Oxidative stress in cancer therapy: Friend or enemy?

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Drugs used in the clinic
Metal-based anticancer drug candidates

Oxidative stress

suppress cancer

promote cancer

Apoptosis
Autophagy
Necrosis

Angiogenesis
Tumourigenesis
Metastasis
Abstract: Excessive cellular oxidative stress is widely perceived as a key factor in pathophysiological conditions and cancer development. Healthy cells use several mechanisms to maintain intracellular levels of reactive oxygen species (ROS) and overall redox homeostasis to avoid damage to DNA, proteins, and lipids. Cancer cells, in contrast, exhibit elevated ROS levels and upregulated protective antioxidant pathways. Counterintuitively, such elevated oxidative stress and enhanced antioxidant defence mechanisms in cancer cells provide a therapeutic opportunity for the development of drugs with different anticancer mechanisms of action (MoAs). In this review, oxidative stress and the role of ROS in cells are described. The tumour-suppressive and tumour-promotive functions of ROS are discussed to compare these two different therapeutic strategies (increasing or decreasing ROS to fight cancer). Clinically approved drugs with demonstrated oxidative stress anticancer MoAs are highlighted before describing examples of metal-based anticancer drug candidates causing oxidative stress in cancer cells via novel MoAs.

1. Introduction

Cancer is the second leading cause of death and a global burden accounting for 9.6 million deaths in 2018 worldwide.\(^1\) It is the most important barrier for increasing life expectancy in every country of the world in the 21st century.\(^2\) There is a need for the development and screening of novel anticancer therapeutics with non-conventional modes of action (MoAs), offering unique antiproliferative and tumour-inhibiting properties. Current strategies for the treatment of cancer include targeting the cellular redox balance. The main objective of this review is to discuss the role of redox modulation in cancer therapy. The key question raised is how oxidative stress influences cancer cells and whether the function of reactive oxygen species (ROS) is cancer promotive or cancer suppressive. There has been a great deal of debate regarding the function of ROS in cancer cells, with the two conflicting aspects of (i) oxidative stress plays an important role in tumour initiation and progression, and (ii) oxidative stress can be used for the initiation of cancer cell death. This in turn raises arguments among the scientific community in terms of how redox modulation can be used as an anticancer therapeutic strategy. The use of antioxidants as a means to decrease intracellular ROS and protect cells from tumour development and ROS-induced toxicity stands against the use of ROS-inducing anticancer agents which can elevate ROS levels with the aim to promote cancer cell death. Both concepts are very complex and involve various important factors present in the tumour environment and its cellular processes. Factors related to ROS such as their nature, concentration, and location, have been suggested to play a significant role. High levels of ROS can induce cellular death and be detrimental to tumours. On the contrary, low ROS levels can contribute to the initiation and development of cancer. This greatly contributes to the idea that ROS in cancer are double-faced. This review provides an overview of the tumour-suppressive and tumour-promotive functions of ROS. Apoptosis, autophagy, and necrosis, as major ROS-induced types of cell death, are discussed. In addition, the role of ROS in tumorigenesis, angiogenesis, and metastasis, is described. Current strategies for the treatment of cancer involving ROS are also reviewed. Finally, we have provided examples of organic and metal-based anticancer drugs causing oxidative stress, with the aim to support the idea that induction of high levels of ROS is a promising strategy for the treatment of cancer.

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2. Oxidative stress in a nutshell

2.1. What is oxidative stress?

ROS are molecules that contain oxygen and are constantly produced as byproducts from various cell functions such as cellular respiration and energy production. ROS can also be generated after exposure to irradiation, air pollutants, or toxic substances. Species such as the superoxide (\(O_2^-\)) and hydroxyl
Molecular oxygen ($O_2$) contains two unpaired electrons with parallel spins located in two separate π* orbitals, in its ground state (Figure 1). This is due to the fact that there are two molecular orbitals with the same energy to be occupied, and each electron occupies one according to Hund’s rule. Having two electrons with the same spin makes oxygen less reactive as the molecule can undergo reactions using only one electron at a time. This restricts oxygen from reaction with organic molecules because most of them have paired electrons. Such reactions are possible but very slow because a spin inversion will be required which in this case is not allowed by the spin conservation rule. However, if one of the electrons changes its spin due to excitation (e.g., energy received from light or excited molecules), this results in singlet oxygen ($^1O_2$). This is a very reactive oxidant which has two electrons with opposite spins (therefore no spin restriction) and these can react easily with other pairs of electrons.[8]

Molecular oxygen can undergo redox reactions involving one electron, for instance with molecules capable of single-electron transfer (e.g., flavin enzymes and coenzymes, organic radicals, transition metal ions).[5] This can result in the formation of intermediates such as $O_2^-$ which is far more reactive than ground state molecular oxygen and can serve as a base for the formation of other ROS and mediate oxidative chain reactions (Figure 2). One-electron reduction of $O_2^-$ (known as dismutation) results in the formation of $H_2O_2$, which when further reduced can yield OH. This hydroxyl radical is highly reactive and can easily damage macromolecules. This species cannot be eliminated by an enzymatic reaction but can undergo a reduction reaction leading to either hydroxide OH or water. Hydrogen peroxide can undergo decomposition to water and oxygen, this process is catalysed by the enzymes catalase and glutathione peroxidase.

Figure 1. Molecular orbital diagrams for ground state molecular oxygen ($O_2$), singlet oxygen ($^1O_2$), the superoxide anion $O_2^-$, and peroxide ion $O_2^{2-}$ (resulting species from deprotonation of $H_2O_2$). Reproduced with permission.[5]

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Generation of ROS is a normal biological process which has a role in metabolism, cell proliferation, differentiation, signal transduction, host defense, and cell death.[9] The most redox active parts in the cell include mitochondria, peroxisomes, and endoplasmic reticulum (ER). The major intracellular source of ROS in cells takes place in the mitochondrion, where 2% of its consumed oxygen is reduced to form superoxide.[10] Peroxides, found in the cytoplasm of eukaryotic cells, are able to produce as well as to scavenge ROS, and ER has an oxidizing environment where ROS levels are elevated through protein oxidation.[10][11] ROS can be either beneficial or detrimental to cells. In low-to-moderate levels, ROS are important for cell proliferation and differentiation, and for activation of stress-responsive survival mechanisms.[12] However, when this level is increased, ROS can induce cellular damage such as DNA single-strand breaks, mitochondrial membrane disruption, lipid peroxidation, and changes in secondary protein structures.[13] Therefore, living organisms modulate the levels of ROS by maintaining a complex system with various pathways for the production and elimination of ROS in order to keep a tight balance for the oxidant-antioxidant systems. Once this balance is disturbed, cells suffer from oxidative stress which in turn leads to cell damage and cell death.

