Scaffold-Based Selective ROS Generation as Viable Therapeutic Strategies Against Cancer

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Abstract

Reactive oxygen species (ROS) are by-products of normal cellular metabolism and play a crucial part in cell signaling and common cellular functions. An increasing field of evidence suggests that cancer cells contain an abnormally high content of ROS, and this biochemical attribute can be utilized for selective

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killing. Diverse chemotherapeutic agents have been developed that attack cancerous cells through several mechanisms, such as by amplifying the cells' intrinsic oxidative stress, by directly generating ROS, or by inhibiting antioxidant enzymes. This occurs due to their vulnerability to further ROS insults. ROS modulation cancer therapy is a young and sustained research realm for medicinal chemistry community. This chapter reviews evidence linking specific scaffolds to reactive oxygen species generation in cancer treatment and the present status of the preclinical and clinical phases of promising synthetic/natural drugs.

Keywords

Reactive oxygen species · Oxidative stress · Chemotherapeutics · Anticancer drugs · Cancer

Introduction

The World Health Organization (WHO) defines cancer as a disease encompassing "the uncontrolled growth and spread of cells." Current statistics show that nearly one-third of the world's population will develop some type of cancer in their lifetimes. It is quite a lethal disease, responsible for about 13% of the deaths worldwide (Jemal et al. 2011). While cancer treatment in the first half of the twentieth century was dominated by radiotherapy and surgery, the 1940s saw the rise of chemotherapy as a viable alternative when nitrogen mustard derivatives were used in the treatment of lymphomas. Since then, several drugs have been developed that oppose malignant cell proliferation and have improved the life expectancy of cancer patients significantly (DeVita and Chu 2008). Chemotherapeutic intervention helps to achieve spectacular survival rates by providing relief against the dreadful cancer phenotype (Kaelin 2005). However, progress has been sluggish in the development of synthetic therapeutics for the treatment of various important malignancies such as glioblastoma, metastatic melanoma, and pancreatic carcinoma (Kamb et al. 2007).

Development of therapeutics targeting biological events is a generous contribution from medicinal chemists. From experimental evidence, it is proven that, with respect to normal cells, cancer cells experience greater oxidative stress with enhanced generation of reactive oxygen species (ROS). ROS are chemically vigorous; their increased amount in cancer cells lead to varying effects, including increased cellular proliferation, alteration of sensitivity of cells against anticancer drugs, and rise in mutations and genetic instability. However, the higher oxidative stress in cancer cells tends to make them more susceptible to further ROS insults. This feature provides an opportunity for development of newer therapeutics. For that, the foremost task is to identify the cellular mechanism of ROS production and their immediate effect on cancer and normal cells. Some ROS-based chemotherapeutics work through synthetic lethality, that is, the drug is toxic to only those cells that have lost the tumor suppressor genes due to mutations, or whose

oncogenic expression has been upregulated. Only cancerous cells have these features.

Several clinical trials are examining the therapeutic efficiency of novel redox targeting drugs in cancer patients. This shows that redox chemotherapy has started its "bench-to-bedside" transition (Wondrak 2009).

Background

Molecular Basis of ROS Production

Cancer cells exhibit metabolically high activity, thus need the supply of high levels of adenosine triphosphate (ATP). These maintain cells' prolific biochemical functions necessitated by their increased cell growth and proliferation rates. Generally, glucose in presence of oxygen during aerobic respiration is metabolized within cells to convert into water and carbon dioxide and release ATP (Babcock and Wikström 1992). However, in cancer cells, the increased energy demand further stresses the mitochondrial respiration chain, causing incomplete electron transport and subsequently, increased ROS generation. In cells under oxidative stress, oxygen instead of complete reduction into water forms partially reduced superoxide ($O_2^{\bullet -}$) radical. This forms either by accepting one electron from the electron transport chain or by the action of NADPH oxidase (NOX) enzyme. This reactive $O_2^{\bullet -}$ ion causes damage to the iron-sulfur (Fe-S) cluster proteins. As a result, Fe(II) is released from the extracellular matrix and causes inactivation of the Fe − S cluster proteins (Fig. 1).

Another way $O_2^{\bullet -}$ species are metabolized is through their dismutation into hydrogen peroxide (H_2O_2) by enzymes known as superoxide dismutases (SOD). H_2O_2, a very reactive molecule, is able to attack several functional groups in cellular

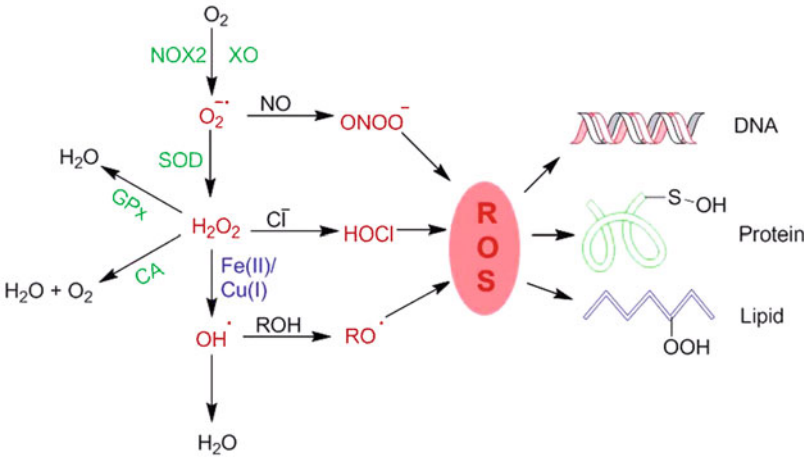

Fig. 1 Mechanism of intracellular ROS production and effect to biomolecules

biomolecules, causing their inactivation. For example, the reaction of $H_2O_2$with the thiol-containing proteins leads to the oxidation of cysteine to sulfenic acid. Consequent reactions with more H_2O_2 causes production of sulfinic and sulfonic acids that could permanently inactivate the function of protein (Dickinson and Chang 2011).

