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Pharmacology Section

Understanding Redox Homeostasis and Its Role in Cancer

JAYSHREE SHRIRAM DAWANE, VIJAYA ANIL PANDIT

ABSTRACT

When a cell is damaged or altered without repair to its system, it usually dies. But if such damaged or unrepaired cells do not die and proliferate with uncontrolled growth; a mass of cancer cells develop. Cancer has a complex aetiology with multiple risk factors that involve the interplay between genetic and environmental influences. The redox dysregulation originates from metabolic alterations and it is dependent on mitogenic and survival signalling through reactive oxygen species. There is a delicate balance between the production and the destruction of reactive species; for this, we need to understand the role of redox homeostasis in cancer. So, the specific vulnerability of the malignant cells can be selectively targeted. This article will outline the redox homeostasis and the relationship between the disturbances in it and cancer.

Key Words: Redox state, Antioxidants

INTRODUCTION

Redox (reduction and oxidation) reactions are a family of reactions that are concerned with the transfer of electrons between molecules. Normally, the redox reactions ensure that the cells respond properly to endogenous and exogenous stimuli. During the cellular redox process, Reactive Species – Oxygen (ROS) and nitrogen (RNS)- are liberated as by-products. Lot of research has been directed to understand the beneficial and the deleterious effects of the reactive oxidizing molecules on the human body. An excess of these molecules is commonly referred to as 'Oxidative stress', which can lead to cell damage [1]. This may be one of the factors in the occurrence of many diseases like atherosclerosis, cataract, cardiovascular diseases, Alzheimer's, etc [2-6]. Efforts are also on to design strategies to overcome oxidative stress by using antioxidants.

Reactive oxidizing molecules are the molecules with a strong oxidizing property. Free radicals, also called as radicals, are atoms which have unpaired electrons in their outer orbits. Because of this property, they are highly reactive. There are other molecules which lack free electrons in their outer orbits but are highly reactive. These molecules are called as non free radicals.

Because of their high reactivities, the free and the non free radicals are collectively called as Reactive Species.

Under the normal physiologic conditions, moderate levels of these reactive molecules allow their incorporation into the structure of the macromolecules in a reversible fashion. Such reversible oxidative modifications of lipids, proteins, or DNA play a crucial role in the physiologic processes such as the differentiation, maturation, and trafficking of the intracellular vesicles.

ROS and RNS are formed under normal physiological conditions as the products of the cellular metabolism [7]. ROS can be (i) generated during UV light irradiation and by X-rays and gamma rays (ii) produced during metal catalyzed reactions (iii) present in the atmosphere as pollutants (iv) produced by neutrophils and macrophages during inflammation, and (iv) the by-products of mitochondrial catalyzed electron transport reactions, and various other mechanisms [8].

Though many reactive molecules are formed in the body, the Reactive Oxygen Species (ROS) and the Reactive Nitrogen Species (RNS) are the Important ones [Table/Fig-1]. A delicate balance between these 2 antagonistic effects is very important for the proper functioning of cells. This balance is termed as redox homeostasis. The delicate balance between the ROS generation and elimination is maintained by many complex mechanisms.

The reactive oxygen species can be beneficial, as they participate in various redox-regulatory mechanisms of the cells in order to protect the cells against oxidative stress and the maintenance of the cellular "redox homeostasis"[Table/Fig-1]. They also act as second messengers, controlling various normal physiological

	Free radicals	Particals, Not free radicals
ROS (Reactive oxygen species)	Superoxide, $O_2^{\bullet}$ -	Hydrogen Peroxide, H_2O_2 (Fenton´S Reaction)
	Hydroxyl Radical, $OH^{\bullet}$	Hypochlorous Acid, Hclo
	Peroxyl, $ROO^{\bullet}$	Ozone, O_3
	Alkoxyl, $RO^{\bullet}$	Singlet Oxygen, $^1O_2$
	Hydroperoxyl, $HO_2^{\bullet}$	
RNS (Reactive nitrogen species)	Nitrogen(II) Oxide, NO.	Nitrosyl, NO+
	Nitrogen(IV) Oxide, NO_2.	Nitrous Acid, HONO
		Nitogen(III) Oxide, N_2O_3
		Peroxynitrite, ONOO –
		Alkylperoxinitrite, ROONO

[Table/Fig-1]: Examples of reactive species [9].

functions of the organism [10].

