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REVIEW

Induction of reactive oxygen species: an emerging approach for cancer therapy

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Abstract Reactive oxygen species (ROS), a group of ions and molecules, include hydroxyl radicals (·OH), alkoxyl radicals, superoxide anion (O_2·−), singlet oxygen ($^1O_2$) and hydrogen peroxide (H_2O_2). Hydroxyl radicals and alkoxyl radicals are extremely and highly reactive species respectively. Endogenous ROS are mainly formed in mitochondrial respiratory chain. Low levels of ROS play important roles in regulating biological functions in mammalian cells. However, excess production of ROS can induce cell death by oxidative damaging effects to intracellular biomacromolecules. Cancer cell death types induced by ROS include apoptotic, autophagic, ferroptotic and necrotic cell death. Since abnormal metabolism in cancer cells, they have higher ROS content compared to normal cells. The higher endogenous ROS levels in cancer cells endow them more susceptible to the ROS-induction treatment. Indeed, some anticancer drugs currently used in clinic, such as molecular targeted drugs and chemotherapeutic agents, effectively kill cancer cells by inducing ROS generation. In addition, photodynamic therapy (PDT) is mainly based on induction of ROS burst to kill cancer cells. The mechanism of cell death induced by radiotherapy using ionizing radiation also refers to ROS production. Moreover, ROS play an important role in tumor immune therapy. Altogether, combining above traditional treatments with ROS-induced agents will be considered as a promising strategy in cancer therapy. In this review, we focus on our current understanding of the anticancer effects of ROS.

Keywords ROS · Cancer · Cell death · Therapy

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Introduction

Reactive oxygen species (ROS) are broadly defined as chemically reactive molecules containing oxygen, and include hydroxyl radical (·OH), superoxide anion (O_2·−), singlet oxygen ($^1O_2$) and hydrogen peroxide (H_2O_2) [1]. Under normal physiological condition, the intracellular ROS are maintained at a constant low level by the balance between ROS production and elimination. ROS react with molecules by reversible oxidative modifications and play a critical role in cellular signaling pathways, such as metabolism, growth, differentiation and death signaling [2, 3]. Low levels of ROS promote cell proliferation and survival. Indeed, slight increase of ROS is associated with the initiation and progression of cancer [4]. However, excess production of ROS can induce cell senescence and death by oxidative damaging effects to intracellular biomacromolecules, such as protein, lipid, RNA and DNA (Fig. 1) [4]. Hydroxyl radical is an extremely reactive type of ROS with high oxidizing potential, which is main responsible for oxidative stress-induced DNA damage [5]. Excessive amounts of endogenous ROS can be attenuated by non-enzymatic antioxidants such as vitamins E and A, as well as enzymatic antioxidants such as superoxide dismutase (SOD), catalase and glutathione (GSH) [6, 7]. Tumor cells exert lower antioxidant enzyme activity relative to normal cells, therefore ROS levels in tumor cells is higher. In addition, higher ROS levels in tumor cells may partly be due to defective mitochondrial oxidative metabolism [1]. Thus, tumor cells with increased

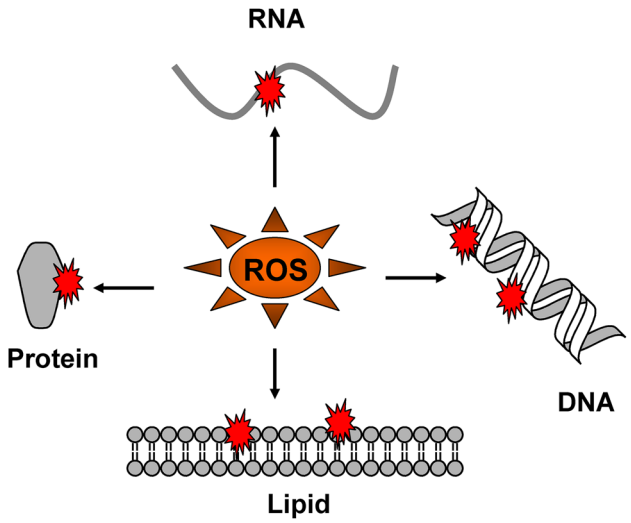


Fig. 1 Interaction between ROS and biomacromolecules

endogenous ROS levels are possibly to be more vulnerable to exogenous ROS-induced agents [8]. Indeed, studies showed that upregulation of ROS levels by redox modulation can selectively kill cancer cells with low toxicity to normal cells [9, 10]. Therefore, induction of ROS is a promising approach for cancer therapy. In this review, we mainly focus on the anticancer role of ROS, and present several potential therapeutic strategies that promote ROS burst to further increase oxidative stress and induce cancer cell death.