Another highly reactive ROS species, hydroxyl radical (OH•), is generated through Fenton reaction of H_2O_2 catalyzed by Fe(II) or Cu(I). This radical directly and irreversible reacts with nucleotide bases, leading to permanent changes to the DNA sequence (Dharmaraja 2017) (Fig. 1).

ROS Paradox in Cancer

A large amount of data gathered over several years have given rise to two opposing inferences: one conclusion being that an altered ROS content leads to enhancement of the tumor, i.e., it is pro-tumorigenic; another conclusion is that the enhanced ROS made the cancerous cells more vulnerable to cell death. This confusing conclusion shows that the effect of ROS on cancer is dependent on various factors such as cell type. Basically, the increase in ROS allows the molecular changes that lead to tumor initiation, progression, and subsequent chemoresistance. Further increasing the ROS may break down the equilibrium and would lead to sensitization of the cells to chemotherapeutic drugs. Therefore, this paradox provides an opportunity to develop two opposing therapeutic approaches to receive the same ultimate effect (Chio and Tuveson 2017; Galadari et al. 2017).

Modulation of ROS as a Therapeutic Target

ROS modulation-based cancer therapy is a young branch of research and attracts sustained research interest (Gupta et al. 2012; Gorrini et al. 2013; Pelicano et al. 2004). To leverage the effect of changes in ROS leading to the development of new and effective therapies, it is necessary to understand the sophisticated workings of redox biology and apply biophysical and biochemical approaches to functionally elucidate the oxidative modifications in cancer versus normal cells. As stated earlier, mounting evidence indicates that cancer cells are characterized by abnormally increased ROS and that this biochemical feature can be exploited for selective killing.

As both ROS induction and decline below a threshold could lead to the killing of cancerous cells, both prooxidant and antioxidant approaches have been utilized (Fig. 2) (Trachootham et al. 2006; Wang and Yi 2008). The highly intrinsic ROS levels have been utilized for the development of novel therapeutic approaches to preferentially kill cancerous cells (Pelicano et al. 2004; Hileman et al. 2004; Trachootham et al. 2009; Tandon et al. 2005; Tsang et al. 2003; Lopez-Lázaro 2007; Fang et al. 2009; Peng and Gandhi 2012).

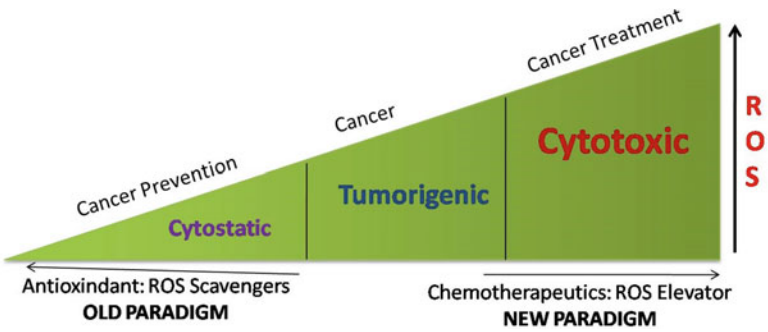

Fig. 2 ROS modulation strategy as anticancer therapeutics of the malignant cells.

Reduction of ROS Levels: Antioxidants and Nutraceuticals

According to one report, only a small portion of cancers are caused by genetic defects, while more than 90% of cases are caused by lifestyle-related factors (Anand et al. 2008). This indicates that cancer can be prevented largely by lifestyle changes such as hygiene and diet. Nutraceuticals and antioxidants are beneficial in both the prevention and treatment of cancer. Plant-derived nutraceuticals and antioxidants provide various advantages such as cost effectiveness, efficacy, safety, and immediate availability, as well as their effect on multiple targets. Therefore, they have gained active research status over the last 20 years. Nutraceuticals behave as either prooxidant or antioxidant depending on the cancer type and concentration used. Curcumin is a commonly studied nutraceutical that has been used traditionally against various diseases and has shown potential against numerous cancers. Various clinical trials have been conducted, implicating the potential of curcumin for cancer prevention and its safety. Lycopene is a carotenoid that is present in especially high amounts in reddish colored fruits such as carrots, tomatoes, and watermelon. Lycopene exerts its anticancer effect through scavenging of ROS.

Vitamin C (ascorbic acid) was shown to decrease oxidative stress among patients suffering from atrophic gastritis (Pisoschi and Pop 2015; Prasad et al. 2017). Epigallocatechin gallate, a polyphenolic compound from green tea, contributes to the potential health benefits. Pomegranate (*Punica granatum*) is a well-known fruit that has been used for its various medicinal purposes since centuries as it contains a high level of flavonoid compounds, such as luteolin, kaempferol, and quercetin.

Conversely, some of the antioxidants used in clinical trials led to an increased cancer incidence, possibly because of the abrogation of intrinsic ROS-mediated apoptosis within the tumors. Similarly, antioxidants led to a decrease in ROS-mediated antitumor activity of anticancer agents such as paclitaxel and radiation therapy (Peng and Gandhi 2012).

