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RESEARCH ARTICLE

Role of metal complexes in oxidative stress and ROS generation leading to Cancer

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ABSTRACT

Reactive oxygen species (ROS) are recognized as essential participants in normal cellular processes, while their intricate involvement in the emergence of various diseases, notably cancer, has garnered significant attention. Elevated levels of ROS isassociated with pro-tumorigenic signalling, heightened cell survival, increased proliferation, and DNA damage, thereby making substantial contributions to the genetic instability. Intriguingly, at elevated levels, ROS paradoxically initiate anti-tumorigenic signalling pathways, thereby instigating cell death through oxidative stress. In this comprehensive review, a focus is given on ROS generation, which encompasses both endogenous and exogenous sources that collectively referred to as oxidative stress. To provide a comprehensive understanding, an exploration of the structural, chemical, and biochemical aspects of free radicals is undertaken.Diverse sources contributing to ROS generation, including metal-mediated free radical formation is also discussed. This review additionally conducts an in-depth examination of oxidative stress within the context of cancer. Moreover, noteworthy contributions of key antioxidant enzymes, namely, superoxide dismutase, catalaseand glutathione peroxidase over the multifaceted landscape of carcinogenesis have been discussed, drawing insights from a multitude of studies. Understanding the intricate interplay between pro- and anti-tumorigenic ROS signalling pathways offers a multitude of potential avenues for cancer therapy. The disrupted redox balance observed in cancer cells presents promising opportunities for ROS manipulation, thereby emerging as a viable and innovative treatment strategy. This present review may serve as an invaluable resource, offering profound insights into the multifaceted roles of ROS in cancer while simultaneously highlighting their therapeutic potential, thereby paving the way for novel and effective cancer interventions.

Keywords: ROS, Oxidative stress, Metal complexes, Cancer.

1. Introduction

In experimental and clinical medicine, there has been a remarkable surge in interest regarding the role of oxygen-free radicals, especially the reactive oxygen species(ROS)[1]. ROS have diverse sources, e.g., it is generated during irradiation from UV light, X-rays, and gamma-rays. It is also produced as by-products of metal-catalysed reactions, in presence as pollutants in the atmosphere, and release by neutrophils and macrophages during inflammatory responses. They are also produced as by-products of mitochondria-catalysed electron transport reactions and other cellular mechanisms[2]. ROS plays a dual zrole within biological systems, displaying the capacity to either benefit or harm living organisms[3]. ROS have physiological roles in cellular responses to harmful stimuli, such as defending against infectious agents and participating in various cellular signalling systems. One example of ROS functioning constructively at low concentrations is their induction of mitogenic responses. At high concentrations, ROS can serve as significant mediators of damage to cellular structures, including lipidsmembranes, proteins, and nucleic acids-a phenomenon referred to as oxidative stress[4]. To mitigate the harmful effects of ROS, cells employ an antioxidant defence system consisting of both non-enzymatic antioxidants and antioxidant enzymes[5]. Despite the presence of these cellular antioxidant defence mechanisms to counteract oxidative damage from ROS, accumulated oxidative damage over an organism's life-span has been proposed to play a critical role in the development of age-related diseases. Such diseases may include cancer, arteriosclerosis, arthritis, neurodegenerative disorders, and various other conditions[1].ROS play a significant role in inducing apoptosis in various cancer cells, particularly those that are drug-resistant[6-8]. Oxidative stress is characterized by an imbalance between the production of ROS, and their removal by protective mechanisms, known as antioxidants. This imbalance can result in damage to critical biomolecules and cells, potentially affecting the entire organism[9]. ROS is natural by-products of cellular metabolism and serve essential roles in triggering signalling pathways in both plant and animal cells in response to changes in the internal and external environments[10]. During normal metabolic processes, aerobic cells produce ROS, including superoxide anion (O_2^-), hydrogen peroxide (H_2O_2), hydroxyl radical ($OH^•$), and organic peroxidesas part of the biological reduction of molecular oxygen[11]. This electron transfer to molecular oxygen primarily takes place within the respiratory chain, situated in the mitochondria's membranes[12-13]. Under hypoxic conditions, the mitochondrial respiratory chain can also produce nitric oxide (NO), which can lead to the formation of reactive nitrogen species (RNS)[14]. RNS, in turn, can generate other reactive species, such as reactive aldehydes like malondialdehyde and 4-hydroxynonenal, by promoting excessive lipid peroxidation[15].If these products were remained in the cell for longer time, they will damage the cells, especially the biomolecules.Modification of these biomolecules can increase the risk of mutagenesis[16]. When environmental stressors persist over extended periods, ROS production continues which may lead to significant damage to cellular structures and functions. This sustained oxidative stress can

induce somatic mutations and contribute to neoplastic transformation[17-18]. Notably, oxidative stress has been linked to cancer initiation and progression due to its role in increasing DNA mutations, causing DNA damage, promoting genome instability, and enhancing cell proliferation[19]. It has also been noted that several pro-oxidants are available in the environment. The common body tissuelike lung and skin are constantly exposed with this pro-oxidant along with several other endogenous oxidants.To safeguard against the harmful effects of these oxidant species, the body relies on a well-organized system of chemical and enzymatic antioxidants[20-21]. The body's defence against these harmful pro-oxidants involves a complex system of enzymatic antioxidants like superoxide dismutase (SOD), glutathione peroxidase (GPx), glutathione reductase, catalase, and non-enzymatic antioxidants such as glutathione (GSH), and vitamins C and D[22].Drug-resistant cancer cells typically exhibit very low ROS levels, primarily due to their elevated levels of intracellular reduced glutathione (GSH) and enhanced activities of antioxidant enzymes such as GPx, catalase (CAT), and SOD[23-25]. Aging is also characterized by the gradual accumulation of molecular damage in DNA, proteins, and lipids. It is accompanied by increased intracellular oxidative stress due to the progressive decline in ROS scavenging mechanisms[26]. Additionally, GSH is essential for phase II detoxification reactions, where enzymes like glutathione S-transferase (GST) require GSH to conjugate electrophilic drugs and xenobiotics[27]. Consequently, high levels of GSH and GST have been implicated in drug-resistant tumours[23,28-29].Chronic inflammation, triggered by biological, chemical, and physical factors, is associated with an elevated risk of several human cancers[30]. The connection between inflammation and cancer is supported by epidemiological and experimental evidence and has been confirmed by the effectiveness of anti-inflammatory therapies in cancer prevention and treatment[31-33].The effectiveness of therapeutic agents in inducing apoptosis in cancer cells often relies on the cells' ability to generate ROS[34]. Interestingly, low levels of ROS are conducive to the expression of ABC transporters like P-glycoprotein (P-gp)[35]. The concept that prolonged irritation can lead to cancer dates back to ancient Ayurvedic medicine, which has been practiced for over 5000 years[36]. Whether this irritation corresponds to what Rudolf Virchow described as inflammation in the 19th century remains uncertain[37]. Virchow was the first to observe inflammatory cells within tumours and noted that tumours often develop at sites of chronic inflammation[38]. Today, inflammation is recognized as a "silent killer" in diseases like cancer. For example, inflammatory bowel diseases like Crohn's disease and ulcerative colitis increase the risk of colon adenocarcinoma[39-40], and chronic pancreatitis is associated with a higher incidence of pancreatic cancer[41]. The precise mechanisms by which the wound-healing process transforms into cancer are subject of intensive research[33,42]. Possible mechanisms include the induction of genomic instability, changes in epigenetic events leading to inappropriate gene expression, increased proliferation of initiated cells, resistance to apoptosis, aggressive

tumour neovascularisation, invasion through tumour-associated basement membranes, and metastasis[43]. This review focuses on the major source of the ROS and their role in inflammation induces carcinogenesis.