2.2. How cells cope with oxidative stress

In order to fight oxidative stress, living organisms have developed a well-coordinated system that is able to cope with stressors and high levels of generated ROS. For this purpose, cells have several antioxidant systems which are capable of dealing with oxidative stress and preventing redox changes in the cellular environment. These can be divided into enzymatic and non-enzymatic antioxidants. The key enzymatic antioxidants include the superoxide dismutase family (SOD1, SOD2, SOD3), catalase (CAT), and peroxidases.[13] Superoxide dismutases catalyse the conversion of the superoxide $O_2^-$ radical to either $O_2$ or $H_2O_2$. The generated hydrogen peroxide is further converted into $H_2O$ by CAT or peroxidases.[14] Non-enzymatic antioxidants react directly with the oxidants and among the most important ones in cells are the tripeptide glutathione (GSH), thioredoxin (Trx), and ascorbate (the monodeprotonated form of ascorbic acid) (Figure 3). Both ascorbate and GSH can be found...
in millimolar concentrations in cells, where GSH is naturally occurring in cells, and ascorbate is a nutrient which has to be ingested via food. Ascorbate is an important reducing agent which can scavenge ROS species such as OH\(^{-}\), RO\(^{•}\), ROO\(^{•}\), and GS\(^{-}\), followed by formation of the less reactive semidehydroascorbate free radical which is then reduced by GSH. Ascorbate is also known to reduce a number of redox-active metals such as iron and copper, however the situation of ascorbate interfering with metalloanticancer drugs is unclear.

"Figure 3. Non-enzymatic antioxidants described herein. Structures of a) glutathione, b) ascorbate, c) reduced form of thioredoxin. Adapted with permission." Copyright 2008, ASBMB.

GSH is another important cellular antioxidant which plays a major role in maintaining the redox balance in cells. It is a tripeptide, synthesised in the cytosol, and it is known to scavenge hydroxyl and superoxide radicals. Apart from acting as an antioxidant within cells, directly interfering with ROS species, GSH has several other functions of biological significance. Depletion of GSH pools has been linked to cell death since this event leads to increase in ROS levels, excessive oxidative stress and apoptosis. GSH contains coordination sites for different metal ions, for instance arsenic, cobalt, copper, zinc, and platinum. A number of metal complexes containing Pt\(^{II}\), Co\(^{II}\), and Ru\(^{II}\), were reported to undergo GSH-mediated reduction of the metal ion which is crucial for unlocking their anticancer activity. In contrast, elevated GSH levels have been linked to resistance of cells to metallo-drugs. Indeed, increase of GSH pools has been shown to lead to detoxification and resistance to some Pt\(^{II}\) and As\(^{III}\) drugs, including cisplatin.

The Trx system is the third important antioxidant defense system in cells. Trx are small polypeptides with an active disulfide site, which have several biological functions including regulation of redox balance, protection from apoptosis, delivery of electrons for the substrate turnover cycle of ribonucleotide reductase, and influencing the activity of other antioxidant defense systems such as peroxiredoxins, antioxidant heme oxygenase-1, ascorbate, and flavonoids.

2.3. Role of ROS in cancer cells

In general, cancer cells have higher levels of ROS as compared to healthy tissues (Figure 4). This is due to the fact that cancer cells require more energy as they grow and spread uncontrollably. This results in alterations of various signaling pathways which affect the cellular metabolism and lead to an imbalance between ROS being produced and eliminated, causing excessive oxidative stress. The role of ROS in cancer cells is still controversial owing to their oncogenic and tumour-suppressive properties. Alterations of redox balance can lead to DNA mutations and tumour formation and/or progression. Studies have shown that 45-80% of patients diagnosed with breast cancer use antioxidant supplements (e.g. vitamins or natural products) as some clinicians have suggested using them for cancer prevention, as well as to help decrease unwanted side effects from chemotherapy. However, cancer cells have developed diverse protective mechanisms in order to keep the level of accumulating ROS moderate and as a contrary belief, antioxidants promote cancer cells survival and proliferation. Therefore, inhibiting advanced antioxidant systems or increasing the production of ROS in cancer cells, or a combination of both, present interesting therapeutic strategies for selectively killing tumorous cells over healthy tissues.

"Figure 4. ROS levels in cancer and healthy cells and their regulation in cancer therapy. Adapted with permission." Copyright 2017, Frontiers.

Immunogenic cell death (ICD) is a type of cell death when the immune system is activated in a dysfunctional way. Damage-associated molecular patterns (DAMPs; e.g. surface-exposed calreticulin (CRT), secreted ATP and high mobility group protein B1 (HMGB1)) are released from dying tumour cells into the tumour micro-environment, and this results in the activation of tumour-specific immune responses. This ICD therefore shortcuts the action of anticancer drugs. Furthermore, DAMPs may activate disease-associated microglia (DAM), which in turn leads to ROS generation and persistent inflammation.

Interestingly, chronic exposure to DAMPs also leads to the transition of immature dentritic cells to a mature phenotype, thereby facilitating the processing of phagocytic loads in dentritic cells and the engulfment of antigenic components by the cells. Such antigen presentation causes specific T cell responses with the ability to kill cancer cells. Induction of ICD can therefore result in protection against tumour via development of
antitumoural immunity. Emerging methods for the development of ICD inducers were recently surveyed in the literature.\textsuperscript{31}

A number of natural products and pharmacological agents have been demonstrated to exhibit such a cancer-preventive activity. As an example, non-steroidal anti-inflammatory drugs are potent and well-studied ICD inducers. Such molecules induce an antitumoural activity involving both mitochondrial apoptotic pathways and improvement of T-cell function.\textsuperscript{28} Anticancer metal drugs are also promising tools for immunotherapeutic intervention.\textsuperscript{33} Radiation therapy, although designed to trigger DNA damage and cell death, is also recognised as a method providing systemic antitumour immunity, via modulation of the peptide repertoire.\textsuperscript{34}

