showed a close correlation with the 8-OH-G excretion rate, even though moderate exercise seems to have no current effect. Studies failed to show an influence on the oxidative DNA modification, by diet composition, including energy restriction and antioxidant supplements⁹². Along with ROS, reactive nitrogen species (RNS), such as peroxynitrites and nitrogen oxides have also been involved in DNA damage⁹³. During reaction with guanine, peroxynitrite has been shown to form 8-nitroguanine. This adduct has the potential to induce G:C→T:A trans versions due to its structure. While the stability of this lesion in DNA is low, in RNA, however, this nitrogen adduct is stable. The potential connection between 8-nitro guanine and the process of carcinogenesis is under research.

In cancer progression

In the cancer cells ROS act as messengers in cellular signaling transduction pathways, promote cellular growth and proliferation, and finally contribute to cancer development^{94,95}. ROS contribute to the uncontrolled cell proliferation and compromise the cell cycle regulatory function. Direct ROS interaction with specific receptors and modulation of the redox states of signaling molecules are the mechanisms responsible for stimulation of cell proliferation. Any alteration in the redox status of a signaling molecule may lead to stimulation of cell growth and cell proliferation. Oxidative modifications of redox-sensitive transcription factors may also be involved in ROS-mediated modulation of cell growth and cell survival^{94,96,97}. Most important characteristics of tumor cells are their increased ability to survive compared with the normal cells. ROS are reported to be tumorigenic by virtue of their ability to increase cell proliferation, survival, and cellular migration. ROS can develop DNA damage, leading to genetic lesions that initiate the tumorigenicity and subsequent tumor progression. Simultaneously, ROS is also capable for cellular senescence, cell death and can therefore, function as anti-tumorigenic agents. ROS promote tumor cell survival or act as anti-tumorigenic agents completely depends on the cell and tissues, the location of ROS production and the concentration of individual ROS.

The up-regulation of multiple intracellular signaling pathways, including cascades involved in survival, proliferation and cell cycle progression are required for uncontrolled tumor cell proliferation. The noticeable effects of oxidants on signaling pathways have been observed in the MAPK/AP-1 and NF- κ B pathways⁹⁸. Cell division needs tremendous energy requirements therefore, the production of metabolites from the energy generating reactions must be buffered to prevent oxidative damage and ultimately cell death. Hence the induction of redox sensitive pathways during tumor cell proliferation is essential⁹⁹.

Oxygen radicals may overcome tumor invasion and metastasis by increasing the rates of cell migration. Before converting into invasive carcinoma, epithelial cells undergo profound alterations in morphology and adhesive mode, resulting in a loss of normal epithelial polarization and differentiation and a switch to a more motile, invasive phenotype. For example, treatment of mammalian carcinoma cells with hydrogen peroxide

before intravenous injection into mice enhances lung metastasis formation, indicating that an important function for ROS is the seeding of metastatic tumor cells¹⁰⁰. This might be due to a lesser attachment of tumor cells to the basal lamina or otherwise due to more activity or expression of proteins that regulate cellular motility. Oxidative stress regulates the expression of intercellular adhesion protein-1 (ICAM-1), a cell surface protein in endothelial and epithelial cells, most likely because of the activation of NF- κ B. ICAM-1 together with IL-8 regulates the trans endothelial migration of neutrophils and has a potential function in tumor metastasis¹⁰¹.

An angiogenic response by the host blood vessels in solid tumors forms a new vascular network for the supply of nutrients and oxygen¹⁰². This neovascular response is partly responsible for tumor growth and metastatic spread^{103,104}. Angiogenesis in tumors is regulated by the so-called "angiogenic switch" which allows the transition from low invasive and poorly vascularized tumors to highly invasive and angiogenic tumors. Tumor cells express a set of molecules that initiate tumor vascularization to increase further in size.

Numerous cellular stress factors, including hypoxia, nutrient deprivation, and ROS are important stimuli of angiogenic signaling¹⁰⁵. Overexpression of RAS (rat sarcoma viral oncogene) has also been linked to vascularization of tumors¹⁰⁶. Transformation by RAS stabilizes HIF-1 α and up-regulates the transcription of vascular endothelial growth factor-A (VEGF-A). Chemical antioxidants inhibit the mitogenic activity of RAS, indicating that ROS participate directly in malignant transformation. ROS stabilize HIF-1 α protein and induce production of angiogenic factors by tumor finally.