2. Reactive Oxygen Species: Sources and reactions

Free radicals are molecules or molecular fragments containing unpaired electrons, conferring a high degree of reactivity upon them. In living systems, the most important class of free radicals is derived from oxygen, known as ROS[44]. ROS can originate from both endogenous and exogenous sources. The primary source of endogenous ROS is mitochondria. Mitochondria also produce superoxide radicals, which are efficiently converted into hydrogen peroxide and then water by antioxidant enzymes like SOD[45]. Xanthine oxidase (XO), an enzyme that catalyses reactions involving purines, leading to the production of superoxide anions and hydrogen peroxide[46]. Activated immune cells such as neutrophils, eosinophils, and macrophages increase oxygen uptake, resulting in the generation of various ROS, including superoxide anion, nitric oxide, and hydrogen peroxide[47]. Cytochrome P_{450} enzymes can also produce ROS when induced, particularly superoxide anion and hydrogen peroxide[48]. Microsomes and peroxisomes are additional endogenous sources of ROS, with microsomes being responsible for a significant portion of H_2O_2 production under hyperoxic conditions[49]. Peroxisomes primarily produce H_2O_2 but not $O_2^{\bullet-}$ under normal physiological conditions, and their contribution to overall H_2O_2 production is notable in the liver and other organs containing peroxisomes, especially during prolonged starvation[48]. Activated Kupffer cells, the resident macrophages of the liver, release biologically active molecules like cytokines, which have been linked to hepato-toxicological and hepatocarcinogenic events. Recent research suggests a connection between products released by activated Kupffer cells and the tumour promotion stage in carcinogenesis[49]. On the other hand, exogenous sources also significantly contribute in the ROS generation. Oxidative stress and damage have been observed following exposure to various xenobiotics, such as chlorinated compounds, metal complexes (both redox and non-redox), radiation, and barbiturates. For example, 2-butoxyethanol indirectly produces ROS, leading to cancer in mice[50]. Some frequently encountered ROS are shown in Table-1.

Table1: List of frequently encountered ROS in biological systems

ROS	Chemical formula	Reactivity
Superoxide radical anion	$O_2^{\bullet-}$	i) Selectively reactive, sparing most biological molecules ii) Reduces transition metals (Fe^{3+}, Cu^{2+}) with rate depending on metal ion ligand iii) Rapid reaction with nitric oxide (k2 > 109 M^{-1} s^{-1}) forming peroxynitrite: $O_2^{\bullet-} + NO^{\bullet} \rightarrow ONOO^-$ iv) Can damage enzymes with Fe–S clusters v) Reacts with other radicals to form hydroperoxides: $O_2^{\bullet-} + R^{\bullet} + H^+ \rightarrow ROOH$
Hydroxyl radicle	$^{\bullet}OH$	i) Highly reactive, indiscriminately reacts with adjacent molecules at near diffusion-controlled rates
Hydrogen peroxide	H_2O_2	i) Unreactive with most biomolecules ii) Slow reaction with most thiols (k ≈ 1 M^{-1} s^{-1} for GSH), faster with certain Cys residues iii) Reacts with transition metal ions to produce $^{\bullet}OH$ (rate constants 10^{2-107} M^{-1} s^{-1}) iv) Main reactions with haem, thiols, and peroxidases v) Reacts with CO_2 to form more reactive peroxymonocarbonate (HCO_4^-)[51]
Hypohalous acids	HOCl, HOBr	i) Strong oxidants, react predominantly with thiols and methionine ii) Reactions with amines generate secondary chloramines/bromamines, which are less potent iii) React rapidly with thiocyanate (SCN^-) to form HOSCN, highly specific for thiols
Carbonate radical anion	$CO_3^{\bullet-}$	i) Formed from CO_2 reacting with peroxynitrite and HCO^{-3} reacting with $^{\bullet}OH$ ii) Fairly reactive, oxidizes guanine in DNA and cysteine, tyrosine, tryptophan
Singlet oxygen	$^1O_2$	i) Reactive singlet state of O_2, formed by photosensitization reactions or chemical reactions with peroxyl radicals and HOCl
Nitrogen dioxide radical	$NO_2^{\bullet}$	i) Major atmospheric pollutant, rapidly oxidizes electron-rich compounds; forms nitrated products like 3-nitrotyrosine, nitrotryptophans, nitrolipids, and nitrated DNA bases ii) Some nitrated products have signalling functions

2.1 CHEMISTRY OF REACTIVE OXYGEN SPECIES (ROS)

Superoxide anion, which arises as a result of metabolic processes or when oxygen undergoes "activation" through physical irradiation, is considered the primary ROS. It can further react with other molecules to generate secondary ROS, either directly or through enzyme- or metal-catalysed processes[52]. It is important to note that the superoxide radical doesn't directly interact with polypeptides, sugars, or nucleic acids,

and its ability to peroxidise lipids is a subject of debate. Superoxide is typically removed through a dismutation reaction[53]:

$$2O_2^{\bullet-} + 2H^+ \rightarrow H_2O_2 + O_2$$

In biological systems, SOD enzymes significantly accelerate this reaction, by approximately four orders of magnitude. Additionally, SOD enzymes work in concert with H2O2-removing enzymes like catalases and GPx[54]. The generation of various free radicals is closely associated with the involvement of redox-active metals[44]. The redox state within cells is primarily governed by iron (and occasionally copper) redox couples, which are maintained within strict physiological limits. Under stress conditions, excess superoxide can release "free iron" from iron-containing molecules[55]. The released Fe (II) can participate in the Fenton reaction (Figure-1), generating highly reactive hydroxyl radicals (•OH) as follows:

$$Fe\ (II) + H_2O_2 \rightarrow Fe\ (III) + {}^{\bullet}OH + OH^-$$

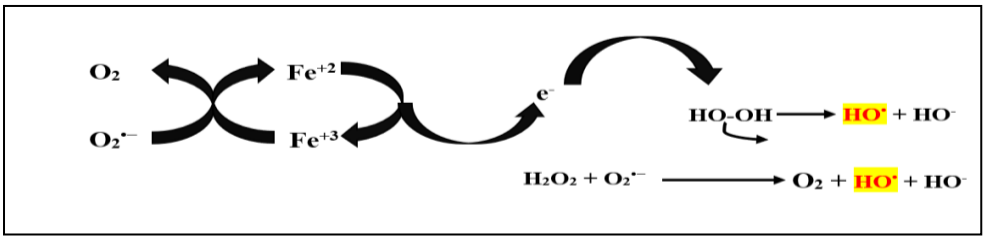

Figure 1: Fenton reaction HO•- Hydroxyl radical; H_2O_2 – Hydrogen peroxide; O_2^{-2} – Peroxide; Fe^{+2}- Iron (II); Fe^{+3}- Iron (III)