Induction of ROS Levels: Scaffold-Based Chemotherapeutics

The origins of chemotherapy dates back to World War I with the use of biological warfare. In 1944, the first patient was treated using mustard gas to target their lymphoma which achieved a temporary remission before they died of bone marrow failure. Sidney Farber et al. introduced the concept of chemotherapy for cancer treatment in 1948 when they used a synthetic folic acid antagonist to treat acute leukemia (Farber et al. 1948). Since then, several chemotherapeutic agents have been developed. The primary mechanism of most of the chemotherapy drugs against cancer cells is due to the generation of ROS, or free radicals. Some of the classes of drugs that induce ROS are camptothecins (topotecan, irinotecan), anthracyclines (doxorubicin, epirubicin), platinum coordination complexes (cisplatin, carboplatin), podophyllin derivatives (etoposide), and alkylating agents (melphalan, cyclophosphamide). Generally, the drugs used for chemotherapy act on cells undergoing mitosis, causing the division to stall, and subsequently causing the induction of apoptosis (Wondrak 2009). Some are approved by the U.S. Food and Drug Administration with some in development stage or in clinical trials (Figs. 3 and 4). These chemotherapeutic agents may be classified based on various scaffolds which are discussed below under respective headings.

Quinone

Primarily, ROS in cells are mainly sourced from quinone scaffolds, which react with intracellular cytochrome P450 (CYP450). This results in generating superoxides through its sequential conversion of semiquinone radicals in presence of NADPH followed by reaction with dissolved oxygen. Some of the well-known quinone-based anticancer drugs associated with ROS-mediated cell death are geldanamycin (heat shock protein 90 (HSP90) inhibitor), mitomycin C (DNA alkylating agent), mitoxantrone (topoisomerase inhibitor), and doxorubicin (DNA intercalator and topoisomerase inhibitor) (Fig. 3).

Geldanamycin: Geldanamycin is a benzoquinone antibiotic that acts on the Hsp90 protein as an inhibitory ligand by masking its ATP-binding site. Some of its derivatives are 17-dimethylaminoethylamino-17-demethoxygeldanamycin (17-DMAG) and 17-allylamino-17-demethoxygeldanamycin (17-AAG) (Fukuyo et al. 2008). The anticancer activity of geldanamycin-derivatives is being examined in numerous clinical trials (Solit et al. 2008). Strong experimental evidence has supported the involvement of ROS production in the antitumor effects of DMAG and 17-AAG. When these compounds bind to BRAF protein-bound HSP90, the ROS so generated facilitates ROS-induced damage to BRAF, causing loss of its activity.

Menadione: Menadione (2-methylnaphthalene-1,4-dione, also known as vitamin K3; Fig. 3) is an experimental redox chemotherapeutic that contains a naphthoquinone pharmacophore. Its mechanism of action involves menadione's reduction by a single electron into the cytotoxic semiquinone free radical which in turn is swiftly reoxidized to its quinone form by electron transfer reaction with molecular oxygen (Verrax et al. 2006). Together with the reducing activity of cellular

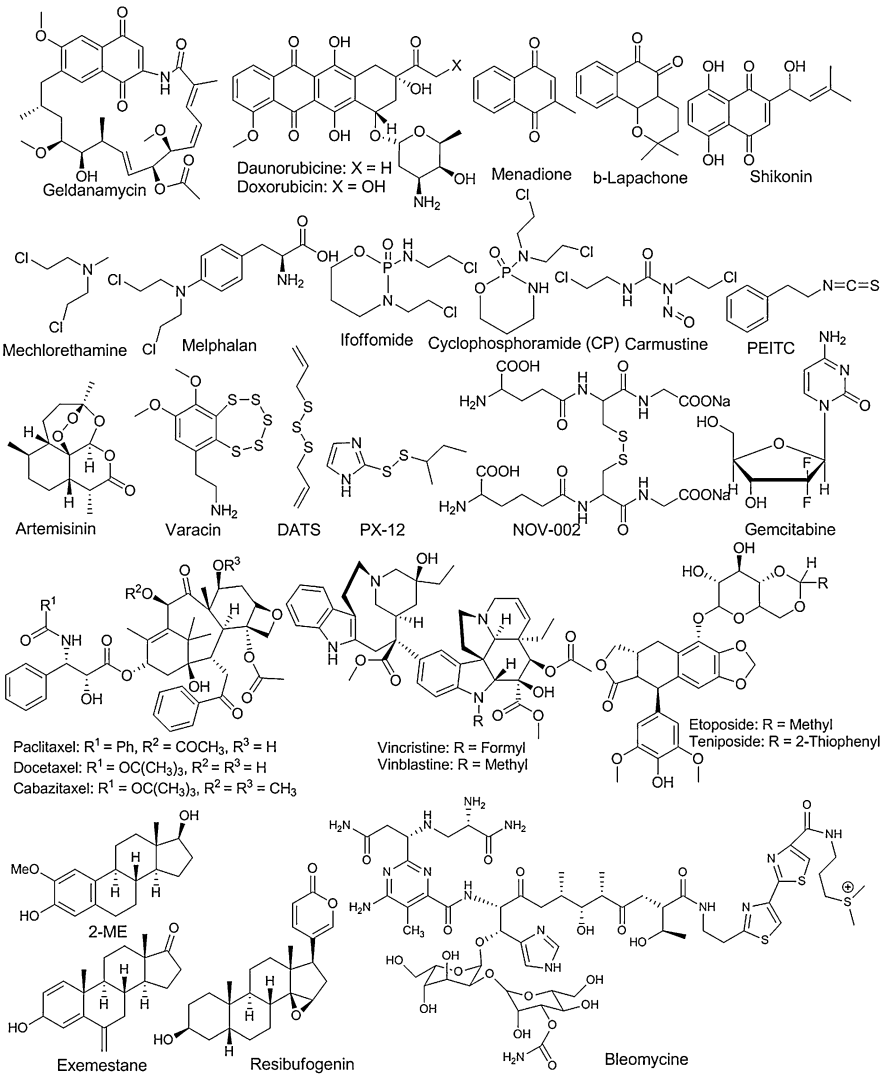

Fig. 3 Representative scaffold of quinone, nitrogen mustard, nitrosourea, endoperoxide, polysulfides, nucleoside, taxane, alkaloid, steroid, and peptide

reductases or redox factors (such as ascorbate), this leads to superoxide formation. Additionally, menadione is developed also as an inhibitor for Cdc25 phosphatase activity.