3. ROS in cancer therapy

ROS are involved in various signaling pathways and for this reason they have a dual role in cancer treatment. Through pro-tumourigenic signaling, ROS promote cancer cell proliferation, survival, and adaptation to hypoxia. On the other hand, via anti-tumourigenic signaling, oxidative stress is induced by ROS and death cell follows. The nature of the free radicals and their concentration influence these two contradicting functions of ROS. For example, a low intracellular level of ROS can contribute to tumour promotion.\textsuperscript{35} On the contrary, a high level of ROS can lead to cellular damage and tumour suppression.\textsuperscript{25} Location is moreover a key factor to keep in mind when determining ROS function; indeed, mitochondrial ROS are known to be able to induce cell death, while nitrogen oxides-generated (NOx) ROS have been linked to cell proliferation and migration.\textsuperscript{36} Antioxidants also have a complex role owing to their capacity to either protect normal cells from toxic radicals, or stimulate tumour growth. While in normal cells, the formation of malignancies is prevented by the presence of antioxidants,\textsuperscript{37} antioxidants contribute in developed tumours to cell growth, enhance resistance mechanisms, and they also interfere with ROS-dependent anticancer therapies.\textsuperscript{38} Modern cancer treatment approaches relying on the modulation of the redox balance include ROS-depleting therapy with the use of antioxidants, or ROS-elevating therapy which allows for an increase of intracellular ROS levels either directly or through the inhibition of antioxidant systems.

3.1. The tumour-suppressive functions of ROS

Anticancer therapies involving the generation of oxidative stress have gained considerable attention in recent years. Excessive levels of ROS and the subsequent oxidative stress result in various forms of cell death. Three major ROS-regulated forms of cell death are discussed herein: apoptosis, autophagy, and necroptosis. Additional cell death types which can be activated by ROS such as ferroptosis, cornification, paraptosis, pyronecrosis, excitotoxicity, mitotic catastrophe, anoikis, entosis, are known\textsuperscript{39} but will not be described in this review.

Apoptosis

Apoptosis is a regulated cell death process of self-destruction and it is one of the three major forms of programmed cell death (PCD). Apoptosis (type I PCD) is the most common form of cell death known and is described as a series of events that lead to the death of a cell in an orderly way.\textsuperscript{40} It is characterised by distinct morphological and biochemical features including cellular and nuclear volume reduction, chromatin condensation, membrane surface blebbing, nuclear fragmentation, cytoskeletal disintegration, and the formation of apoptotic bodies which are membrane-bound fragments formed as a result of the breakdown of the cell and can be eaten by nearby cells.\textsuperscript{41} The degradation of cellular components can occur either through caspase-dependent or-independent pathways. These caspases are cysteine proteases which can be activated through two major pathways, named the intrinsic and extrinsic pathways.\textsuperscript{42} The intrinsic pathway, also known as mitochondria-mediated pathway, can be activated by various cellular stimuli such as mitochondrial DNA (mtDNA) damage, growth factor deprivation, heat shock, hypoxia, ER stress, etc.\textsuperscript{43} Mitochondria-generated ROS can affect different intracellular structures, for instance mtDNA, which upon oxidative stress can have a strong effect on the transcription of proteins involved in the electron transport chain and thus cause disruption of the respiratory chain function, followed by additional ROS production, loss of mitochondrial membrane potential and disturbed ATP synthesis.\textsuperscript{43} ROS such as H$_2$O$_2$ and superoxide have been shown to cause the release of cytochrome c from the mitochondria into the cytosol and as a result induce apoptosis through the mitochondrial pathway.\textsuperscript{44} The extrinsic pathway, also described as the death-receptor-dependent apoptotic pathway, is governed by specific transmembrane receptors which belong to the tumour necrosis factor (TNF) receptor superfamily.\textsuperscript{45} The cytokine TNF-α is an important regulator in cellular signaling processes and plays a role in both cell survival or apoptotic cell death. TNF-α and ROS can actually influence each other, where low ROS levels are essential for TNF-receptor 1 signaling pathway, and high ROS levels lead to DNA damage and the subsequent cell death.\textsuperscript{46}

Autophagy

Autophagy is a tightly regulated catabolic process of cellular self-degradation which involves the recycling of damaged components and organelles as a consequence of diverse stress stimuli such as nutrient deprivation, viral infection, and genotoxic stress.\textsuperscript{47} The cellular functions of autophagy can be regarded as both being promotive as well as suppressive, and as such its role in cancer is considered to be dual.\textsuperscript{48} On the one hand, autophagy is necessary for the maintenance of homeostasis and cell survival due to its essential function of eliminating harmful cellular organelles which could otherwise cause tumour initiation. Evidence comes from the fact that a decrease in the expression levels of autophagy-related proteins such as autophagy-related gene (Atg) proteins, can play a role in promoting the initiation and development of cancer.\textsuperscript{49} On the other hand, autophagy can positively influence established tumours which use this cellular process as a survival mechanism in response to starvation, hypoxia, and chemotherapeutic agents.\textsuperscript{50} Studies show that ROS have a role as signaling molecules in inducing autophagy in cancer cells. For instance, increased levels of H$_2$O$_2$ have been linked to the induction of autophagic cell death in human colon cancer cells.\textsuperscript{51} mTOR, the mammalian target of rapamycin, which is a major negative regulator of autophagy, can be negatively influenced by ROS such as H$_2$O$_2$ which inhibit the activity of mTOR and thereby initiate autophagy.\textsuperscript{52}

Necroptosis

Necrosis was initially considered as an accidental and unregulated form of cell death caused by severe injuries and adverse conditions. However, recent studies have revealed that
necrosis can occur in a genetically controlled and highly regulated manner, and since then this process was termed “necroptosis”. This form of programmed cell death is characterised by a number of morphological and biochemical features such as loss of plasma membrane integrity, swelling of the cytoplasm and organelles, spillage of cellular organelles in the microenvironment, and depletion of ATP levels. Tumor necrosis factor (TNF) is an important cytokine which has been associated with necroptosis. Studies demonstrated that TNF induces mitochondrial ROS which in turn promote the formation of necrosome. When ROS are scavenged or the electron transport chain is inhibited, this leads to inhibition of the TNF-induced necroptosis. In general, apoptosis is the default cell death pathway and necroptosis is the back-up system that deals with the cells which were not affected by apoptosis. It is hypothesised that high levels of ROS induce apoptosis while massive levels lead to necroptosis. Moreover, ROS have been reported to induce both apoptotic and necrotic cancer cell death. Evidence comes from a study of the role of H2O2 in Jurkat T-lymphocytes, where low H2O2 levels induced apoptosis by caspase activation, and in the case of high concentration of H2O2 there was no evident caspase activity and necroptosis was caused. Additional studies have reported that ROS promote the change of apoptosis to necroptosis in prostate cancer and hepatoma cells.