In cancer treatment

A state of oxidative stress is created in the body when cancer is treated using anticancer drugs and radiation. Active oxygen triggers apoptosis via p53 and cytochrome release from mitochondria. Ionizing radiations-generates charged particles or electrons that carry the kinetic energy provided by photons (X rays, γ rays), falls directly on DNA producing breaks in phosphodiester bonds. This leads to around 30% of DNA damage^{107,108}. The remaining damage is created by the action of free radicals. In a process known as water "radiolysis" the \bullet OH, which has high biological relevance, is generated by the interaction of ionizing

radiation (*e.g.*, X- or gamma rays) with the water molecule. The deposition of energy from radiation also leads to the formation of hydrogen atoms, hydrated electrons, and other molecular products. It includes molecular hydrogen, hydrogen peroxide, and peroxy nitrite, compounds that generate DNA-damage like 8-hydroxyguanine (8-OH-Gua), 8-OH-dG, 8-oxoguanine and consequently single and double-strand DNA breaks¹⁰⁹. Free radicals are important factors in carcinogenesis^{110,111}. The \bullet OH has an extraordinarily short life in the human body, due to collisions with the different and abundant molecules in the biological environment. Scientists have come to the conclusion that radiotherapy generates the \bullet OH, which is the free radical most associated with cell death during the treatment of cancer. The NO \bullet appears to act as radio sensitizer under conditions of hypoxia, mimicking the effects of oxygen relating on radiation-induced DNA damage¹¹²⁻¹¹⁴. One of the major late complication after radiotherapy in breast cancer is the fibrosis that results from radiation-induced inflammatory responses¹¹⁵.

For triggering apoptosis within the cell, chemotherapy mainly depending on ROS. Chemotherapy agents are divided into different categories like alkylating agents (*e.g.*, cyclophosphamide, ifosfamide), anthracycline antibiotics which affect nucleic acids (*e.g.*, doxorubicin, bleomycin), platinum compounds (*e.g.*, cisplatin), mitotic inhibitors (*e.g.*, vincristine), antimetabolites (*e.g.*, 5-fluorouracil), camptothecin derivatives (*e.g.*, topotecan), biological response modifiers (*e.g.*, interferon) and hormone therapies (*e.g.*, tamoxifen). Among chemotherapeutic agents the level of ROS generation is different¹¹⁶. Agents that produce high levels of ROS include anthracyclines, platinum coordination complexes, alkylating agents, epipodophyllotoxins and camptothecins¹¹⁷. Nucleoside and nucleotide analogs, antifolates, taxanes and vinca alkaloids produce only low levels of ROS. Formation of superoxide radicals during apoptosis induced by chemotherapeutic agents, involving the release of cytochrome c from mitochondria, NADH dehydrogenase and reduced coenzyme Q10 divert electrons from the electron transport system (ETS) to oxygen¹¹⁷. Anthracyclines develops the highest levels of ROS. They divert electrons from the ETS of cardiac mitochondria, resulting in the formation of superoxide radicals in addition to generating ROS at other cellular sites. Doxorubicin (Adriamycin), the most studied

anthracycline, is one of the most effective chemotherapeutic agent used in several cancers¹¹⁷. The toxic effect of Doxorubicin is compared to its ability to react with cancer cell DNA. It can induce DNA damage, mainly through inhibition of DNA topoisomerase II enzyme after induction of double-strand DNA breaks resulting in covalent attachment between active site residues of topoisomerase II and 5' end of DNA. Doxorubicin also intercalates into DNA and alters helical torsion which also causes DNA damage. Its use is seriously limited by its acute and chronic toxic effects in the heart including cardiomyopathy and congestive heart failure¹¹⁷⁻¹¹⁹. Free radical generation and subsequent oxidative stress mediate its toxic effects. Studies showing that antioxidant supplementation in combination with Doxorubicin protects against oxidative injuries without diminishing its clinical efficacy¹¹⁹.

In recovery

Studies in head and neck and cervix carcinomas concluded that the pattern of a decreased level of antioxidants compared to the controls before radiotherapy, increasing oxidative stress during radiotherapy and again increased antioxidant level after radiotherapy is positively associated with survival in the patients^{120,121}.

The initial decrease in the antioxidant level of blood in carcinoma patients, compared to healthy control is due to the increased oxidative stress due to the tumor formation in the body. During treatment (radiotherapy and chemotherapy), administration of free radicals increase oxidative stress, the decline in the level of antioxidants may be due to both decreased dietary intake and increased utilization of antioxidants by free radicals produced from ionizing radiations. Studies reveal that increased oxidative stress is beneficial during therapy, it promotes the tumor cells destruction. In post radiotherapy, high level of antioxidants in blood was positively associated with the survival of the cancer patient. It directly indicates the recovery. Supplementation of antioxidants after treatment of malignancy may improve the outcome in patients.