Thus, under stress conditions, $O_2^{\bullet-}$ acts as an oxidant and facilitates •OH production from H_2O_2 by making Fe (II) available for the Fenton reaction[56-60]. The superoxide radical also contributes to the Haber-Weiss reaction (Figure-2), which combines a Fenton reaction with the reduction of Fe (III) by superoxide, yielding Fe (II) and oxygen:

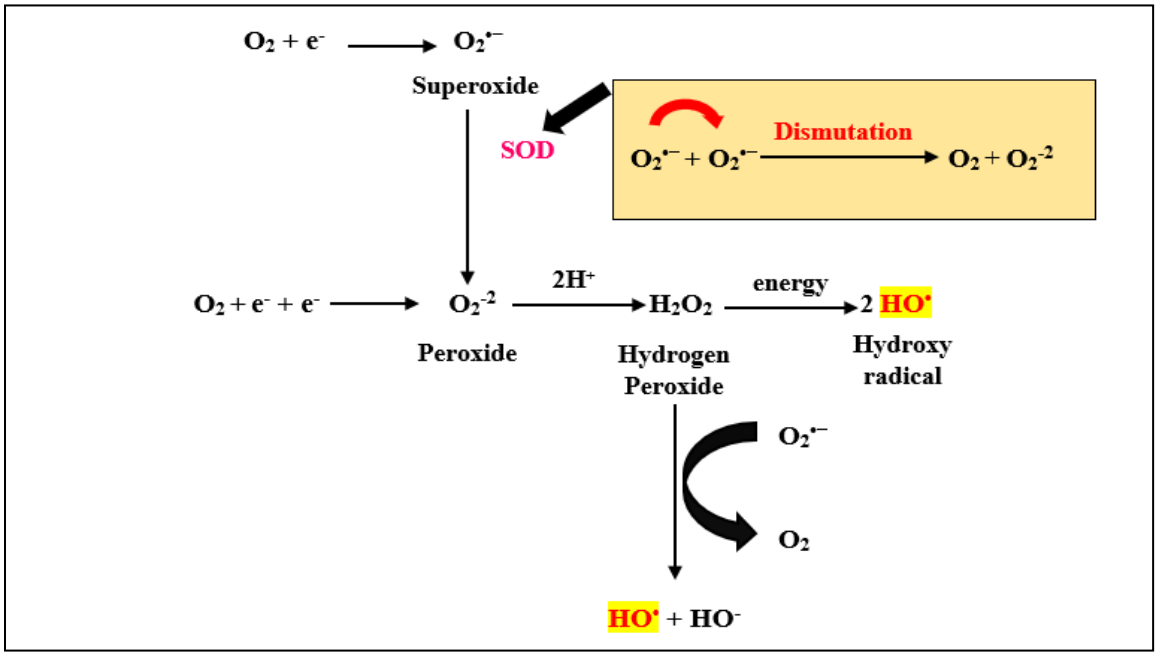

Figure 2: Harber – Weiss reaction (SOD - Superoxide dismutase; HO•- Hydroxyl radical; H_2O_2 – Hydrogen peroxide; O_2^{-2}– Peroxide)

The •OH is highly reactive, with a half-life in aqueous solution of less than 1 ns [60]. Therefore, when produced in vivo, it rapidly reacts near its site of formation. It can be generated through various mechanisms, including ionizing radiation and photolytic decomposition of alkyl hydroperoxides. As mentioned earlier, the majority of hydroxyl radicals generated in vivo result from the metal-catalysed breakdown of hydrogen peroxide via the Fenton reaction:

$$Mn^{+}\ (Cu^{+}, Fe^{2+}, Ti^{3+}, Co^{2+}) + H_2O_2 \rightarrow M^{(n+1)+}\ (Cu^{2+}, Fe^{3+}, Ti^{4+}, Co^{3+}) + {}^{\bullet}OH + OH^{-}$$

This reaction primarily occurs when Mn^{+} represents iron, copper, chromium, cobalt, or certain other metals[59-61]. However, recent findings have raised doubts about the in vivo role of copper in Fenton-like generation of hydroxyl radical due to the limited availability of "free pools" of copper within cells[62]. Although Fenton chemistry is known to occur in vitro, primarily due to the effective sequestration of "free catalytic iron" by various metal-binding proteins[63]. However, in conditions of iron overload, such as hemochromatosis and β-thalassemia, excess "free iron" can have detrimental effects. This free iron is transported into a labile iron pool (LIP), a low-molecular-weight pool of weakly chelated iron that rapidly passes through cells. The LIP likely contains both forms of iron ions Fe (II) and Fe (III)) chelated by various chelators like citrate, phosphate, carboxylates, nucleotides, and others, with experiments suggesting a concentration of 0.2–0.5 M and a predominance of Fe (II). Another class of radicals derived from oxygen in living systems includes peroxyl radicals (ROO•). Peroxyl radicals are high-energy species with a diverse range of reduction potentials, depending on the R group[64]. The simplest peroxyl radical is the di-oxyl (hydroperoxyl) radical HOO•, which is the conjugate acid of superoxide, $O_2^{\bullet-}$. The chemistry of peroxyl radicals varies depending on the R group, the local environment, and the concentrations of oxygen and other reactants[65]. Peroxyl radicals are known to participate in various biological reactions, with lipid peroxidation being a commonly cited example.They are also involved in DNA cleavage and protein backbone modification, and peroxyl radicals can synergistically enhance DNA damage induced by superoxide radicals.

3. Metal induced oxidative stress and carcinogenesis

Numerous studies have extensively examined the connection between metal-induced toxicity and carcinogenicity, highlighting their significant role in generating reactive oxygen and nitrogen species within biological systems[44,56-58]. This metal-mediated generation of free radicals can lead to various modifications in DNA bases, heightened lipid peroxidation, and disruptions in calcium and sulphydryl homeostasis[48,66-68].

3.1 COPPER

Copper's role as an essential component of various endogenous antioxidant enzymes and its potential association with free radicals in the process of carcinogenesis have been subjects of research[68]. The collective body of evidences from both in vitro and in vivo experiments suggests that copper, in the form of copper salts, is not genotoxic[69]. In vitro studies have indicated that cancer cells thrive and proliferate more easily in a high-copper

environment, potentially promoting tumours formation[70-71]. Researchers have also developed a novel copper complex, Copper N-(2-hydroxyacetophenone) glycinate (CuNG) (Figure-3) which has significant role in overcoming drug resistance in Ehrlich Ascites Carcinoma (EAC) cell[72-73]. Copper-dependent oxidative damage can potentially be mitigated through chelation with antioxidants, particularly dipeptides that contain imidazole rings capable of binding copper. Copper-DNA adducts may have the potential to exacerbate oxidative DNA damage[74]. Redox-active metal ion, copper (Cu), is known to contribute to the generation of ROS through processes like the Fenton reaction in biological systems[75]. Furthermore, hydrogen peroxide (H_2O_2) can deactivate the pro-oxidant portion of the enzyme CuZnSOD by loss of copper ions. This means that exposure of human erythrocytes to elevated levels of H_2O_2 results in the inactivation of CuZnSOD. Oxidative stress within cells often leads to DNA damage, particularly when ROS are not effectively neutralized, or when antioxidant defences are overwhelmed. Such DNA damage can manifest as single-strand breaks, double-strand breaks, or chromosomal aberrations. Elevated oxygen concentrations, exposure to factors like cigarette smoke, asbestos, ozone, or carcinogenic metals such as certain nickel compounds at concentrations exceeding normal levels, can all contribute to DNA damage[76-77]. Recent studies have indicated that alpha-tocopherol or catechol can induce oxidative DNA damage in the presence of copper (II) ions by generating $O_2^{\bullet-}$ and $^{\bullet}OH$ free radicals[78-79].