**Increase of ROS levels as a therapeutic strategy**

During the transition of normal healthy tissues to invasive carcinoma, the level of ROS increases due to metabolic aberrations and the high energy demand of cancer cells. As a result, cancer cells naturally have higher levels of ROS and consequently better antioxidant mechanisms. As the level of ROS in cancer cells is already high, pushing these levels even higher can interfere with the ability of cancer cells to cope with such species and in turn make them undergo apoptosis (Figure 5). As such, cancer cells might be more sensitive than normal cells to therapeutic agents which cause oxidative stress. Examples of clinically approved anticancer drugs which are known to induce high levels of ROS as part of their MoAs, are provided in section 4 of this review. In addition, some metal-based anticancer candidates are also capable of increasing intracellular ROS and cause oxidative stress leading to cancer cell death, and such examples are described in section 5.

**Figure 5. ROS levels and their impact on tumour microenvironment.**

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3.2. The tumour-promotive functions of ROS

ROS are continuously generated as a result of various cellular processes and received from many exogeneous sources. ROS play an important role in various cellular functions because they are involved in a number of signaling pathways, however, when their level jumps to excessive amount and can no longer be suppressed, they can cause damage to DNA and proteins. In most cases, repair mechanisms will be activated and the damage will be reduced, otherwise apoptosis will be initiated. In some cases, DNA can be damaged excessively, and this can lead to the formation of cancerous cells. When ROS cannot be neutralised after a period of accumulation, this results in DNA damage and it can contribute to tumour proliferation, survival, chronic inflammation, angiogenesis, and metastasis. In this section, some of the important tumour-promoting functions of ROS are discussed.

**Tumourigenesis**

The production of ROS can lead to genetic as well as epigenetic alterations in the cellular environment. ROS-induced genetic alterations include DNA bases modifications, strand breaks, and DNA-protein cross linkages. These cellular processes lead to an increase of the mutational rates of the cell thus disturbing critical cellular processes, for instance DNA repair, and are all involved in the initiation stages of tumourigenesis. Indeed, such ROS-induced DNA damage in combination with the inability of cells to undergo DNA repair mechanisms, are well-known features of some human cancers as demonstrated in breast cancer cell lines and human breast tumour tissue sample. In such scenarios of oxidative stress, ROS attack DNA leading to the formation of single and/or double strand breaks, as well as pyrimidine and purine lesions. A very important oxidative DNA damage product is the oxidative lesion 8-oxo-7,8-dihydroguanine (8-oxoGuA) which is found in tissues low in antioxidant enzymes and high in ROS levels, and can serve as a reliable biomarker of oxidative stress. Patients with lung, breast, and prostate cancer had almost 50% higher rates of 8-oxoGuA when compared to healthy individuals. Such high levels of 8-oxoGuA alongside other DNA lesions are thought to serve as risk factors in the transition process of benign to malignant tumours.

ROS-induced epigenetic alterations (e.g., changes in DNA methylation patterns and histone modifications) induce the silencing of tumour suppressor genes and activate various oncogenes, both of which promote the transformation of normal to malignant cells. Such epigenetic alterations are related to both hypo- and hypermethylation of DNA. For instance, hydroxyl radical-induced DNA lesions can interfere with the ability of DNA to act as a substrate for DNA methyltransferases (DNMTs) resulting in decreased DNA methylation. On the contrary, high levels of ROS can hypermethylate tumour suppressor gene promoter regions and thereby induce gene silencing which can then lead to the formation of malignant tissues.

**Angiogenesis**

Angiogenesis is the growth of new blood vessels from pre-existing capillaries in order to facilitate oxygen and nutrient supply to cancer cells and promote their growth and rapid proliferation. This process plays a significant role in the progression and metastasis of tumours and it has been considered as one of the six hallmarks of cancer. Some of the key events of angiogenesis include endothelial cells (ECs) proliferation, migration, and tubular structure formation before the formation of mature blood vessels. The process of angiogenesis is mediated by a large network of pro- and anti-angiogenic factors, among which vascular epithelial growth factor (VEGF) is the most important regulator of angiogenesis.
because the VEGF signaling pathway maintains the occurrence and balance of blood vessels. A series of in vitro and in vivo studies have indicated that ROS, such as superoxide and hydrogen peroxide, act as signaling mediators of angiogenesis and metastasis of cancer cells through the induction of transcription factors and genes involved in these processes. For instance, it has been reported that H$_2$O$_2$ increases the expression of VEGF in vascular smooth muscle cells and ECs and therefore contributes to angiogenesis. The role of ROS in promoting the formation of new blood vessels in cancer cells has also been observed in animal models of various cancers: breast cancer, bladder cancer, lung cancer, melanoma, sarcoma, colon cancer, and prostate cancer. Interestingly, even in the presence of VEGF inhibitors, ROS can cause the release of other growth factors, for example fibroblast growth factor (FGF) and angiopoietin, which then bind to their receptors in ECs and induce the process of revascularisation. This demonstrates the ability of ROS to circumvent the activity of VEGF inhibitors and promote angiogenesis through the activation of other important factors in this cellular process.