Anthracycline skeleton associated drugs such as epirubicin and doxorubicin generate ROS that leads to DNA damage, apoptosis in a p53-independent manner, and subsequently antitumor activity (Tsang et al. 2003).

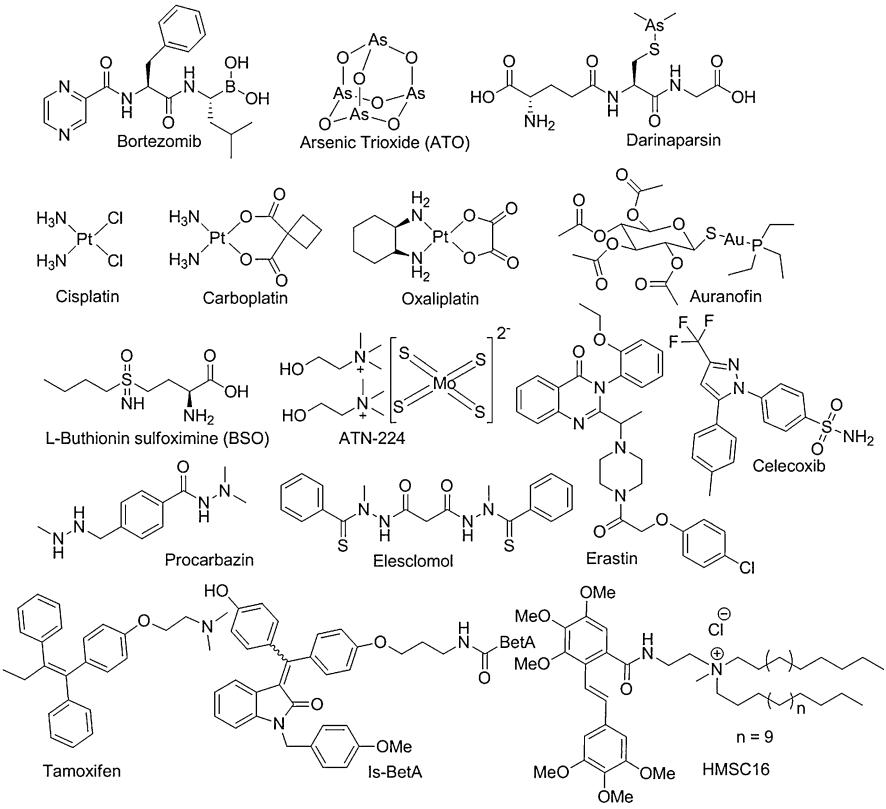

Fig. 4 Structure of some representative nonmetal, metal, and miscellaneous scaffolds

Shikonin: Shikonin is a well-known naphthoquinone and an effective necroptosis inducer in cancer cells. This necroptosis is supported by an overproduction of ROS, which then augments the shikonin-induced expressional upregulation of pro-apoptotic proteins RIP1 and RIP3. Furthermore, it has been shown that ROS plays a crucial part in shikonin-induced glioma cell necrosis (Bin et al. 2017).

Nitrogen Mustard

Nitrogen mustards is an important class of alkylating anticancer drugs and their topical formulation has been widely used as a first-line treatment of patients with early-stage mycosis fungoides (MF, a type of T-cell lymphoma) since 1959. This alkylating agent forms both inter-strand and intra-strand DNA cross-links and has activity in all phases of the cell cycle. Mechlorethamine (or chlormethine) was the first nitrogen mustard to be introduced into clinical use. It is a prototype for several antineoplastic alkylating agents such as cyclophosphamide. Currently, it is used topically in the treatment of cutaneous T-cell lymphoma, and systemically in the

treatment of Hodgkin's disease along with other potent anticancer drugs such as procarbazine, vincristine, and prednisone.

Mechlorethamine is rapidly converted in vivo to the ethylene immonium ion which covalently binds to the N-7 position of guanine, resulting in inter-strand and intra-strand cross-links within the DNA. However, mechlorethamine is swiftly degraded in aqueous solution, as a result of which unchanged mechlorethamine is undetectable in the blood within minutes after intravenous administration (Singh et al. 2018).

Cyclophosphamide (CTX) and ifosfamide: Both drugs are part of oxazaphosphorine class of alkylating agents (Fig. 3). These chemotherapeutic agents are widely used in the treatment of ovarian, breast, and hematological cancers as well as autoimmune disorders. They are inactive in their parent forms (prodrug) that get converted by the CYP450 enzymes in the liver, giving rise to active metabolites phosphoramide mustard derivatives and acrolein, that are then able to block DNA synthesis (Sannu et al. 2017).

These metabolites are the source of ROS which act by causing irreparable genetic damage by forming inter- and intra-strand crosslinks and affecting normal protein formation, which then ultimately leads to apoptosis of the damaged cell. Therefore, cyclophosphamide and ifosfamide have been shown to have more potent antitumor activity since their derivatives are cytotoxic rather than cytostatic (Jeelani et al. 2017).