The free radicals become deleterious when they are not eliminated by the endogenous systems. Oxidative stress represents an imbalance between the production of reactive species and a biological system's ability to readily detoxify the reactive intermediates or to repair the resulting damage. Detoxification of the reactive radicals occurs through antioxidants.

An excess of the reactive molecules cause severe and irreversible oxidative damages. This mini-review deals with the production of the reactive species and their physiological roles; type and location and the role of anti-oxidants along with the role of the reactive species and anti-oxidants in cancer.

Oxidative stress can be caused due to the depletion/reduced activity of the antioxidants or an increase in the reactive species [11].

ANTIOXIDANTS

The antioxidant compounds are exogenous or endogenous in nature. They prevent the generation of/ intercept and inactivate the already formed oxidants. Thus they block the chain of the reactions which is produced by these oxidants. An antioxidant is any substance, when present at low concentrations, significantly delays or inhibits an oxidation reaction'. The antioxidants are of two types, non-enzymatic and enzymatic [12].

TYPES

1) The enzymatic antioxidant defences include

Superoxide Dismutase (SOD), catalase (CAT), Glutathione Peroxidase (GPx) and glutathione reductase.

2) The non-enzymatic antioxidants are

a) Metabolic antioxidants- glutathione (GSH), Lipoid acid, L-arginine, coenzyme Q10, melatonin, uric acid, bilirubin, metal chelating proteins, transferrin, etc.

b) Nutrient antioxidants- ascorbic acid (Vitamin C), tocopherol (Vitamin E), carotenoids, trace metals (selenium, manganese,zinc) flavonoids,omega-6 fatty acids and other antioxidants. [Table/Fig-2] shows the various antioxidants with their locations and brief roles.

REDOX SIGNALLING AND REDOX HOMEOSTASIS

[Table/Fig-2,3 & 4] Redox signalling is the biochemical communication between the free radicals, Reactive Oxygen. Species (ROS), and other electronically activated species such as nitric oxide and other oxides of nitrogen, which act as biological messengers. Similarly, the modulation of the charge-transfer processes and the electronic conduction in the macromolecules is also redox signaling [14].

The redox state, like the pH or the osmotic pressure, represents the chemical characteristics of the intracellular environment. The free radicals and the reactive diamagnetic species which are derived from the radicals operate at low, but measurable concentrations in the cells. Each cell is characterized by a particular concentration of electrons (redox state) which is stored in many cellular constituents. The redox state of a cell and its oscillation determines the cellular functioning [15]. The term 'redox signalling' is used to describe a process in which the signal is delivered through redox reactions. Redox signalling requires that the steady state of the "redox balance" is disturbed, either by an increase in the ROS formation or by a decrease in the activity of the antioxidant system. The process of redox signalling is used for adapting the internal environment to the changes which occur extracellularly [7].

The essential cellular functions such as gene expression are influenced by the balance between the pro- and the antioxidant conditions. The number of reactive molecules is tightly controlled over a narrow range. The ability of the cell to maintain a condition of "steady state" or "redox homeostasis" is determined by the balance between the rate of its production and the rate of its removal by various antioxidants.

The intracellular "redox homeostasis" or "redox buffering" capacity is substantiated primarily by glutathione (GSH) and thioredoxin (TRX). Glutathione (2GSH/GSSG couple) represents the major cellular redox buffer and it therefore serves as an indicator of the redox environment of the cell [15,16].