**Invasion and metastasis**

Metastasis is the spread of cancer cells from the primary tumour to adjacent tissues and distant sites, and it is the final most devastating stage of malignancy. In fact, the majority of cancer deaths from solid tumours are caused by metastasis, making it the leading cause of cancer morbidity and mortality. The process of metastasis is very complex and comprises steps of invasion, migration, entry and survival in the blood circulation, extravasation, and proliferation of the cancer cells in the new location. Among all the cancer cells that enter the blood circulation, a limited number of these survive, and even a smaller fraction manage to grow into new colonies. Interestingly, since the tumour environment lacks oxygen and nutrients, and offers the conditions of hypoxia and inflammation, it has been suggested that metastasis could be a strategy by which cancer cells escape cell death and ROS are involved in various stages of invasion and metastasis. It has been found that intracellular ROS induce epithelial-mesenchymal transition (EMT), the process of epithelial cells losing their polarity and gaining mobility, which is a major cause of metastasis. Several studies have demonstrated that ROS contribute to the initiation of EMT through the upregulation of matrix metalloproteinases (MMPs), which are an important family of enzymes known to degrade the proteins found in basal membrane. In addition, ROS regulate the expression of other factors involved in EMT such as E-cadherin, integrins, and Snail. Moreover, recent studies have shown the role of ROS in the regulation of growth factors related to the resistance of cancer cells to anoikis. Anoikis is a type of apoptotic cell death which occurs when cells lose contact with the extracellular matrix or neighboring cells. ROS induced by the enzyme system NADPH oxidase have been observed to upregulate epidermal growth factor receptor (EGFR) which is involved in cell survival and anoikis inhibition. Such anoikis resistance provides cancer cells with the ability to survive after detaching from their primary tumour site as well as during the process of movement through the circulating system. However, it has been also suggested that ROS are necessary for early stages of cancer development but suppress the process in later periods, a theory supported by recent studies in melanoma.

**Decrease of ROS levels as a therapeutic strategy**

Based on the hypothesis that moderate ROS levels greatly contribute to the molecular and biochemical alterations required for initiation, progression, and survival of tumours, decreasing these intracellular ROS levels was proposed as a novel preventive and therapeutic anticancer strategy. In this context, the use of antioxidants in the treatment of cancer has been extensively studied. Antioxidant-based drugs are believed to act through directly scavenging toxic radicals, promoting enzymes involved in the elimination of ROS, and/or inhibiting NOx activity. Studies supporting this idea demonstrated that N-acetylcysteine (NAC) and vitamin C inhibit carcinogenesis in an animal model by reducing the expression of the hypoxia-inducible factor 1-alpha (HIF-1a) which is known to play a role in the process of angiogenesis. In addition, by administration of mitochondrial antioxidants the process of tumourigenesis in mouse models was effectively decreased. Chemotherapy causes lipid peroxidation which depletes the cellular levels of antioxidant vitamins and minerals; this, in turn, contributes to the generation of oxidative stress in cells. Therefore, the use of antioxidants in this scenario is believed to be beneficial for cancer patients who have been put through continuous cycles of chemotherapy. Supporting evidence comes from a study where vitamin E was reported to reduce toxicity caused by chemotherapy, and in combination with omega-3 fatty acid to increase the survival time of terminal cancer patients. The above mentioned studies seem promising but an increasing number of additional studies as well as clinical trials have demonstrated contradictory results. In clinical trials of head and neck, lung, or prostate cancers, dietary supplementation with the antioxidants β-carotene, vitamins A or E, or NAC, did not result in a decrease in the occurrence of tumours. Indeed, treatment with the these supplements led to an increased incidence of cancer and mortality in patients with lung and prostate cancers. More studies demonstrated that treatment with the antioxidant NAC promotes the progression of lung cancers and melanoma. Possible reasons for such results could be the protective nature of antioxidants in cells as they can interfere with events such as ROS-induced apoptosis which is essential for the elimination of precancerous and transformed cells. Moreover, supplementary antioxidants may be interfering with ROS-inducing radio- and chemotherapies by reducing the tumour-suppressive functions of radical species at the tumour site. Because of these contradicting results, the use of antioxidants in cancer therapy has been a highly controversial topic.

4. Clinically approved drugs causing oxidative stress

4.1. Organic drugs

In terms of chemotherapy options, several organic anticancer drugs which are used in the clinic are known to induce oxidative stress (Figure 6). Examples include taxanes (e.g., paclitaxel, docetaxel), vinca alkaloids (e.g., vinblastine, vinorelbine), and antimitabotiles (e.g., fluorouracil, fludarabine), which have been shown to promote the release of cytochrome c from the mitochondria causing cell death, and to disturb the electron transport chain leading to the production of free radicals. Other therapies affecting the redox balance in cells include anthracyclines (e.g., doxorubicin, epirubicin, daunorubicin). The mechanism of action of doxorubicin involves generation of an
excessive amount of ROS and reactive nitrogen species (RNS), which leads to DNA damage, mitochondrial dysfunction, decrease in protein synthesis, and disturbance of calcium homeostasis. Since doxorubicin is mainly accumulated in the myocardium and the level of antioxidants present in the cardiomyocytes is low, treatment with this agent leads to serious cardiac dysfunction.\textsuperscript{[105]}

Current strategies for increasing oxidative stress in cancer cells include interfering with redox systems, and more specifically with glutathione (GSH) and thioredoxin systems. High level of GSH content in cancer cells is often related to drug resistance (e.g., resistance to platinum-containing drugs, anthracyclines or alkylating agents)\textsuperscript{[106]} and therefore modulating GSH levels through blocking GSH synthesis (e.g., buthionine sulfoximine (BSO) inhibits glutamate cysteine ligase (GCL) which is the rate-limiting enzyme during the \textit{de novo} synthesis of GSH)\textsuperscript{[107]} appears to be a promising approach for improving the therapeutic effect of anticancer drugs. Changes in the ratio of glutathione disulfide (GSSG) and GSH can lead to oxidative stress and cell death; such activity has been shown by the anticancer drug NOV-002 which is a GSSG mimic containing 0.001 mol\% of cisplatin.\textsuperscript{[108]} NOV-002 is a competitive substrate for the membrane-associated enzyme gamma-glutamyl-transpeptidase (GGT) and thus can help increase the activity of GGT. This in turn stimulates the production of ROS leading to S-glutathionylation of proteins, ER stress, and apoptosis.