Nitrosoureas

Nitrosoureas are DNA alkylating anticancer drugs that are able to cross the blood–brain barrier and therefore they are used to treat brain tumors. The nitrosoureas include carmustine (BCNU), lomustine (CCNU), and semustine.

Carmustine: Carmustine (Fig. 3) is one of the nitrosourea-based drugs commonly used in the treatment of various cancers such as brain tumors, multiple myeloma, and lymphomas. It works through mitochondrial membrane depolarization that ultimately leads to induction of intrinsic apoptosis pathway. However, thrombocytopenia (loss of platelets) is a side effect of this drug (Zhang et al. 2015).

Organic Endoperoxides

Artemisinin: Artemisinin (Fig. 3) and its semisynthetic derivatives have been demonstrated to target cancer cells through the intracellular prodrug activation method. Both the anticancer and the antimalarial activity of artemisinin work by the formation of reactive species that is triggered by redox-active iron ions (Singh and Lai 2004). An endoperoxide bridge in the structure of artemisinin is the active moiety that is activated by intracellular iron [Fe(II)]. It forms carbon-based electrophilic radical center and also ROS. Thus, artemisinin and its derivative-associated endoperoxide-pharmacophore not only provides a unique chemical reactivity but also it provides high stability for its resistance to reduction by common reducing agents such as $NaBH_4$.

Organic Di- and Polysulfides: Diallyl Trisulfide and Varacin

Sulfur-based redox chemistry is well-documented and versatile under physiological conditions in the anticancer drug discovery programs. Organic disulfides undergo reductive cleavage, giving rise to two thiol-group containing moieties, while a reducing reaction partner such as a protein undergoes oxidation, leading to the formation of a disulfide bridge. This could potentially lead to the deactivation of the protein.

Varacin and polysulfides: Varacin is a benzopentathiepin-type pentasulfide that attacks cells by formation of electrophilic ROS (Fig. 3) (Jacob 2006). It is an example of a class of polysulfides that are isolated from natural sources. This class also includes the linear compound diallyl trisulfide (Fig. 3). Polysulfides can release ROS and reactive sulfur species (RSS) following their bio-reductive activation. RSS is involved in induction of potent cytotoxicity when targeted to bacteria, fungi, and cancer cells.

PX-12 and NOV-002: PX-12 (Fig. 3) is an investigational small-molecule drug that inhibits thioredoxin-1 (Trx-1), subsequently inhibiting tumor growth in cancer models through stimulation of apoptosis and downregulation of vascular endothelial growth factor (VEGF) and HIF-1α, both important tumor-promoting factors (Galmarini 2006). As high level of Trx-1 has been associated with colorectal, gastric, and lung cancers, PX-12 has been shown as a potential cancer treatment in combination with other chemotherapeutic drugs for treating patients with advanced metastatic cancers. Initial trials showed increased patient survival. NOV-002, (Fig. 3) the glutathione disulfide, phosphorylates two protein kinases ERK and p38, thereby critically regulating cancer cell-associated apoptosis and growth. Additionally, cellular glutathione (GSH) so produced due to glutathione reductase, the cellular reducer of NOV-002, critically maintains cellular redox homeostasis.

Leinamycin is an antibiotic that showed toxicity towards cancerous cells. It is activated by thiol-containing molecules such as cysteine, and the resulting metabolite is able to react with nucleotides in a double-stranded DNA and block cell division (Asai et al. 1996). Another potent genotoxic molecule, calicheamicin promotes ROS-mediated DNA strand scission at pyrimidine-rich recognition sites.

Organosulfur Isothiocyanate: Sulforaphane and β-Phenylethylisothiocyanate

Electrophilic organosulfur, isothiocyanates contain highly reactive isothiocyanate (R-N=C=S) pharmacophore that is responsible for the prooxidant and thiol-adducting reactivity of these molecules. This class includes drugs such as sulforaphane (R-1-isothiocyanato-4-methylsulfinylbutane), 6-methylsulfinylhexyl-isothiocyanate, benzyl-isothiocyanate, and β-phenylethyl-isothiocyanate (PEITC) (Fig. 3) that display superior toxicity against premalignant and cancer cells. This occurs due to their multiple prooxidant effects including ROS formation through mitochondrial damage, depletion of cellular glutathione, and adduct product of cysteine thiols in several important proteins such as STAT3 and β-tubulin.

Sulforaphane: In several cancerous cell lines, sulforaphane is shown to induce apoptosis through the formation of ROS. Furthermore, its systemic administration

caused significant inhibition of murine xenograft models, taking it to further clinical trials (Wondrak 2009).

β-phenylethylisothiocyanate: β-phenylethylisothiocyanate (PEITC), a constituent of cruciferous vegetables such as cauliflower, has been shown to induce ROS-mediated apoptotic cell death in melanoma cell line and leukemias (Wang et al. 2014). Clinically, it was tested in smokers to ascertain if it alters the metabolism of a key carcinogen namely, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) present in cigarettes. Therein, a small but significant change was observed.

Peptide and Nucleoside

Bleomycin: Bleomycin is a non-ribosomal peptide-polyketide hybrid natural product (Fig. 3). It was first discovered in 1962 and got FDA approval in 1973 and is one of the most effective chemotherapeutic agents against cancers of testis, ovary, cervical besides Hodgkin's lymphoma. Bleomycin primarily acts through DNA intercalation via its bithiazole moiety and inhibit DNA metabolism. Also, imidazole ring, pyrimidine ring, as well as primary amine-associated nitrogen atoms chelate superoxide radical forming Iron ions. This increases ROS production with Fas upregulation and elevated activity of apoptosis inducing factors, caspase-8 and caspase-9. This represents activation of both intrinsic and extrinsic apoptosis pathways.