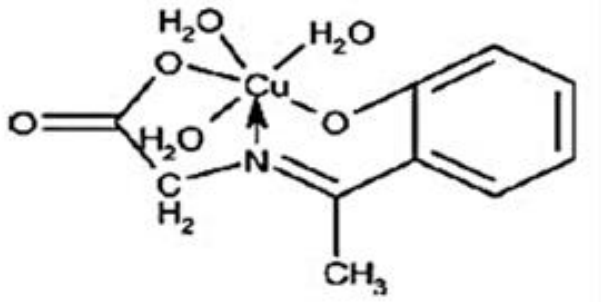

Figure 3: Chemical structure of CuNG[72]

3.2. IRON

Iron is regarded as a vital element for all the living organisms. It has significant role in oxygen transport. Study on analysis of biochemical, animal, and human data has led to the suggestion that elevated levels of iron in the body may be associated with an increased risk of various diseases, including vascular diseases, cancer, and specific neurological conditions[80-81]. The generation ofROS by iron and the subsequent damage to DNA and lipids seem to result from an exaggeration of iron's normal function. Iron-induced free radical damage to DNA is believed to play a significant role in cancer development, as cancer cells are known to thrive in response to increased iron levels[82]. Consequently, pre-menopausal women and children are thought to have a lower risk of common diseases because their body iron levels are less likely to be excessive during these periods. Nelson and Babbs proposed

that exposure of the intestine to ingested iron could be a primary factor in the development of colorectal cancer in highly developed meat-consuming countries[83-84]. They found a dose-dependent relationship between serum ferritin levels and the risk of colon adenomas. Genetic hemochromatosis is linked to an increased risk of hepatocellular carcinoma. The connection between elevated body iron stores and the development of hepatocellular carcinoma in individuals with iron overload unrelated to genetic hemochromatosis, along with experimental evidence supporting iron's co-carcinogenic role, strongly supports the idea that iron is involved in hepatocellular carcinoma development[85-86]. Several studies on the animal model have extensively documented iron-induced carcinogenesis. Intramuscular injections of an iron-dextran complex, commonly used to treat anaemia in humans, have been found to induce spindle cell sarcoma or pleomorphic sarcoma in rats at the injection site[87]. It has been shown to induce renal carcinogenesis when combined with iron (Fe-NTA complex). It acts as efficiently as "free iron" in vitro at physiological pH by catalysing the breakdown of hydrogen peroxide through the Fenton reaction[88].

3.3. CHROMIUM

Chromium, an essential trace element found naturally, plays a vital role in regulating blood glucose levels. However, it is crucial to differentiate between Chromium (III) and Chromium (VI) due to their distinct characteristics. Chromium (VI) can be potentially toxic and carcinogenic when consumed in high doses[89-91]. All chromates, including Cr (VI), have the ability to enter cells through channels designed for the transfer of isoelectric and isostructural anions, such as SO_4^{2-} and HPO_4^{2-}[92]. Insoluble chromates are taken up by cells through phagocytosis. Inside the cell, glutathione quickly forms a complex with Chromium (VI), followed by the gradual reduction of Chromium (VI) to yield Chromium (V). Using an EPR spin-trapping technique, the formation of Chromium (V) species (likely the Chromium (V)–glutathione complex) and the generation of the glutathione-derived thiyl radical ($GS^{\bullet}$) were demonstrated[90]. Once formed, Chromium (V) species were found to alter DNA conformation. In addition to GSH, various other substances, including ascorbate, cysteine, lipoic acid, NAD(P)H, fructose, ribose, and others, have also been shown to reduce Chromium (VI) in vitro[90]. Studies suggest that the in vivo one-electron reductant of Chromium (VI) occurs most likely by NAD(P)H flavoenzymes. Once Chromium(V) is formed, it can react via the Fenton reaction with hydrogen peroxide (H_2O_2), generating a hydroxyl radical capable of causing DNA damage[90]. Recent studies have emphasized the involvement of Cr (III)-dependent pathway in Cr (VI) carcinogenicity and mutagenicity. These studies, led by Zhitkovich and his team, provide evidence that intracellular reduction of Cr (VI) results in the extensive formation of Cr–DNA adducts. Among these adducts, Cr (III)-mediated DNA cross-links involving molecules like glutathione, cysteine, histidine, and ascorbate represent a major class of DNA modifications[93]. Additionally, several studies from the same laboratory have disproven the existence and genotoxic/mutagenic effects of Cr(V) species and the hydroxyl radical. Reduction of carcinogenic Cr (VI) by physiological concentrations of Vitamin C has been shown to generate ascorbate–Cr (III)–DNA crosslinks and binary Cr (III)–DNA

adducts, both of which are potential sources of oxidative DNA damage through intermediate reaction products[93]. These findings suggest that Cr–DNA adducts are responsible for both the mutagenicity and genotoxicity of Cr (VI). Hexavalent chromium is known to be associated with lung cancer in humans[89]. Workers exposed to hexavalent chromium in workplace air have shown significantly higher rates of lung cancer compared to non-exposed individuals. Chromium has also been implicated in an increased incidence of breast cancer[94].

3.4. NICKEL

Nickel is recognized as a human carcinogen, primarily affecting gene expression through mechanisms involving enhanced DNA methylation and compaction, rather than relying on mutagenic pathways[95]. The nickel compounds implicated as potential carcinogens encompass insoluble dusts of nickel subsulphides and nickel oxides, the vapor emitted by nickel carbonyl, and soluble aerosols of nickel sulphate, nitrate, or chloride[96]. Acute nickel toxicity primarily arises from exposure to nickel carbonyl, leading to severe pulmonary and gastrointestinal toxicity. The lung stands out as the primary target organ for nickel toxicity in humans. An epidemiological study conducted in Norway involving workers in nickel refineries provided compelling evidence of a substantial association between cumulative exposure to water-soluble nickel and the increased risk of developing lung cancer[97]. Additional research has demonstrated that inhalation of nickel refinery dust, which contains nickel sub sulphide, has been linked to higher mortality rates from nasal cavity cancers and potentially laryngeal cancer[98]. Furthermore, nickel has the potential to interfere with DNA repair processes, and toxic doses of nickel have been observed to induce lipid peroxidation and the formation of protein carbonyls in animals. It is notable that trace amounts of nickel have been shown to be essential for normal growth and reproduction in certain animal species, suggesting that small quantities of nickel may also play an essential role in human physiology. A very recent report from our team also indicates a novel Nickel chelate complex, Nickel N-(2-hydroxyacetophenone) glycinate (NiNG) (Figure-4)[99]. Blocking the mitogen-activated protein kinase (MAPK) pathways effectively prevents cell death induced by NiNG in both drug-resistant and sensitive cancer cells[99]. An imbalance in redox status serves as the central mediator of NiNG-induced apoptosis in both drug-resistant and sensitive cells[99]