![Figure 6. Structures of organic drugs described in section 4.1.](image)

Poly(ADP-ribose)polymerase (PARP) is an enzyme which is overexpressed in different cancers and has a role in sustaining DNA integrity, as well as in the response to oxidative stress. PARP inhibitors in combination with platinum-containing drugs have been shown to negatively impact the ability of cancer cells to cope with oxidative stress. For instance, patients with triple-negative breast cancer in phase 2 clinical trials, when treated with a combination of veliparib (PARP inhibitor) and carboplatin, benefited from the therapy.\textsuperscript{[109]} Moreover, treatment of \textit{Brca}1- and \textit{Brca}2-deficient mouse models with the PARP inhibitor AZD2281 (olaparib) and platinum-based anticancer drugs led to a delay in tumour growth.\textsuperscript{[110][111]} The synergy between PARP inhibitors and cisplatin in non-small cell lung cancer (NSCLC) cells can cause DNA damage foci, mitochondrial membrane
permeabilisation, and release of cytochrome c, therefore sensitising NSCLC cells to the cytotoxic effect of cisplatin.\textsuperscript{[112]} ER stress can also be useful for the induction of oxidative stress and initiation of cell death. This is achieved through disturbing the ER capacity of protein folding, leading to accumulation of misfolded proteins and subsequent activation of a defense mechanism known as the unfolded protein response (UPR).\textsuperscript{[113]} In the case of high level of such ER stress, cells are not able to cope with it and undergo apoptosis. Several ER stress and ROS inducers have been reported including celecoxib, bortezomib, and nelfinavir.\textsuperscript{[114][115][116]}

4.2. Cisplatin

Platinum-based anticancer drugs (\textit{e.g.}, cisplatin, carboplatin, and oxaliplatin) have also been shown to induce high levels of oxidative stress (Figure 7).\textsuperscript{[117]} Cisplatin is well-known for its direct interaction with DNA\textsuperscript{[118][119]} and thus inducing cytotoxicity by blocking transcription and/or DNA replication processes. In addition, it also leads to permanent cell death by inducing apoptosis through the activation of a number of signal transduction pathways (\textit{e.g.}, calcium signaling, death receptor signaling, activation of mitochondrial pathways).\textsuperscript{[120]}

Cisplatin targets nuclear DNA and therefore cannot distinguish between cancerous and healthy cells, leading to systemic toxicity.\textsuperscript{[121]} Additionally, the Pt\textsuperscript{II} complex reacts with other biomolecules in the cellular environment as platinum has high affinity for sulfur and selenium atoms from many proteins and peptides. The drug has been found to disturb the GSH and thioredoxin reductase (TrxR) antioxidant systems. The mechanism of cisplatin resistance involves a reaction of cisplatin with GSH, where GSH easily coordinates to the metal center forming cisplatin-conjugates. This leads to detoxification of cisplatin but also to depletion of the intracellular GSH levels and as a result regulation of the redox balance and production of high levels of ROS.\textsuperscript{[122][123]} It has been found that there is a direct interaction between cisplatin and the reduced form of TrxR and thus the Pt-drug inhibiting the activity of TrxR.\textsuperscript{[124]} Interestingly, the same inhibition effect has been shown for GSH-cisplatin conjugates.\textsuperscript{[125]} Cisplatin has also been found to have an effect on the mitochondrial redox status by causing depletion of NADPH pools,\textsuperscript{[126]} and to induce ER stress followed by apoptosis.\textsuperscript{[127]} These processes taken together have a destructive effect on the mitochondrial membrane structures as vital lipids and proteins are being damaged leading to cell death.\textsuperscript{[18,126]} Such oxidative stress is part of cisplatin cytotoxicity not only towards cancer but healthy cells as well.\textsuperscript{[129]} This can lead to nephro- and hepato-toxicity which are only two of the many possible side events caused by cisplatin.\textsuperscript{[130][126]}

4.3. Auranofin

Targeting thioredoxin metabolism can also be used as an effective therapeutic strategy. Auranofin, a gold-containing antirheumatic drug (Figure 8) which is a thioredoxin inhibitor,\textsuperscript{[131]} when administered in combination with BSO to head and neck squamous carcinoma cells, leads to an increase in sensitivity to epidermal growth factor receptor inhibitors, and oxidative stress. Indeed, this effect was reversed by pre-treatment of the cells with NAC, a known reductant which protects cells from ROS, therefore evidencing oxidative stress.\textsuperscript{[132]} More studies have demonstrated that treating ovarian cancer cells with auranofin results in cytochrome c-mediated cell death, and the generation of H$_2$O$_2$ which again is inhibited by the activity of NAC.\textsuperscript{[133]}

5. Metal-based anticancer drug candidates causing oxidative stress in cancer cells

After the tremendous success of cisplatin in the clinic, ruthenium complexes have been among the most studied and advanced metal-based anticancer therapeutics. Their biological activity depends on a number of factors including the metal ion and its oxidation state, the number and type of coordinated ligands, and the coordination geometry.\textsuperscript{[121]} At the time when DNA was proposed as being the primary biological target for platinum-containing drugs, ruthenium-based drugs were designed to mimic this MoA. However, it was soon realised that these precious-metal complexes offer much more potential as anticancer agents following a non-conventional (non-DNA binding) mechanism of anticancer action. Such novel anticancer agents which induce oxidative stress in cancer cells, containing either ruthenium or osmium as a metal center, are discussed herein. Some examples of complexes containing platinum, copper, rhodium, and iridium, are also included.

5.1. Ruthenium complexes

Ruthenium complexes are a well-studied group of metallodrugs for the treatment of cancer. In particular, octahedral Ru\textsuperscript{III} and piano-stool Ru\textsuperscript{II} complexes have attracted much interest in the past 30 and 20 years respectively (Figure 9).\textsuperscript{[134]} Three Ru\textsuperscript{III} compounds have entered clinical trials – NAMI-A ((ImH)[trans-RuCl$_4$(dmso-S)(Im)]), Im = imidazole), KP1019 (IndH[trans-RuCl$_4$(Ind)$_3$], Ind = indazole), and NKP-1339 (the sodium salt of KP1019). KP1019 demonstrates cytotoxicity towards cisplatin-resistant colorectal cancers, and KP1339 has been designed to improve the poor solubility of KP1019.\textsuperscript{[135]} NAMI-A is a non-cytotoxic antimitastatic drug which has been shown to scavenge nitric oxide (NO\textsuperscript{-}) species in cancer cells. NO\textsuperscript{-} is a highly reactive specie which plays a role as a messenger in a number of physiological processes including neurotransmission,
homeostasis and tumour invasion. It has been proposed that the ability of NAMI-A to scavenge NO• species, and its inhibitory
effect on endothelial cell migration, are strongly related.\[136\] Unfortunately, NAMI-A recently failed phase I/II clinical studies
as it was found to be ineffective when administered in combination with the organic anticancer drug gemcitabine.\[137\]

Figure 9. Structures of ruthenium complexes described in section 5.1.