The redox-mediated mechanisms in the regulation of the cellular processes

1. Transcriptional regulation
2. Direct oxidative modification
3. Regulation of the redox-sensitive interacting proteins
4. Regulation of the redox-sensitive modifying enzymes
5. Regulation of the protein turnover

The alterations in the redox homeostasis which are caused by exogenous stimuli or endogenous stress or both, can result in increased oxidative stress with elevated levels of cellular ROS. The

Location and Roles of antioxidants [13].

Antioxidants	Location/Sources	Role
Superoxide dismutase (SOD)	Cytosol, mitocondria, nucleus, plasma	Dismutation of superoxide to hydrogen peroxide (H_2O_2)
Catalase (CAT)	Peroxisomes	Dismutation of H_2O_2 to molecular oxygen and water
Glutathione peroxidase (GPx)	Cytosol, mitochondria	Reduction of H_2O_2 and other hydroperoxides, lipid peroxides, lipoxygenase products
Glutathione reductase (GR)	Cytosol, mitochondria	Reduction of low molecular weight disulfides
Glutathione (GSH)	A tripeptide is present in high concentrations in most eukyrotic cells. Present within the cytosol of cells and is the major intracellular non protein thiol compound	Substrate in GSH redox cycle, act as a reductant, reducing H_2O_2 directly to water with the formation of GSSG. It also reacts with superoxide anion,hydroxyl, and alkoxyl radical directly by a radical transfer process and inhibits tissue damage. GSH is capable of scavenging ROS directly or enzymatically via GPx

[Table/Fig-2]: Enzymatic Antioxidants

Uric acid)	Wide distribution	Uric acid is a powerful antioxidant and is a scavenger of singlet oxygen and radicals like superoxide anion, hydroxyl, and alkoxyl radical and binds transition metals.
Cysteine	Wide distribution	Cysteine is also a vital component for the synthesis of glutathione and can reduce organic compounds by donating e- from SH groups. N-acetyl-L-cysteine (NAC) is a derivative of cysteine act as glutathione precursor and Scavenges of H2O2 and peroxide
CoQ 10	Synthesized in human cells and also found in wheat bran, fish, meat.	It inhibit lipid peroxidation, reduces mitochondrial oxidative stress, and also able to recycle vitamin E.
Transferrin	It is a major iron transporting protein in the body	It bind free iron salts, which can leads to the generation of reactive oxygen species.
Lactoferin	It is a milk protein found extracellularlly	Similar action like transferring to helps in iron binding
Ceruloplasmin	A metal binging protein present extracellularlly	It is a copper binding protein. It catalyses the oxidation of Fe^{2}+ to Fe^{3}+ while oxygen is reduced to water
Bilirubin	Blood stream, tissue, plasma and extravascular place. It is Principal component of RBC.	It is an end product of heme catabolism, generally viewed as cytotoxic, lipid-soluble waste product. But at micromolar concentrations in vitro, efficiently scavenges peroxyl radicals and protects albumin-bound linoleic acid from peroxyl radical-induced oxidation.

[Table/Fig-3]: Metabolic Antioxidants

Vitamin E	Present in high concentrations in both cells and mitochondrial membranes. Found in amla , lemon, oranges, groundnut, oil, olive oil, palm,oil, cashew nuts,germinated pulses.	Direct scavenging of superoxide,hydroxyl radicals, upregulation of antioxidant enzymes, breaks lipid peroxidation chain reactions.
Vitamin C	ICF, ECF, and also found in lemon,oranges, olive oil, palm oil, cashew nuts, germinated pulses.	Cysteine is also a vital component for the synthesis of glutathione and can reduce organic compounds by donating e- from SH groups. N-acetyl-L-cysteine (NAC) is a derivative of cysteine act as glutathione precursor and Scavenges of H2O2 and peroxide
Carotenoids: α-carotene, β-carotene, γ-carotene, crocin, β-cryptoxanthin, lycopene, lutein, zeaxanthin, bixin, astaxanthin, capsorubin, canthaxanthin	apples, banana, berries, grapes, jackfruit, kiwi, lemon, mango, pineapple, orange, papaya, plum, watermelon, sweet, beet,potato, carrot, asparagus, brinjal, broccoli, brussels tomatoes, sprouts, spinach, cauliflower, corn, onions, cabbage, beans, pumpkin, cucumber, mushroom, chillies, red palm oil, Milk, yogurt, eggs and medicinal plants.	Scavenges superoxide, hydroxyl radicals, neutralize oxidants from stimulated neutrophils, regenerates vitamin E.