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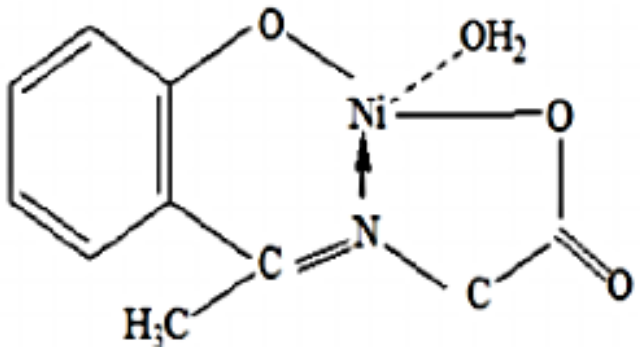

Figure 4: Structure of NiNG[99]

4. Oxidative stress and cell signalling

Cells employ a sophisticated system known as cell signalling or signal transduction to communicate with one another and respond to extracellular stimuli[100]. This signalling process is initiated by external signals, including hormones, growth factors, cytokines, and neurotransmitters[101]. These signals are relayed to the transcription machinery responsible for the expression of set of specific genes, primarily through a group of proteins called transcription factors. These signal transduction pathways can trigger a wide range of biological activities, including muscle contraction, gene expression, cell growth, and nerve transmission[102]. WhileROS are primarily associated with causing cellular damage, they also play a crucial physiological role in various aspects of intracellular signalling and regulation[103]. Research has clearly demonstrated that ROS can influence the expression of numerous genes and signal transduction pathways[100]. Due to their oxidizing nature, ROS have the ability to impact the redox status of cells, which, depending on their concentration, can lead to either a positive response (such as cell proliferation) or a negative cellular response (such as growth arrest or cell death). As mentioned earlier, high concentrations of ROS can lead to cell death or necrosis, but at low or transient levels, ROS can stimulate proliferation and enhance the survival of various cell types. Thus, ROS can serve as essential secondary messengers in various physiological processes[104]. For instance, they can regulate the cytosolic calcium concentration (which, in turn, regulates the biological activities mentioned above), modulate protein phosphorylation, and activate specific transcription factors like NF-κB and the AP-1 family of factors[105]. ROS and metal ions primarily inhibit phosphoserine/ threonine, phosphotyrosine, and phospholipid phosphatases, often by interacting with the sulfhydryl groups on their cysteine residues, leading to the formation of intramolecular or intermolecular disulfide bonds[100]. These structural changes alter the conformation of proteins, thereby upregulating several signalling cascades. Notably, these cascades include growth factor kinase-dependent, src/Abl kinase-dependent, MAPK-dependent, and PI3-kinase-dependent pathways. These signalling cascades ultimately result in the activation of various redox-regulated transcription factors, such as AP-1, NF-κB, p53, HIF-1, and NFAT (Figure-5).

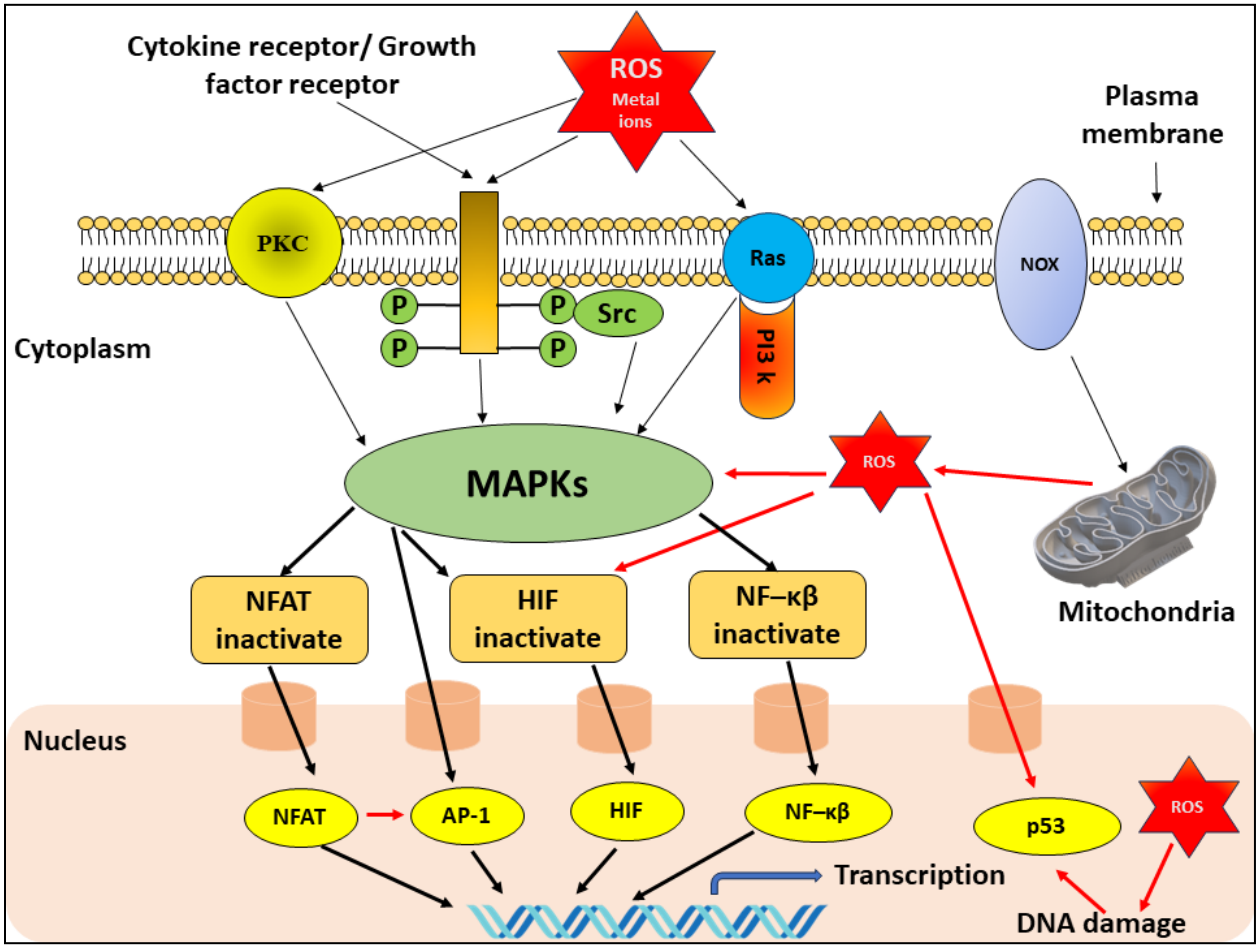

Figure 5: Role of ROS and metal ions in cell signalling; ROS and metal ions primarily exert their inhibitory effects on phosphoserine/threonine, phosphotyrosine, and phospholipid phosphatases. This inhibition is likely due to their interaction with the sulfhydryl groups on cysteine residues within these enzymes, leading to the oxidation and subsequent formation of intramolecular or intermolecular disulfide bonds. These structural modifications result in altered protein conformation, subsequently triggering the upregulation of several signalling cascades, notably including growth factor kinase, src/Abl kinase, MAPK, and PI3-kinase-dependent pathways. These signalling cascades ultimately activate various redox-regulated transcription factors, such as AP-1, NF-κB, p53, HIF-1, and NFAT. super oxides are also generated by the activation of NADPH oxidase (NOX).