Several years back, it was believed that ruthenium complexes exhibit their cytotoxic activity in a similar manner to Pt-containing
drugs - through DNA binding.\[138\] However, there is an increasing amount of evidence that many Ru\(^{III}\) and Ru\(^{II}\) compounds have a
non-conventional mode of action and do not interact with DNA directly, but rather interact with proteins such as serum albumin
which can be used for cellular uptake.\[139\] Recent studies suggest that direct binding to DNA might not be the mechanism
by which NKP-1339 induces cell death. It has been proposed that
NKP-1339 induces the production of excessive levels of ROS in HCT116 colon cancer cells, leading to translocation of
TrxR1 enzyme containing thioredoxin reductases are potential targets for novel metal
anticancer drugs.\[147\] Moreover, Ru\(^{II}\) complexes, and more specifically RAPTA compounds, were also found to be inhibitors
of thioredoxin reductase. These results taken together suggest that the inhibitory activity of ruthenium complexes does not
happen as a result of a reduction of the metal centre oxidation state.\[148\] Other examples of Ru\(^{III}\) complexes that inhibit the
TrxR1 enzyme contain N-heterocyclic carbenoids ligands, with the
M-C\(_{n-1}\)C bond being particularly stable.\[149\]

Photodynamic therapy (PDT) is a highly promising cancer therapy that relies on photosensitizer harvesting light and
oxygen to produce ROS. Traditionally, organic molecules with
tetrapyrrolic system have been used as photosensizers; more
recently ruthenium complexes have gained interest in this field,
for instance the Schiff base complex named RAS-1T (Figure 9). In this study, Gaiddon and co-workers\[142\]
demonstrated that by altering the arene ligand in the structure of the
metal complex, the way the complex induces cell death can be changed – either through ROS-mediated ER stress pathway
or ROS-independent pathway. RAS-1T, which induced the
former, was found to cause early time-point ROS, followed by
an increased expression of Nrf-2 (regulator of cellular resistance
to oxidants), and induction of ER stress. Indeed, pre-treatment of
cells with NAC led to an increase of cell viability, as well as to
low levels of Nrf-2 and suppression of ER stress biomarkers,
therefore evidencing a mechanism of action via the production of
ROS and subsequent ER stress. Barry and co-workers demonstrated that electron-deficient metal complexes can also induce the production of high levels of ROS in cancer cells with
the complex [(p-cymene)Ru(maleonitriledithiolate)] increasing
ROS levels in ovarian A2780 cancer cells and the ROS-scavenger NAC inhibiting the cytotoxicity of this metal complex
(Figure 9).\[143\]

Ru\(^{II}\) complexes have been reported to affect the glutathione system both in cell-free systems and in cancer cells. For
instance, Ru\(^{II}\) azopyridine complexes have been shown to have a
catalytic activity in cancer cells through a ligand-based redox reaction. In this case, the azo ligand from the complex is
reduced, leading to oxidation of GSH to GSSG which disturbs the
redox balance, resulting in oxidative stress. Interestingly, the azo ligand itself is not readily reduced, therefore
the catalytic activity is attributed to the full ruthenium complex.\[144\] Ru\(^{II}\) polypyridyl complexes have also been reported
to induce the production of ROS in the nuclei of NSCLC cells,
followed by the involvement of the produced ROS in the
cleavage of DNA.\[145\] In the case of RM175, a Ru\(^{II}\)
ethylenediamine complex, binding to guanine in DNA is facilitated by a reaction between the complex and GSH, followed
by oxidation, therefore demonstrating how affecting the redox balance in cancer cells can be part of a mechanism involving
binding to DNA.\[146\]

Targeting the thioredoxin system as a possible mechanism of
anticancer action of Ru\(^{II}\) and Ru\(^{III}\) complexes has been reported. More specifically, PMR227, a Ru\(^{II}\) complex bearing a 2-amino-5-
methylthiazole ligand, has been shown to inhibit the activity of the
selenoenzyme TrxR1 and therefore suggesting thioredoxin reductases are potential targets for novel metal-based
anticancer drugs.\[147\] Moreover, Ru\(^{II}\) complexes, and more specifically RAPTA compounds, were also found to be inhibitors
of thioredoxin reductase. These results taken together suggest that the inhibitory activity of ruthenium complexes does not
happen as a result of a reduction of the metal centre oxidation state.\[148\] Other examples of Ru\(^{III}\) complexes that inhibit the
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tetrapyrrolic system have been used as photosensizers; more
recently ruthenium complexes have gained interest in this field,
for instance ruthenium complexes with porphyrin,
phthalocyanine or polypyridine ligands.\[150\] TLD1433 (Figure 9) is the first Ru(II)-based photosensitizer to enter a human clinical trial for treating cancer with PDT, with phase I on patients with
non-muscle-invasive bladder cancer being successful, and
currently undergoing phase II clinical trials for efficacy in a much
larger patient population.\[151\]
5.2. Osmium complexes

Osmium complexes have also been of interest for the design of novel anticancer therapeutics. Os‖ complexes have been shown to affect the cellular redox balance in a similar manner to ruthenium-based anticancer agents (Figure 10). Examples include Os‖ azopyridine complexes such as FY26 which has demonstrated very promising cytotoxicity results, and have been found to induce the production of ROS, and more specifically O₂⁻ species.\(^{[152]}\)

![Os(II) sulfonamide complex](Image)

**Figure 10.** Structures of osmium complexes described in section 5.2.

It has been suggested that such an increase in ROS levels could be due to a ligand-based reduction by GSH, followed by localisation of the complex in the mitochondria which was mapped by synchrotron X-ray fluorescence (Figure 11).\(^{[153]}\)