[Table/Fig-4]: Nutritional Antioxidants

ability to adapt to such ROS stress determines the overall fates of the cells [17].

Their exists a fine inter-relation between all antioxidants [Table/Fig-5].

THE PHYSIOLOGICAL ROLE OF THE REACTIVE SPECIES

Numerous physiological functions are controlled by the redox-responsive signalling pathways. The redox regulation involves the regulated production of Nitric Oxide (NO) or ROS by Nitric Oxide Synthases (NOS) or NAD(P)H oxidase respectively, and the effects of these compounds on the specific signalling cascades.

- $NO^{\bullet}$ is generated in the biological tissues by specific NOSs which exist in 3 isoforms-neuronal (nNOS), endothelial (eNOS) which is constitutive and inducible (iNOS) which is not constitutive and can be induced. iNOS is expressed in the macrophages and if it is stimulated by lipopolysaccharides or cytokines, it can liberate NO as a defence mechanism. The cytotoxic effects of' NO have been shown to be an important defence against parasitic fungi, protozoa, helminths and mycobacteria but not against the extracellular pathogens [18].

- **Their Role (ROS) in Phagocytosis-** Large amounts of superoxide radicals and other ROS can be produced by the neutrophils and the macrophages during the process of phagocytosis [Table/Fig-6].

- **Their role in the regulation of the cardiac and the vascular cell functioning-** The non phagocytic cells like- fibroblasts, vascular smooth muscle cells, cardiac myocytes and endothelial cells can produce ROS intracellularly by NAD(P)H oxidase to regulate the intracellular signalling. ROS has been mainly shown to play a role in the regulation of the cardiac and the vascular cell functioning.

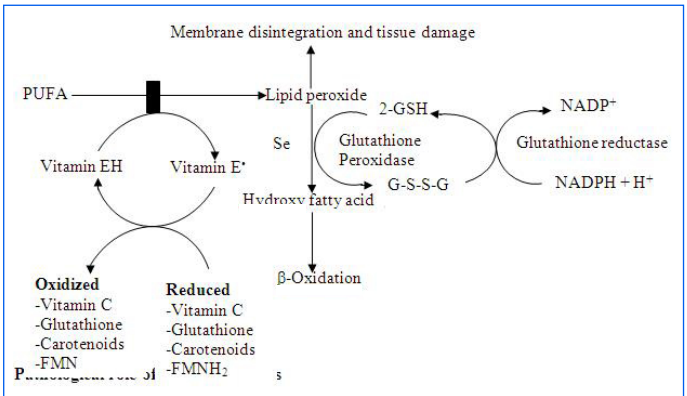
- **Their role in the inhibition of platelet adhesion-** H_2O_2 and $NO^{\bullet}$ which are produced in the endothelial cells activate the enzyme, guanylate cyclase (sGC), which in turn, converts GTP to cGMP. cGMP modulates the function of the protein kinases and the ion channels and it regulates the smooth muscle tone and inhibits the platelet adhesion [20].

- **Their role in the oxygen homeostasis-** This is done by tight regulation of the RBC mass and the respiratory ventilation. The changes in the O2 concentration are sensed by several ROS producing proteins and they are regulated by some hormones like erythropoietin, the Vascular Endothelial Growth Factor (VEGF) and the insulin-like growth factor (IGF-II).