5. Antioxidant defence mechanism in cancer

5.1. SUPEROXIDE DISMUTASE (SOD)

Superoxide dismutase is the enzyme responsible for catalysing the dismutation of $O_2^{\bullet-}$ (superoxide) into O_2 (oxygen) and the less-reactive species H_2O_2 (hydrogen peroxide). Although SOD was isolated as early as 1939, it wasn't until 1969 that McCord and Fridovich provided conclusive evidence of its antioxidant activity[106]. Superoxide dismutase exists in several isoforms, each differing in the nature of the active metal centre, amino acid composition, number of subunits, cofactors, and other characteristics. In humans, there are three forms of SOD: cytosolic Cu, Zn-SOD, mitochondrial Mn-SOD, and extracellular SOD (EC-SOD)[107]. SOD effectively neutralizes $O_2^{\bullet-}$ at high reaction rates through a "Ping-

Pong" type mechanism, involving successive oxidation and reduction of the transition metal ion at the active site[108]. Cytosolic Cu, Zn-SOD is an enzyme composed of two identical subunits (homodimer), with a molecular weight of approximately 32 kDa[108]. This SOD specifically catalyses the dismutation of the superoxide anion into oxygen and water. Each subunit contains a dinuclear metal cluster consisting of copper and zinc ions. Enzyme activity remains relatively independent of pH within the range of 5–9.5. Mitochondrial Mn-SOD, a homotetramer with a molecular weight of 96 kDa, contains one manganese atom per subunit[108]. This enzyme undergoes a cycle from Mn (III) to Mn (II) and back to Mn (III) during the two-step dismutation of superoxide. Mn-SOD is known to be one of the most effective antioxidant enzymes with anti-tumour activity. Studies on various cell lines have shown that over expression of Mn-SOD can lead to the retardation of tumour growth[109]. However, the role of Mn-SOD as a tumour suppressor protein is not universally clear, as some tumours exhibit reduced Mn-SOD activity. In certain tumour cells, the activity of total SOD (Cu, Zn-SOD and Mn-SOD) has been found to be diminished[110]. Intriguingly, some cancers of the gastrointestinal tract have displayed marked over expression of Mn-SOD, which correlates with poor prognosis, advanced stages of progression, and an invasive and metastatic phenotype. These observations suggest that while excessively high levels of Mn-SOD can suppress cell growth, they may simultaneously increase the invasive potential of cancer cells. Over expression of Mn-SOD has been associated with the activation of enzymes from the zinc-dependent matrix metalloproteinase family (MMP), particularly MMP-1 and MMP-2. MMPs play diverse roles in cellular remodelling processes, with some family members being critical for tumour invasion. Activation of MMPs is likely mediated through the redox-sensitive transcription factors AP-1 and NF-κB, triggered by elevated levels of hydrogen peroxide induced by Mn-SOD activity[111]. In conclusion, it is hypothesized that an imbalance between superoxide radical formation and hydrogen peroxide degradation in cells over expressing Mn-SOD may activate the metastatic potential of cancer cells. The exact role of Mn-SOD in inducing the loss of matrix functions in metastasis requires further investigation. Extracellular superoxide dismutase (EC-SOD) is a secretory, tetrameric glycoprotein containing copper and zinc, with a high affinity for specific glycosaminoglycans such as heparin and heparin sulphate[108]. Its regulation in mammalian tissues is primarily coordinated by cytokines, rather than as a response of individual cells to oxidants. A completely distinct class of SOD containing nickel (Ni-SOD) was recently discovered in Streptomyces and cyanobacteria. Ni-SOD is a small protein consisting of 117 amino acids with no sequence homology to other SODs[112].

5.2.CATALASE

Catalase is an enzyme found in the cells of plants, animals, and aerobic bacteria that require oxygen for the cellular respiration[108]. It is primarily located within a cell organelle known as the peroxisome. Catalase plays a highly efficient role in facilitating the conversion of hydrogen peroxide into water and molecular oxygen. Catalase boasts one of the highest turnover rates among all enzymes.

To illustrate, a single molecule of catalase can convert approximately 6 million molecules of hydrogen peroxide into water and oxygen every minute.

$$2H_2O_2 \xrightarrow{\text{Catalase}} 2H_2O + O_2$$

The reduced capacity of various types of tumours to detoxify hydrogen peroxide is attributed to a decrease in catalase levels.

5.3. GLUTATHIONE

The primary thiol antioxidant in biological systems is the tripeptide glutathione (GSH). It plays a crucial role as a multifunctional non-enzymatic antioxidant within cells[113]. Glutathione serves as the predominant thiol-disulphide redox buffer in the cell and is highly abundant in various cellular compartments, including the cytosol (1–11 mM), nuclei (3–15 mM), and mitochondria (5–11 mM)[113]. This antioxidant exists in two forms. The reduced form, GSH (glutathione) (Figure-6), and the oxidized form, GSSG (glutathione disulphide) (Figure-7). In the nucleus, GSH plays a vital role in maintaining the redox state of critical protein sulphydryls, which are essential for processes such as DNA repair and gene expression. Oxidative conditions can lead to rapid modifications of protein sulphydryls, including the formation of sulphenic acids (protein-SOH) through two-electron oxidation and thiyl radicals (protein-S$^\bullet$) through one-electron oxidation[114]. These partially oxidized products react with GSH to form S-glutathiolated proteins (protein-SSG). The glutathione cycle, involving enzymes like glutathione reductase, glutaredoxin, and thioredoxin, subsequently reduces protein-SSG back to protein sulphydryls (protein-SH). However, if oxidation of protein sulphydryls is not intercepted by GSH, further oxidation can lead to the irreversible formation of sulphinic (protein-SO_2H) and sulphonic (protein-SO_3H) acids[114]. The potent antioxidant capacity of thiol compounds like glutathione primarily arises from their sulphur atoms, which can readily accommodate the loss of a single electron[115]. Furthermore, the lifetime of sulphur radical species, particularly the thiyl radical (GS$^\bullet$), can be considerably longer than many other radicals generated during oxidative stress.