Furthermore, involvement of ROS in the mode of action has been evidenced by a co-treatment with BSO. As mentioned above, BSO is a redox modulator which blocks the synthesis of GSH, therefore reducing GSH levels and in turn enhancing the cytotoxic activity of FY26.\(^{[154]}\) For such Os‖ azopyridine complexes, and more specifically in the case of the iodido species, it has been found that GSH catalyses their hydrolysis in cells, and therefore activating them. These hydroxide complexes then react with GSH and produce thiolato and sulfenato adducts, and react with H₂O₂ to generate hydroxyl radicals.\(^{[155]}\)

Os³ sulfonamide complexes have been shown to be less active towards cancer cells than Os‖ azopyridine compounds but still exhibiting moderate activity and able to induce the production of ROS in vivo in a zebrafish model (Figure 12). The ROS production induced by the Os³ sulfonamide complex was localised mainly in the area of the swim bladder, whereas for the Os‖ azopyridine complex it was spread over the whole organism.\(^{[156]}\)

![Figure 12.](Image)

**Figure 12.** ROS (in green) in zebrafish treated with either complex 1 (Os‖ sulfonamide complex) or complex 10 (Os‖ azopyridine complex). Reproduced with permission. Copyright 2018, The American Chemical Society.

5.3. Others

Complexes containing other metals have also been reported to have an effect on the cellular redox status, either exhibiting their cytotoxic activity mainly through inducing oxidative stress, or this contributing to their overall mode of action (Figure 13). Studies on copper complexes and their cellular redox activity include Cu‖ which is reduced to Cu¹ by molecules in cells which contain thiols, for instance GSH. In an oxidising environment the reduced Cu center can cause the formation of O₂⁻ which in turn stimulates the production of ROS via a Fenton-like reaction.\(^{[157],[158]}\)

In the case of the Cu‖ complex CuNG containing N-(2-hydroxyacetophenone)glycine as a ligand, GSH pool depletion has been observed as well as increased ROS levels, but no antitumour effect. Such an activity can be used as a strategy to overcome GSH-dependent drug resistance. In a xenograft study with EAC-Dox cells which are characterised by a high resistance to doxorubicin, this CuNG Cu‖ complex was administered in combination with doxorubicin. This led to an enhanced sensitivity of the cells to doxorubicin and improved the survival of treated mice from 19 to 87 days.\(^{[159],[160]}\)
A number of rhodium complexes also exert their anticancer activity through inducing changes in the cellular redox balance. Examples include Rh\(^{10}\) complexes with isoquinoline derivatives which were found to induce apoptosis via mitochondrial pathways by elevating the levels of ROS and Ca\(^{2+}\), triggering S or G2 phase cell cycle arrest and causing the release of cytochrome \(c\).\[^{[161]}\] Dinuclear Rh\(^{10}\) organometallics based on 3-hydroxy-4-pyridinone derived ligands were designed with spacers between the two bidentate ligands ranging from four to twelve \(\text{CH}_2\) groups (Figure 13). This approach, based on the success of BBR3464, allowed for the tuning of their lipophilicity. The longer the carbon chain was, the better the anticancer activity was, with the compounds inducing significant ROS formation at low concentrations.\[^{[162]}\] In the case of the multi-targeting Rh\(^{3}\) complex containing a naturally occurring caffeine derived N-heterocyclic carbene (NHC) ligand, the complex was found to inhibit the activity of TrxR, which in turn induced the production of ROS, leading to both a decrease in the mitochondrial membrane potential and potential DNA damage, and as a result cell cycle arrest and apoptosis. Moreover, this Rh\(^{3}\) compound was found to inhibit the migration of the highly-invasive breast cancer MDA-MB-231 cells in a wound healing assay, and thus unveiling its antimetastatic activity.\[^{[163]}\]

Iridium-based complexes have also been found to disturb the cellular redox status. For instance, Ir\(^{3+}\) azopyridine complexes such as ZL109 can provide a multi-targeting therapeutic strategy as these were found to induce cell death by redox modulation and DNA damage. Studies revealed that treatment with such compounds leads to activation of the antioxidant defence mechanisms, and upregulation of ROS levels in ovarian cancer cells. Moreover, cell cycle analysis by flow cytometry showed S/G2 arrest and late-stage DNA damage response.\[^{[164]}\] Similarly to the Rh\(^{3}\) dimer complexes with pyridinone ligands mentioned above, the Ir\(^{3+}\) analogues were synthesised. The Ir\(^{3+}\) induced ROS formation in a concentration-dependant manner, as observed for Rh\(^{3}\), but caused less DNA damage, most certainly owing to the inertness of Ir compared to Rh.\[^{[165]}\] Other Ir\(^{3}\) complexes with a \(\beta\)-carboline alkaloid ligand (IrC1 in Figure 13) induced the production of ROS in the mitochondria both in the dark and under light irradiation, as well as exhibited anticancer activities in the dark with IC\(_{50}\) values around 1 \(\mu\text{M}\). This combined chemo and photodynamic therapy could be of interest for the design of future metal drugs.\[^{[166]}\]

**Conclusions**

Oxidative stress is a key biological process. Normal cells possess regulation systems that allow for the appropriate level of reactive oxygen species to be maintained via antioxidative defence mechanisms. Cancer cells, with their unique redox balance, can thrive under low and intermediate levels of ROS (higher than in normal cells), leading to tumourigenesis, angiogenesis, metastasis, and survival. However when ROS levels increase above a threshold, cancer cells cannot cope anymore and the tumour-suppressive functions of ROS appear, leading to apoptosis, autophagy or necrosis of the cancer cells. Therefore, it is essential, when designing anticancer drugs with a mechanism of action based on ROS activity, to comprehensively investigate the effects of ROS as a function of their concentration in order to overcome drug resistance and spare normal tissues. Anticancer metal complexes (such as platinum, gold, ruthenium, osmium) have been shown to interact with the redox homeostasis of cells, leading to a few complexes in clinical use or in human clinical trials. Whilst the mechanism of action of cisplatin is well understood, essential knowledge about the mechanism of action of other metal complexes needs to be gained; this will allow for the development of new anticancer metaldrugs with less toxicity and more cancer-specific targets.

**Keywords:** antitumour agents • bioinorganic chemistry • redox chemistry • ROS • metal complexes

**References:**


Oxidative stress and the role of ROS in cells is described before focusing on the therapeutic strategies focused on ROS levels for cancer therapy. Clinically approved drugs with oxidative stress anticancer mechanisms of action are described before highlighting examples of metal-based anticancer drug candidates with such mechanisms of action.

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