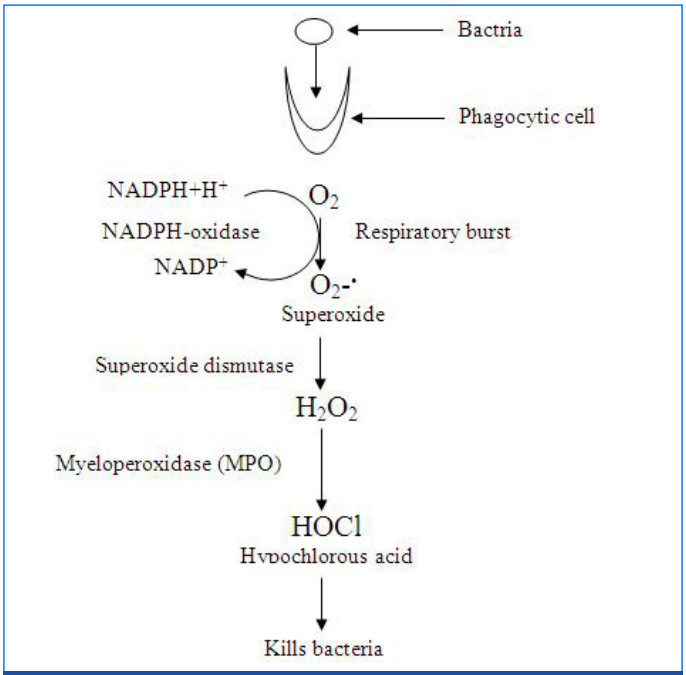
- **Their role in cell adhesion-** They play a role in the embryogenesis, wound repair, differentiation, etc. These processes are tightly redox regulated. The expression of the cell adhesion molecules are stimulated by various cytokines- Tissue Necrosis Factor (TNF), interleukin-1 (IL-1)and interleukin-1β.

- **Their role in the activation of the immune response-** Even small numbers of environmental pathogens can activate the immune response by involving the lymphocyte receptor for the antigens, the receptors for the co-stimulatory signals and various types of cytokines. The IL-2 production by the lymphocytes is increased by ROS [21,22].

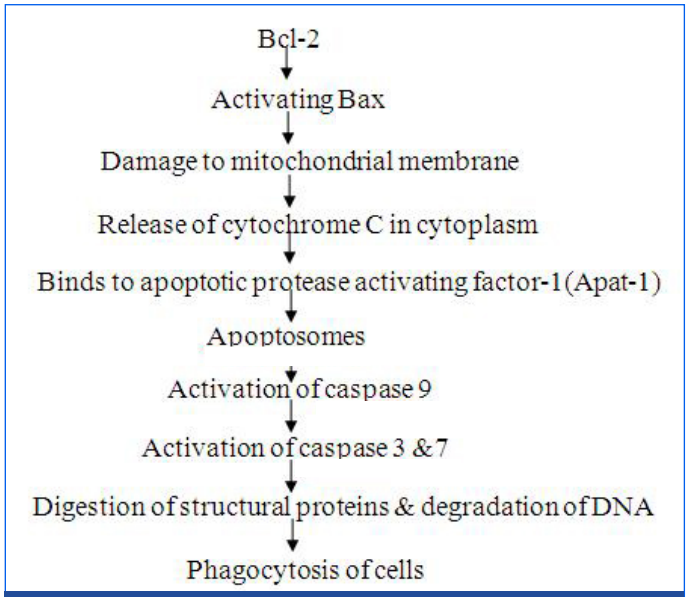
- **Their role in the programmed cell death (apoptosis)-** Apoptosis is required for the proper development/destruction of the cells that represent a threat to the integrity of the organism. It depends upon the balance between the withdrawal of the positive signals- growth factors, interleukins, etc- and the receipt of negative signals- increased levels of oxidants, DNA damage, irradiation, etc. The ROS induced intracellular damage causes Bcl -2-a protein which is located in the outer membrane of the mitochondria, to set off a chain of reactions which leads to apoptosis, as shown in [Table/Fig-7].