The reaction between GSH and a radical R$^\bullet$ can be described as follows:

$$GSH + R^\bullet \rightarrow GS^\bullet + RH$$

Thiyl radicals (GS$^\bullet$) may dimerize to form oxidized glutathione (GSSG):

$$GS^\bullet + GS^\bullet \rightarrow GSSG$$

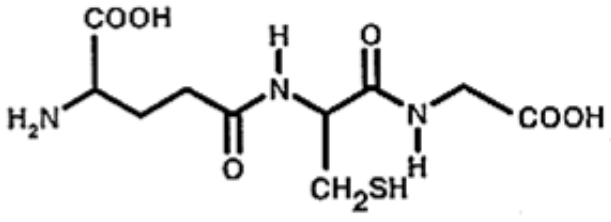

Figure 6: GSH (Reduced form)

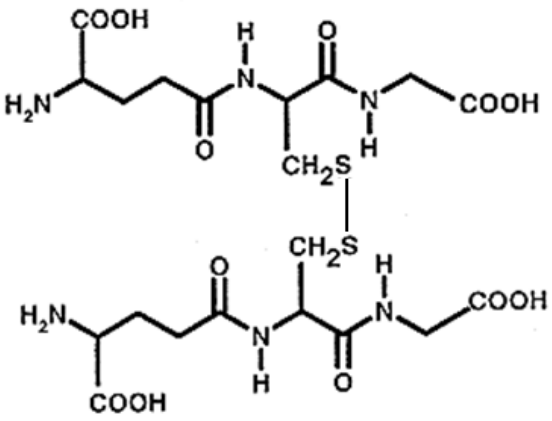

Figure 7: GSSG (Oxidised form)

Accumulated GSSG inside cells, and the GSH/GSSG ratio, serve as indicators of oxidative stress within organisms[116]. Excessive GSSG levels can potentially damage numerous enzymes via oxidative mechanisms. GSSG can also react with protein sulphydryl groups, forming protein–glutathione mixed disulphides:

GSSG + protein-SH ↔ protein-SSG + GSH

These mixed disulphides (protein-SSG) exhibit longer half-lives, likely due to protein folding processes. The main protective roles of glutathione against oxidative stress encompass several aspects[113]. Acting as a cofactor for detoxifying enzymes, including glutathione peroxidase (GPx) and glutathione transferase. Participating in amino acid transport through the plasma membrane. Direct scavenging of hydroxyl radicals and singlet oxygen, leading to the detoxification of hydrogen peroxide and lipid peroxides through the catalytic action of GPx. Regenerating essential antioxidants such as vitamins C and E, returning them to their active forms. Glutathione can directly reduce the tocopherol radical of Vitamin E or indirectly reduce semidehydroascorbate to ascorbate. The capacity of glutathione to regenerate vital antioxidants is closely related to the redox state of the glutathione disulphide–glutathione couple (GSSG/2GSH)[117]. This couple's half-cell reduction potential varies based on the redox environment it operates in. For instance, the redox potential is -180 mV in the endoplasmic reticulum and -232 mV in the cytosol[117]. Compartmentalization of GSH is linked to the distinct redox environments within these compartments. The glutathione system has a significant role in promoting overall health and preventing a range of diseases[118].Numerous studies, including our own researchhave revealed that a deficiency in cellular glutathione, whether due to reduced biosynthesis or increased depletion, can lead to various health challenges, such as oxidative stress, impaired immune function, susceptibility to viral infections, and an increased risk of cancer[99, 119-120]. Recent biomedical literature has also underscored that a lack of glutathione (GSH) is considered a leading factor in explaining the higher susceptibility to COVID-19 infection among the elderly population and individuals with comorbidities such as diabetes, cardiovascular, or pulmonary diseases[121]. As people age, their endogenous GSH levels naturally decline, rendering cells in older individuals, especially in lung tissue, more vulnerable to oxidative damage caused by environmental factors and viral attacks. There is compelling evidence indicating that glutathione deficiency, a common factor in many chronic diseases, can exacerbate oxidative damage in COVID-19 patients[121].

5.4. GLUTATHIONE PEROXIDASE

Glutathione peroxidase (GPx) exists in two forms: one is selenium-independent (glutathione-S-transferase or GST), while the other is selenium-dependent (GPx)[108]. These two enzymes differ in molecular structures and catalytic mechanisms. Glutathione metabolism plays a critical role in one of the most essential mechanisms of antioxidative defence. Humans possess four distinct selenium-dependent glutathione peroxidases[109]. All GPx enzymes are capable of adding two electrons to reduce peroxides by forming selenoles (Se-OH). These selenoenzymes' antioxidant properties enable them to neutralize peroxides, preventing them from becoming substrates for the Fenton reaction.

GPx functions in conjunction with the tripeptide glutathione (GSH), which is present in cells at high (micromolar) concentrations. GPx's catalytic reaction involves the substrate H_2O_2 or an organic peroxide ROOH. In the process, GPx decomposes peroxides into water (or alcohol) while simultaneously oxidizing GSH:

$$2GSH + H_2O_2 \xrightarrow{GPx} GSSG + 2H_2O$$

$$2GSH + ROOH \xrightarrow{GPx} GSSG + ROH + H_2O$$

Significantly, GPx competes with catalase for H_2O_2 as a substrate and serves as the primary defence against low levels of oxidative stress.

6. Cellular redox environment and oxidative stress

Oxidation and reduction reactions in biological systems are commonly referred to as redox reactions and form the foundation for numerous biochemical processes. In the context of biological systems, it's more suitable to use the terms antioxidant and pro-oxidant instead of reductant and oxidant, respectively[122]. A reductant, or reducing agent, donates electrons, while an oxidant, or oxidizing agent, accepts electrons. An oxidation process (oxidation reaction) involves a loss of electrons, while a reduction process (reduction reaction) entails the gain of electrons. This phenomenon is described by the redox (reduction/oxidation) theory of cellular functioning. Each cell maintains a specific concentration of electrons, known as its redox state. The redox state of a cell and its fluctuations play a crucial role in cellular differentiation[123-124]. Normally, the redox state of a biological system remains within a narrow range, akin to the regulation of pH. Under pathological conditions, however, the redox state can deviate towards lower or higher values. Notably, a 30mV change in the redox state corresponds to a tenfold change in the ratio between reductant and oxidant species[125]. The primary components responsible for intracellular "redox buffering" are glutathione and thioredoxin (TRX). Glutathione, represented by the 2GSH/GSSG couple, serves as the principal cellular redox buffer and an indicator of the cell's redox environment[125]. The intracellular concentration of glutathione is 500 times higher than that in the extracellular space, playing a crucial role in the cell's detoxification processes. In the endoplasmic reticulum, where the 2GSH/ GSSG ratio is relatively low, mixed disulphide formation and disulphide exchange are significant components of protein folding. However, under conditions of enhanced oxidative stress, the GSSG content increases through a specific reaction, leading to an increase in the content of protein mixed disulphides. A notable aspect is that GSSG seems to act as a non-specific signalling molecule, affecting various proteins involved in signalling processes. High ratios of reduced to oxidized GSH and TRX are maintained by the activities of GSH reductase and TRX reductase, respectively. Both these redox thiol systems counteract intracellular oxidative stress by reducing hydrogen peroxide and lipid peroxides through reactions catalysed by peroxidases. For instance, GSH peroxidase catalyses the reaction $H_2O_2 + 2GSH \rightarrow 2H_2O + GSSG$. Besides their antioxidant functions, GSH and TRX also participate in cell-signalling processes. Other crucial elements influencing redox regulation include exogenous molecules