Proteasome inhibitor I: Also chemically known as *N*-benzyloxycarbonyl-Ile-Glu (*O*-t-butyl)-Ala-leucinal. It causes proteasomal inhibition that leads to ROS induction and mitochondrial dysfunction (Papa et al. 2007). It has been approved by the FDA for the treatment of leukemia (Gorrini et al. 2013).

Gemcitabine: Gemcitabine is a nucleoside analog and well-known antimetabolite used for chemotherapy (Fig. 3). This drug is used for the treatment of various cancers including breast cancer, pancreatic cancer, non-small cell lung cancer, and bladder cancer, and used experimentally in lymphomas. However, its use in esophageal cancer is still being investigated. Just like the pyrimidine base analog 5-fluorouracil (5-FU), this drug replaces the cytidine base during DNA replication. As new nucleosides cannot be attached to the "faulty" nucleoside, DNA synthesis block occurs followed by apoptosis and subsequently causes tumor growth arrest. In pancreatic cancer cells, however, an undesired effect of gemcitabine was observed. The ROS that is induced upon gemcitabine treatment causes pro-inflammatory and pro-tumorigenic factors NF-κB (nuclear factor of κ-light-chain-enhancer of activated B cells) and HIF-1α (hypoxia inducible factor) to accumulate in the nucleus. This leads to the upregulation of CXCR4 that contributes to the invasiveness of cancerous cells (Arora et al. 2013).

Taxane, Alkaloid, and Steroid

Paclitaxel and docetaxel are well-known taxanes and commonly used as chemotherapeutic agents for the treatment of several cancers such as breast cancer, prostate cancer, and stomach cancer (Fig. 3). They work by inhibiting the microtubule formation that affects cell division process. Paclitaxel is biologically synthesized by the plant *Taxus brevifolia*, or Pacific Yew. As it was difficult to get sufficient

quantities of paclitaxel from this rare plant, an analog was sought that could be synthesized artificially. Docetaxel was discovered as a semisynthetic drug, where a product from the readily available plant *Taxus baccata*, or European Yew, is modified. The taxanes were shown to induce apoptosis in chronic myelogenous leukemia cells through generation of ROS. The mitotic blockage caused an increase in apoptosis that was abrogated when the antioxidant N-acetyl-L-cysteine (NAC) was added exogenously (Meshkini and Yazdanparast 2012).

Vincristine is a compound that is part of the family of drugs known as vinca alkaloids (Fig. 3). They work by inhibiting the polymerization of tubulin, thereby blocking microtubule formation and stopping cell division process. Though it can be obtained biologically from *Catharanthus roseus*, or Madagascar periwinkle, the yield is extremely low. Hence, a total synthesis technique has been developed that is now commonly used for its production. Vinca alkaloids were found to use ROS to induce apoptosis in cells. In lung adenocarcinoma cells, oxidative stress was observed in cells undergoing aberrant JNK-mediated mitochondrial dysfunction that was reduced upon ROS inhibition (Chiu et al. 2012). Vinblastine and vinorelbine also belong to the class of vinca alkaloids. Vinorelbine was shown to deplete intracellular GSH, which led to an increase in intracellular ROS content.

Podophyllotoxin is an antimitotic natural product that works by destabilizing microtubules, therefore affecting cell division. Its semisynthetic derivatives such as etoposide, teniposide, and etoposide phosphate have shown good clinical activity in the treatment of various cancers such as small cell and non-small cell lung carcinomas, Hodgkin's disease and non-Hodgkin's lymphoma, germ cell tumors, and acute leukemias (Fig. 3) (You 2005). Etoposide stimulates ROS generation that leads to necrosis (Shin et al. 2016).

Steroids are important cell signaling agents. Their potent affinities for various nuclear receptors are extensively utilized for drug development particularly for receptor mediated diseases. In the recent years, there has been an extensive focus on modification of steroids that has led to the development of several important anticancer lead molecules such as exemestane, fulvestrant, and 2-methoxyestradiol (Fig. 3) (Gupta et al. 2013). 2-methoxyestradiol acts as an inhibitor of SOD enzyme that can lead to an increase in superoxide radical levels. Currently, it is in phase I and II clinical trials for the treatment of metastatic breast and prostate cancer. Also, in leukemia cells but not in normal cells such as lymphocytes or neuroblastoma, it induces ROS-mediated apoptosis (Lakhani et al. 2003). Exemestane works by blocking the biosynthesis of estrogen through the inactivation of the aromatase enzyme, consequently leading to apoptosis. Therefore, it was used for the prevention and treatment of breast cancer (Bhuyan et al. 2017).

Resibufogenin, a member of bufadienolide family, was shown to induce ROS in colorectal cancer (CRC) cells, that were subsequently killed in a RIP3-dependent cell death pathway, which is the signature of necroptosis (Han et al. 2018).

Non-metal and Metal

Several nonmetal-based anticancer agents with their ability to induce cellular ROS generation are currently used for cancer treatment. Examples include arsenic trioxide, darinaparsin, and bortezomib.

Arsenic trioxide (ATO): Arsenic trioxide (Fig. 4) is commonly used for the treatment of acute promyelocytic leukemia (APL). Although exact mechanism remains largely unknown but recent studies indicated arsenic trioxide to act through increased production of superoxide by impairing the function of the mitochondrial respiratory chain. This led to leakage of electrons from the respiratory complexes.