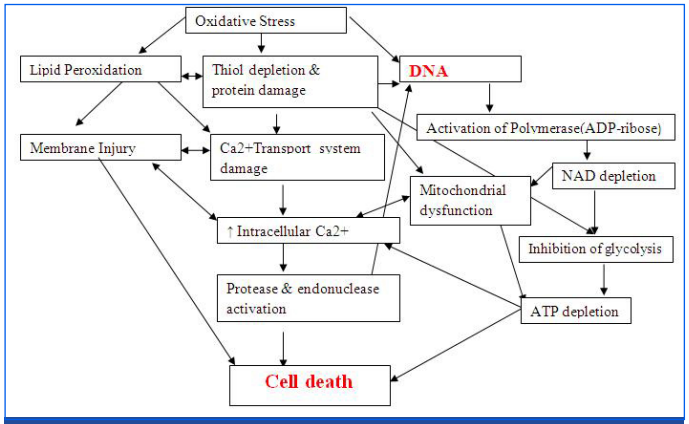
[Table/Fig-5]: Interrelationship between the antioxidant systems [19].



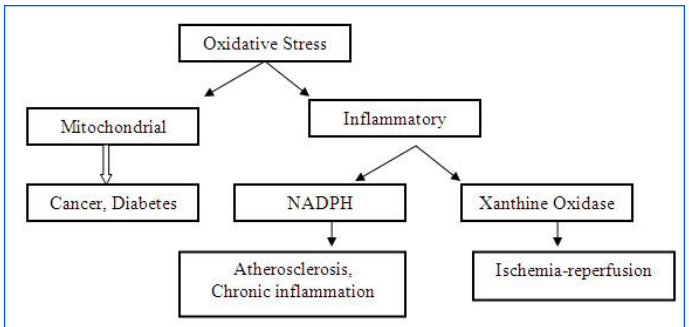
[Table/Fig-6]: The respiratory burst during phagocytosis which is caused by neutrophils [19].



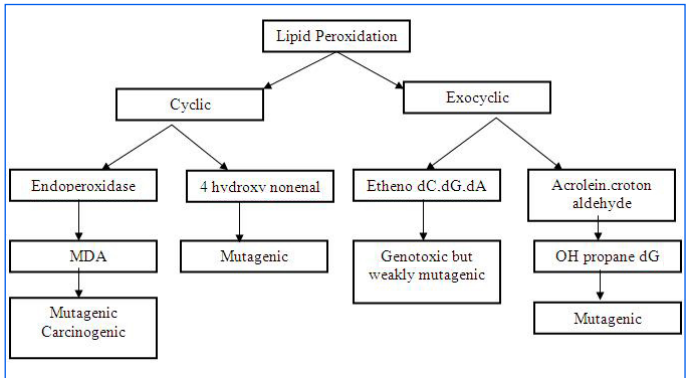
[Table/Fig-7]: Mechanism of Apoptosis



[Table/Fig-8]: Mechanism of Cell death which is caused by Oxidative stress [24].



[Table/Fig-9]: Oxidative stress has been implicated in various pathological conditions [24].



[Table/Fig-10]: Effects of the end products of lipid peroxidation

- Their role in reproduction. During fertilization, the spermatozoa produce ROS for their maturation and membrane fusion with the oocyte. However, the excess generation of ROS by the spermatozoa or a reduced antioxidant defence have been implicated in male infertility [23].

- ROS in ageing17

Increasing evidence has suggested that ROS are involved in the process of biological ageing.

At low concentrations, the reactive species have a physiological role. At high concentrations, however, the reactive species may lead to permanent changes in the signal transduction and gene expression, which are typical for disease states. A high concentration of the reactive species is called as oxidative stress. At high concentrations, the reactive species mainly damage the proteins, nucleic acids and the lipids, as shown in [Table/Fig-8].