such as ascorbic acid, carotenoids, and selenium. In recent years, the term "redox state" has expanded to describe not only the state of a redox pair, such as GSSG/2GSH, $Asc^{\bullet-}$/$AscH^{-}$, and others but also to characterize the broader redox environment of a cell[125]. Determining the molar concentrations of major redox couples is relatively straightforward in homogeneous fluids like plasma, enabling the evaluation of the redox environment. However, in cellular compartments, issues such as compartmentalization and non-equilibrium conditions may complicate determining the molar concentrations of specific redox couples. Since various cellular compartments like the cytosol, extracellular space, mitochondria, and nucleus lack significant gradients of various redox couples involving glutathione, it is possible to estimate the overall concentration of glutathione in each compartment[125]. The cellular redox environment is instrumental in signal transduction, enzyme activation, DNA and RNA synthesis, cell proliferation, differentiation, and apoptosis[124, 126]. Generally, cell death initiation is associated with an oxidative cellular environment, whereas a reducing environment supports increased cell proliferation. An example of this involves stimulated proliferation of certain tumour cells exposed to high thiol concentrations. Accordingly, antioxidants have been shown to prevent apoptosis[127]. Studies have demonstrated that the cell cycle is characterized by fluctuations in the redox environment, primarily mediated by changes in glutathione concentration within the cell[125]. Oxidizing molecules such as H_2O_2 and thioredoxin are present outside the cell and can enter cells, leading to alterations in the intracellular redox environment. Various levels of oxidants and antioxidants in the cell appear to be associated with the induction or inhibition of cell proliferation. A more reducing cell environment promotes proliferation, while a slight shift towards a mildly oxidizing environment initiates cell differentiation. Further progression towards a highly oxidizing environment in the cell results in apoptosis and necrosis. Apoptosis is triggered by moderate oxidizing stimuli, whereas necrosis is induced by intense oxidizing effects[127]. Each stage of the cell cycle has a distinct redox state characterized by a specific cellular reduction potential. In line with these findings, reduced glutathione (GSH) has been identified as playing a role in protecting cells from apoptosis. The depletion of GSH, leading to a more oxidizing cellular environment, has been observed to coincide with the onset of apoptosis. GSH loss is facilitated by specific membrane translocators that extrude GSH from the cell. Moreover, the release of mature Cytochrome c from mitochondria has been linked to glutathione depletion. It is important to note that the depletion of intracellular glutathione is not the sole factor influencing the decision to undergo apoptosis[125]. Nevertheless, the redox environment stands as a critical determinant for triggering apoptosis. In light of these findings, cancer cells are characterized by a more reducing cellular environment, representing an imbalance skewed towards cell proliferation. Extensive evidence suggests that redox balance is

disrupted in cancer cells compared to normal cells, potentially due to oncogenic stimulation. Altered levels of antioxidant enzymes (SOD, catalase, glutathione peroxidase) and non-enzymatic antioxidants (GSH, Vitamin C, thioredoxin), as well as changes in associated signal pathways, have been observed in various human cancers[128]. The cumulative production ofROS in many cancer cells is linked to altered redox regulation of signalling cascades. The reducing intracellular environment in the nucleus and mitochondria, maintained by elevated levels of glutathione and thioredoxin, not only prevents apoptosis but also promotes cell survival through the activation of redox-sensitive nuclear transcription factors[129]. The human DNA repair enzyme APE/Ref-1 is a dual-function protein that plays a crucial role in responding to oxidative stress and in DNA base excision repair. Additionally, APE/Ref-1 facilitates the DNA-binding activity of several transcription factors (AP-1, NF-κB, p53, and others) through both redox-dependent and redox-independent mechanisms[130]. Several studies have shown that upregulation of Ref-1 protects cells from various apoptosis triggers, including oxidative stress and radiation. Conversely, down-regulation of Ref-1 is associated with apoptosis and cell sensitization. Ref-1 is implicated in various stages of carcinogenesis (initiation, promotion, and progression), primarily through the maintenance of the intracellular redox balance, activation of cell survival signals, and repair of damaged DNA lesions. Elevated expression of Ref-1 has been observed in cervical, prostate, and ovarian cancers, with Ref-1 exhibiting both cytoplasmic and nuclear enzymatic activities[131]. Apoptosis is closely related to the Bcl-2 protein[132]. Bcl-2 can inhibit the release of Cytochrome c, preventing a decrease in glutathione concentration, thus shifting the cellular redox environment away from apoptosis. Cancer cells typically exhibit over expressed Bcl-2, which can enhance resistance to ROS-induced apoptosis. Notably, a defective redox regulation of progression from G1 to S in non-malignant cells has been found in cancer cells. While thiol antioxidant-induced modulation of the intracellular redox state results in G1 arrest in non-malignant cells, tumour cells continue to cycle. Recent studies indicate that understanding the mechanisms through which TRX, GSH, and Ref-1 maintain intracellular "redox buffering" capacity can aid in the development of targeted cancer prevention and therapeutic drugs[133].

7. Conclusion

The production of ROS is a natural consequence of aerobic life and is an unavoidable process. ROS continually pose challenges to the integrity of our genetic material. Its effects can be influenced by various factors such as diet, hormones, and environment. Excessive production of ROS due to internal or external factors can lead to oxidative stress, which can be detrimental to living organisms. ROS not only have the potential to damage DNA but can also affect other cellular components like proteins and lipids, leaving behind reactive by-products that can interact with DNA bases. One extensively studied DNA lesion is the

formation of 8-OH-G, which is significant because it forms relatively easily, possesses mutagenic properties, and can serve as a potential biomarker for cancer development. DNA mutations play a crucial role in carcinogenesis, and elevated levels of oxidative DNA damage have been observed in various types of tumours, suggesting a possible link between such damage and cancer development. While the exact extent of oxidative DNA damage's contribution to carcinogenesis remains uncertain, it appears to be primarily associated with the initiation phase of this process. Additionally, ROS participate in cell-signalling pathways related to cell growth regulation, making them instrumental in carcinogenesis. The regulation of cell growth is a complex process, and the role of ROS in this context depends on the type and concentration of the specific radicals involved. Activation of transcription factors, including MAP-kinase/AP-1 and NF-κB pathways, has a direct impact on cell proliferation and apoptosis, with oxidative stress being a common factor in all these events. Although the involvement of oxidants at various stages of malignant transformation is evident, many details regarding the specific role of ROS-induced damage in multifactorial diseases like cancer remain to be elucidated. The effect of oxidative stress on carcinogenesis depends on the type, reactivity, and concentration of the radicals involved. To confidently identify valid biomarkers for cancer incidence, extensive studies involving the long-term monitoring of DNA in healthy individuals are required to identify those who eventually develop cancer. The detrimental effects of oxidative stress are countered by both antioxidant enzymes and non-enzymatic antioxidants. Among these, manganese superoxide dismutase (Mn-SOD) is considered one of the most effective antioxidant enzymes with potential anti-tumour activity. Experimental evidence suggests that abnormally high levels of Mn-SOD, while suppressing cell growth, may also enhance the invasive potential of cancer cells. It is conceivable that an imbalance between superoxide radical formation and hydrogen peroxide degradation in cells over expressing Mn-SOD could activate the metastatic potential of cancer cells. However, antioxidant protection therapy against free radicals should be administered with caution, as its effectiveness depends on the cancer stage at which it is introduced. When used during the cancer progression stage, antioxidant therapy might inadvertently stimulate tumour growth by promoting the survival of cancer cells. Additionally, it's essential to consider that some antioxidants can exhibit pro-oxidant properties depending on their concentration and the oxygen levels in their environment. To prevent cancer related to oxidative stress, a crucial approach is to minimize exposure to both internal and external sources of oxidative stress, including the elimination of environmental carcinogens and carcinogenic metals to the extent possible. In this context, prevention is a more effective strategy than treatment.

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