Darinaparsin: Darinaparsin (S-dimethylarsino-glutathione; ZIO-101; Fig. 4) is a synthetic arsenic-based compound. While its mechanism of action is not clear, it is shown to induce oxidative stress with more potency than arsenic trioxide, As_2O_3 (ATO). It exhibits anticancer activity towards ATO-resistant and MRP1/ABCC1-overexpressing cell lines as ATO was shown to be efficiently exported by the MRP1/ABCC1 protein (multidrug resistance-associated protein 1), suggesting increased therapeutic efficacy of darinaparsin in ABCC1-overexpressing tumors. Several clinical trials have examined the effect of darinaparsin for treating various cancers such as advanced hepatocellular carcinoma and hematological cancers (Wondrak 2009).

Bortezomib: In cancer cells, apoptosis-causing proteins rapidly undergo proteasome-mediated degradation. Bortezomib, the boron-based compound (Fig. 4), acts by inhibiting this aberrant proteasomal activity by blocking the 26S subunit. The contribution of ROS in the activity of bortezomib was revealed in mantle-cell lymphoma. In several patient samples of MCL, all the hallmarks of apoptosis were shown upon bortezomib treatment but were abrogated when ROS scavengers were used (Pérez-Galán et al. 2006).

Anticancer metal complexes (e.g., platinum, gold, copper, vanadium, cobalt, manganese, etc.) function based on the hypothesis of "activation by reduction" as well as the "hard and soft acids and bases" theory. Generally, metal complexes act as prodrugs that undergo transformation through the process of ligand substitution and redox reactions as they reach the target site. Anticancer metal-based drugs mainly target the glutathione and thioredoxin redox systems. The serendipitous discovery of a platinum-based compound by Barnett Rosenberg in 1960s shaped the history of cancer treatment through the advent of the use of metal-based compounds for the first time, thus providing the basis of modern-era use of metal-based anticancer drug (Jungwirth et al. 2011; Romero-Canelon and Sadler 2013). Nowadays, cisplatin and its analogues carboplatin and oxaliplatin (Fig. 4) find wide use as efficient chemotherapeutics against multiple and widespread varieties of cancers. Riding on the success of cisplatin, multiple coordination complexes of ruthenium, gold, copper, cobalt, titanium, etc. were developed and tested for their anticancer activity with many more undergoing preclinical evaluations (Jungwirth et al. 2011).

The exact mechanism of action is unknown. However, in the case of cisplatin, cisplatin detoxification is thought to be the leading mechanism involved during its anticancer action. Cisplatin forms conjugates with GSH, leading to depletion of the intracellular GSH pool, thus disturbing the redox homeostasis and subsequently increasing the level of ROS. Similarly, even the NADPH pools were found to be depleted, possibly through the same action.

Auranofin: Auranofin, [tetra-O-acetyl-β-D- (glucopyranosyl)thio] (triethylphosphine) gold(I) (Fig. 4), was approved in 1985 for the treatment of rheumatoid arthritis as an orally available drug. It was seen to be less toxic, but less

efficient too. A continuing study shows that patients suffering from rheumatoid arthritis treated with Au(I) compounds such as auranofin had a lower rate of malignancies than those treated with other drugs, leading to a comprehensive search for Au(I) and Au(III) complexes against cancer. Auranofin was shown to cause significant cell death in gastrointestinal stromal tumors by inhibiting thioredoxin reductase, leading to increased ROS formation (Teppo et al. 2017). Beside auranofin, no other gold-based compound has been approved so far for the treatment of any disease. Aurothiomalate, however, was investigated against advanced non-small cell lung cancer in a phase I study (clinicaltrials.gov identifier: NCT00575393) (Han et al. 2018).

Miscellaneous Scaffolds (Procarbazine, Elesclomol, Erastin, Celecoxib)

Procarbazine: Procarbazine (Fig. 4) is one of the first drugs that is known to kill cancer cells by directly generating ROS. In an oxygen-rich environment, it is converted into its azo derivative that leads to generation of ROS (Renschler 2004).

Elesclomol: Elesclomol (Fig. 4) is an FDA-approved orphan drug that is known to interact with the electron transport chain (ETC) in the mitochondria and causes increase in the ROS (Blackman et al. 2012). It is used for the treatment of multiple myeloma.

Erastin and ferroptosis: Erastin (Fig. 4) blocks VDAC2 and VDAC3 and functionally inhibits the cystine-glutamate antiporter system Xc^-, which causes the cells treated with erastin to be deprived of cysteine and unable to synthesize the antioxidant glutathione. Also, it inhibits the glutathione peroxidase 4 (GPx4) antioxidant enzyme. Inhibition of both system Xc^- and GPx4 causes a different kind of cell death known as ferroptosis that is mediated by iron (Lu et al. 2017).

Celecoxib: Celecoxib (Fig. 4) is a commonly used nonsteroidal anti-inflammatory drug (NSAID) that works by inhibiting cyclooxygenase. But it was also found that it can be used as a cytotoxic drug against metastatic cells, where it increased mitochondrial superoxide production while dissipating the mitochondrial membrane transmembrane potential (Pritchard et al. 2018).

ATN-224: Anticancer agents can also work through the inhibition of the antioxidant defense system causing the enhancement of ROS stress in cancer cells. In this respect, SOD has emerged as an important target. ATN-224 (Fig. 4) is a molybdenum-containing SOD inhibitor. It was shown to exhibit cancer cell toxicity in recurrent prostate cancer patients in a phase II clinical trial (Gupta et al. 2012).