Oxidative stress induces a redox imbalance which may lead to an oncogenic stimulation [Table/Fig-9]. The ROS-mediated mutations and the loss of the critical regulatory mechanisms may lead to defective cell death and aberrant proliferation. These may contribute to the development of cancer [17]. The major events which contribute to carcinogenesis and the spread of cancer are-

1. DNA damage

2. Signal transduction abnormalities and

3. Remodelling of the extracellular matrix.

1. DNA damage- can occur because of- a protein damage (which includes the nuclear proteins) and lipid peroxidation Protein damage 7 - is generally the first step in carcinogenesis. Oxidative stress is responsible for the oxidation of the DNA proteins. This leads to mutations in the DNA, which are commonly seen in various tumours. The DNA damage involves single or double stranded DNA breaks and modifications in the DNA bases and/or DNA cross links. This leads to arrest or induction of the transcription; induction of the signal transduction pathways; replication errors and genomic instability- all of which can cause carcinogenesis.

In addition to ROS, RNS also can cause DNA damage. They mainly change guanine to 8-nitroguanine, which induces transversions of G: C to T: A in the DNA, which are unstable.

Mitochondrial DNA damage is also thought to be one of the factors in carcinogenesis. The ROS activate the transcription and the translation of the mitochondrial genome, which leaks out of the mitochondria and gets inserted in the nucleus, thus activating the oncogenes.

In addition to ROS, redox metals, due to their ability to generate free radicals or non-toxic metals and due to their ability to bind to critical thiols, are implicated in carcinogenesis. e.g.

Fe (Iron) – Colorectal Ca

Asbestos (30% Fe), Chromium – Lung Ca

Cadmium – Pancreatic/renal Ca

As (Arsenic) – acts by inhibiting the enzymes by binding to the SH groups and it is a well established co-carcinogen i.e. not causing cancer directly but increasing the ability of the other factors to cause DNA mutations, like- cigarette smoke, UV radiation, etc.

Lipid peroxidation- The various end products of lipid peroxidation like- malondialdehyde (MDA), 4-hydroxynonenal (HNE), acrolein, crotonaldehyde and 8-OH-G react with the DNA and produce mutagenic effects [25] [Table/Fig-10].

2. Signal transduction abnormalities-

Signal transduction is the process by which the extracellular substances produce an intracellular response. The most fundamental process which is regulated by signal transduction is cell growth. So, deviations in the normal regulation of growth can lead to cancer. ROS interfere with the expression of a number of genes and the signal transduction pathways and they can thus cause cancer. The abnormalities of signal transduction are mainly because of the abnormal functioning of the receptors or of various nuclear transcription factors.

Abnormal functioning of the receptors: Several growth factor receptors are affected by ROS and carcinogenic metals like- nickel, arsenic, cobalt, etc. Activation of the extracellular growth factor is seen in lung and urinary carcinomas. Overexpression of the Platelet Derived Growth Factor (PDGF) is seen in lung and prostate cancers.

Nuclear transcription factors: The role of three factors is now well documented- AP-1, NF-kB and p53 [26]. AP-1- This factor is essentially responsible for the cell growth and differentiation. It also functions as a pro or an anti apoptotic agent, depending upon the balance between the pro and the anti-apoptotic genes which stimulate or inhibit AP-1. The duration of the stimulus is also important. In addition, AP-1 can interact with activated oncogenes and produce oncogenic transformation.

NF-kB- regulates several genes which are involved in cell transformation, proliferation and angiogenesis. During cell proliferation, NF-kB can exert dual effects- promotive as well as inhibitory. NF-kB is generally present in the cytoplasm in an inactive form because of its attachment to the inhibitory kB (IkB). ROS, Tumour Necrosis Factor (TNF) and interleukin-1 (IL-1) which are present during oxidative stress, stimulate NF-kB. On stimulation, it gets dissociated from the IkB, unmasks the nuclear localization sequence and enters the nucleus. It then binds to the kB regulatory elements and produces its effects [27].

p53- The tumour suppressor protein, p53, plays a key role in the development of malignancy. p53 exerts its effects by preventing the DNA damaged cells from dividing until a chromosomal repair has taken place. If the damage is not repaired, the cell undergoes apoptosis. A mutation in this gene which leads to its inactivation, is found in more than half of the tumours which are caused due to a direct action of the ROS or carcinogenic metals.