According to them, antioxidant supplementation decreases the oxidative stress level especially in regard to protein damage¹²². It maintains the hemoglobin level of the body, and improve the efficacy of radiotherapy by supplying enough oxygen to the tumor. Quality of life of this group is also improved. But the risk lies in

the recurrence of the tumor after some period of time. Further long-term studies are required for authenticity.

Controversial views of antioxidant use in cancer therapy

Free radicals are used for the treatment of cancer in conventional therapies like chemotherapy and radiotherapy. The supplementation of antioxidants during the treatment always remains a matter of controversy. Since ROS plays a role in drug induced apoptosis, one might suspect that antioxidants may inhibit ROS and prevent apoptosis of cancer cells. There is an intense argument on the concurrent use of antioxidants with the conventional cancer treatments. Numerous patients have turned to such complementary treatments with antioxidants. Many oncologists have moved against antioxidants and warned their patients not to use them during conventional cancer therapy^{123,124}. The base of this argument was on the fact that radiation therapy and some chemotherapy drugs generate ROS and antioxidants may prevent cancer cells to be killed by ROS. As a result, the parallel use of supplemental antioxidants should be avoided during conventional cancer treatment¹²⁵. Not only in the scientific circles, but also in media as well this argument is still continuing¹²⁶. Literature has been reviewed, in order to find the result if there is any interference between the concurrent use of antioxidants and conventional cancer therapy¹²⁷. Except for three specific interferences (tangeretin with tamoxifen, NAC with doxorubicin and β -carotene with 5-fluorouracil), considerable data exists demonstrating increased effectiveness as well as decreased side effects of chemotherapeutic agents when given with antioxidants¹²². There are situations in which antioxidant supplementation would be undesirable for cancer patients, but the issue is more complicated than indicated. Even though many chemotherapy drugs induce the formation of free radicals, their anticancer effects not only depend on free radicals. Antioxidant may enhance the effects of chemotherapy, while preventing free radical induced side effects. Considerable existing data show increased effectiveness of many chemotherapeutic agents, as well as a decrease in toxic adverse effects, when given concurrently with antioxidants^{116,123,128-132}.

Oxidative stress interferes with cellular processes that are necessary for antineoplastic agents to exert their optimal cytotoxicity on cancer cells. Modest levels of oxidative stress have been shown to reduce

the cytotoxicity of anticancer drugs^{117,127}. Thus, it was claimed that the formation of ROS that occurs when anticancer drugs are administered may diminish the effectiveness of the treatment^{117,127,133}. In addition, since some side effects caused by antineoplastic agents appear to be prevented by certain antioxidants, administering these supplements during chemotherapy may diminish the development of side effects as well as improve the response to therapy¹¹⁷. Although limited clinical studies on the effect of antioxidants in cancer treatment are available. However, experimental studies showed that in cancer cells, antioxidant vitamins selectively induce apoptosis, prevent angiogenesis and metastatic spread, suggesting a potential role for antioxidants as adjuvants in cancer therapy¹³⁴.

Conclusion

Abundant knowledge of diverse mechanisms of oxidative stress involved in carcinogenesis and cancer therapies are available, but the current understanding is still not sufficient to cure cancer. The state of oxidative stress in carcinogenesis and tumor-bearing conditions is a complicated mechanism in which various complex interactions are involved. The deleterious effects of oxidative stress include injury to cells, induces gene mutation, involved in carcinogenesis by influencing intracellular signal transduction and transcription factors directly or indirectly via antioxidants. Research has been proven that free radicals can induce proliferation of cancer cells and protect them by increasing their adaptive response, thus leading to cancer progression and even metastatic diseases. Oxidative stress, a marker of tissue damage is found to increase with the progression of cancer. Oxidative stress can be measured by the levels of Lipid peroxidation, protein products and various antioxidants in the serum of cancer patients. Alternatively, free radicals are also recognized as the main mechanism of most of the conventional cancer therapies, leading cancer cells to apoptosis and hence to cancer regression. Antioxidants have also been shown to exhibit dichotomous effects when used as a supplement to cancer therapy. More research is needed for better understanding of the mechanisms and specific apoptotic pathways involved in ROS-induced cell death and to determine the most rational and effective combination of redox active agents. Further, it may reveal the missing mechanism of oxidative homeostasis in normal *vs.* malignant cells, and help

understanding the role of antioxidants along with conventional therapies in an integrative, personalized medical fight against cancer.

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