L-buthionine sulfoximine (BSO): BSO (Fig. 4) is an inhibitor of glutamyl synthetase enzyme that causes blockage in the synthesis of glutathione (GSH). It therefore targets the GSH antioxidant system and depletes cellular levels of GSH. Administration of BSO and melphalan (an alkylating agent) was found to be safe in a Phase I trial. GSH content is significantly reduced in cancer patients (Gupta et al. 2012).

Tamoxifen: Tamoxifen (Fig. 4) was initially synthesized in 1962 by chemist Dora Richardson as a tetra substituted stilbene derivative. As a selective

estrogen-receptor modulator (SERM), it finds widespread use to treat estrogen receptor (ER) expressing breast cancer, primarily due to its antiproliferative action through induction of apoptosis regulated by modulation of ER-responsive genes (Bekele et al. 2016). In this regard, we developed a stilbene-based hybrid molecule that offered simultaneous detection and ROS-mediated killing of cancer cells. Is-BetA (Fig. 4), the hybrid of cancer cell-selective ROS generator betulinic acid (Bet A) and bis-arylidene oxindole (isatin-based stilbene, Is) was developed. Through efficient generation of ROS, the molecule triggered apoptosis, while exhibiting potent cytotoxicity in cancer cells selectively (Pal et al. 2015). To further expand the scope of stilbene moiety in biological use, we developed twin chain cationic lipid conjugated, methoxy-enriched stilbene derivatives HMSC16 (Fig. 4). The molecule generated ROS and simultaneously induced apoptosis and autophagy by affecting the mitochondrial, lysosomal, and nuclear pathways (Yousuf et al. 2020). Owing to it maintaining a favorable hydrophilic-lipophilic balance, the molecule exhibited unique self-aggregating property. As a result, the molecule-aggregate showed encapsulation and delivery of another drug, thus exhibiting the potential to use this unique ROS generating aggregate for combination therapy against cancer.

Prodrug (Masking-Demasking)

In anticancer drug development process, one of the best approaches is to target the intrinsic biochemical properties of the tumor microenvironment. Typically, decreased pH, abnormal ionic concentrations, higher ROS content, etc. within tumor can be targeted to create compounds that are specifically toxic to cancer cells. In this concern, synthesizing prodrugs that are converted into the active compounds only within the tumor is a great advantage (Peng and Gandhi 2012). As a prodrug approach, boronic acids and their esters are highly suitable as the H_2O_2 readily cleaves prodrugs to release drugs directly within the site. For example, boronic ester was incorporated into the metal binding moiety of matrix metalloproteinase (MMP) inhibitors so that they were able to be activated only in the presence of H_2O_2 (Major Jourden and Cohen 2010). Similarly, arylboronate, originally masking the toxicity of nitrogen mustard, is liberated in the presence of H_2O_2 to induce its DNA cross-linking, thus leading to efficient cell death in renal and lung cancers (Kuang et al. 2011).

Another ROS-targeting based strategy involves a dual stimuli-responsive hybrid prodrug (namely, QCA) that consists of moiety generating quinone methide and cinnamaldehyde. In here, cinnamaldehyde generates ROS. Within the typical tumor microenvironment with the presence of H_2O_2 and acidic pH, QCA could generate quinone methide that alkylates GSH thus inhibiting the antioxidant system and amplifying the oxidative stress condition, specifically within cancer cells. This triggers apoptotic cell death (Noh et al. 2015). Using the same idea, aminoferrocene-based prodrug was also developed. The molecule, upon H_2O_2 exposure, produced quinone methide and iron ions. Both the products act to cause

cell death where quinone methides works to amplify oxidative stress, and iron ions induce generation of $H^{\bullet}$ free radicals (Hagen et al. 2012).

Taken together, induction of ROS levels having promising avenue to selectively treat cancer with least host toxicity is clearly demonstrated.

Conclusion and Future Direction

ROS modulation caused by chemotherapeutics works either individually or in combinations of direct increase of ROS production, or through the inhibition of antioxidant defenses. In this chapter, several chemical scaffolds were discussed that induce toxicity in cells through the direct or indirect induction of ROS. The use of scaffolds points to the fact that they can be modified in subtle ways that causes a change in some of their properties such as solubility and immunogenicity, while the central scaffold structure remains the core of moiety that induces toxicity. Toxicity is a major issue for chemotherapeutics that should be properly addressed before human application. The toxicity of chemotherapeutics is generally considered by studying the effect on metabolic rate and structural changes in body organs leading to various side effects. Moreover, one should exercise caution while designing and using ROS-generating agents. This is important as ROS is found for the basis of cisplatin-induced multiple organ-related side effects and toxicity such as nephrotoxicity, ototoxicity, and chemoradiotherapy-associated lung damage.

In the context of cancer, while some compounds such as prodrugs can be directly used safely owing to their specific activation within tumors, most of the compounds are limited by their non-specificity. They need to be specifically delivered to the region of interest with the use of a drug delivery system such as liposome. This system would consist of a targeting agent like specific ligand or a monoclonal antibody that would cause the liposome to home into the tumor.

Cross-References

- ► Mitochondria-targeted Antioxidants and Cancer
- ► Modulators of ROS/NF-κB Signaling in Cancer Therapy
- ► Phytoestrogens Modulate Oxidative Stress
- ► ROS Modulation by Iron Chelators and Lipids: A Developing Anticancer Strategy
- ► Therapeutic Effect of Natural Compounds in Targeting ROS-Induced Cancer
- ► Two-Faced Role of ROS in the Regulation of Cancer Cell Signaling
- ► Understanding ROS-Induced DNA Damage for Therapeutics

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