3. Remodelling of the extracellular matrix [7].

An important step in the growth of any tumour beyond a few millimetres, depends upon the development of new blood vessels. Angiogenesis and the development of metastasis are closely connected. Remodelling of the extracellular matrix is required for cancer cell invasion. Cancer cell invasion involves an interaction between the tumour cells and the surrounding stromal cells,

leading to loss of the matrix function and the matrix boundary. Though many proteases may have a role in the remodelling, the activation of the zinc-dependent Matrix Metalloproteinases (MMPs) is the primary response. The MMPs degrade the components of the extracellular matrix. The MMPs are expressed only when a tissue remodelling occurs. Aberrant expressions of various MMPs have been correlated with tumour cell invasion and metastasis.

Angiogenesis involves an interaction between the endothelial cells, the surrounding pericytes and the smooth muscle cells and the ECM angiogenic cytokines. The MMPs degrade the basement membrane and other ECM components, thus allowing the endothelial cells to detach and migrate to new tissues. The MMPs also release pro-angiogenesis factors- the basic fibroblast growth factor (bFGF), VEGF and Transforming Growth Factor Beta (TGFβ). The MMP inhibitors have been shown to inhibit angiogenesis in various models.

The increased ROS stress can induce DNA mutations and genetic instability, which include a loss of the tumour-suppressor genes such as p53. The loss of the p53 function can in turn, further contribute to a mitochondrial dysfunction, ROS generation, and genomic instability, thus forming a vicious cycle. ROS stress may also induce the expression of the pro-survival factors and certain ROS scavenging proteins, which would enable the cells to adapt and survive under oxidative stress. The ROS-induced mutations and genetic instability further enhance the chance for the selection of the cells with malignant phenotypes (an increase in the proliferation, survival capacity, cell motility, and angiogenesis), leading to the development of cancer [28,29].

CONCLUSION

ROS, the antioxidants status and cancer- [30,31].

The antioxidants produce effects by scavenging the free radicals and by modulating the cell-signal pathways. Thus, antioxidants work in malignancy by many mechanisms such as -

a. Maintaining a normal cell cycle regulation

b. Inhibiting the cell proliferation and inducing apoptosis

c. Inhibiting the tumour invasion and angiogenesis

d. Reducing inflammation

e. Activating the detoxifying enzymes

Many studies have established the association of the disordered GSH-related enzyme functions and cancer. The Glutathione S-Transferases (GSTs) have been more frequently reported. The GSTs utilize glutathione in a wide range of reactions which involve carcinogens, drugs and products of oxidative stress [32]. As a measure of the redox homeostasis, the GSH/GSSG ratio is measured in many diseases. In colon and breast cancers, this ratio is significantly reduced, thus implying the presence of oxidative stress.

However, an antioxidant protection therapy should be used with caution in patients of malignancy. Free radicals are important in inducing apoptosis in cancers and the inhibition of this apoptosis might actually stimulate the survival of the damaged cells and their proliferation into a neoplastic state [33]. Increased glutathione levels in the late stages may give immunity to the cancer cells against chemotherapeutic agents. Another important issue is the pro oxidant character of some antioxidants, which may depend on the concentration and the environment. So, the usefulness of the antioxidant therapy needs proper evaluation before its initiation.

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AUTHOR(S):
1. Dr. Jayshree Shriram Dawane
2. Dr. Vijaya Anil Pandit

PARTICULARS OF CONTRIBUTORS:
1. Assistant Professor, Department of Pharmacology,
2. Professor, Department of Pharmacology,
Bharati Vidyapeeth Medical College, Pune India.

NAME, ADDRESS, E-MAIL ID OF THE CORRESPONDING AUTHOR:
Dr. Jayshree Shriram Dawane,
Assistant Professor, Department of Pharmacology,
Bharati Vidyapeeth University Medical College,
Pune-411043, India.
Phone: 9850817154
E-mail: jayshreedawane@gmail.com

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