Sources and signaling regulation of ROS in cancer therapy

Sources of ROS

Endogenous ROS are by-products during cellular metabolism. It is constantly produced through chemical reactions in enzyme and non-enzyme dependent manners in mammalian cell [11]. Mitochondrial respiratory chain is the main endogenous source of ROS. Some electrons escape from the mitochondrial respiratory complexes (MRC) I and III in the mitochondrial electron transport chain, and react with oxygen to generate superoxide anion (Fig. 2). $O_2{\cdot}-$ are transferred to the mitochondrial matrix through inner mitochondrial membrane (IMM). Subsequently $O_2{\cdot}-$ is converted to H_2O_2 by the superoxide dismutase 2 (SOD2). In the presence of metal cations, such as Fe^{2+} and Cu^{+}, H_2O_2 is converted to ·OH via the fenton reaction. In addition, H_2O_2 is also directly converted to H_2O by glutathione peroxidase (GPX) and catalase (CAT). Some of the superoxide are shifted to the cytosol and then dismutated to H_2O_2 by the SOD1. Although most of $O_2{\cdot}-$ is produced in the mitochondria, a small fraction of $O_2{\cdot}-$ also is produced in the cytosol. In the cytoplasm, $O_2{\cdot}-$ can be induced by enzymatic reactions involving NADPH oxidases (NOXs), xanthine oxidase, uncoupled endothelial nitric oxide synthase (eNOS) and arachidonic acid [12]. Moreover, H_2O_2 can also be generated as a by-product of oxidation in peroxisomes and protein oxidation in the endoplasmic reticulum (ER) [13].

ROS-related cellular signaling

Although ROS is derived from mitochondria, the ROS level is also regulated by some proteins in the cell. In cancer drug therapy, some drugs may regulate ROS level by regulating the activity and function of the proteins, and ultimately affect cancer cell apoptosis. ROS not only directly damage biological macromolecule such as DNA, but also activate apoptotic signal pathway to induce cancer cell apoptosis.

Oncogenic signaling regulation of ROS

The PI3K/AKT and its downstream signaling pathway, is thought to play a vital role in the control of cell proliferation, apoptosis and oncogenesis. AKT can promote ROS production by positively regulating energy metabolism in the mitochondrial. Besides, AKT also can increase the levels of ROS by inhibiting the activity of AMPK (Fig. 3), which is a serine/threonine protein kinase and serves as an energy sensor in mammalian cells. It has been shown that AMPK strongly suppresses cell proliferation in tumour cells. AMPK-mediated inactivation of ACC1/2 is required to maintain the NADPH and ROS levels [14]. Conversely, AKT can also attenuate ROS, via depressing FoxO transcription factor to downregulate SOD2, catalase and sestrin3 expression (Fig. 3).

FOXM1, a transcription factor, is overexpressed in a majority of tumors and involved in cancer cell growth, migration and invasion [15, 16]. Increased FOXM1 expression can downregulate ROS levels in cancer cells by enhancing the expression of detoxifying enzymes, such as catalase, SOD2 and PRDX3 (Fig. 3) [17]. Additionally, oncogenic K-Ras mutations promote the development of pancreatic, colorectal and lung cancer. Recent study shows that mutant K-Ras increases generation of mitochondrial ROS by increasing mitochondrial metabolism in pancreatic acinar cells (Fig. 3) [18]. Notably, radical species such as superoxide and nonradical species such as hydrogen peroxide often lead to the increase of oxidative products of protein in cells, resulting in activation or inactivation of some signaling pathways.

Apoptotic and autophagic signaling upon ROS

Oxidative modifications of proteins by ROS result in their changes in structure and function [19]. ROS regulate

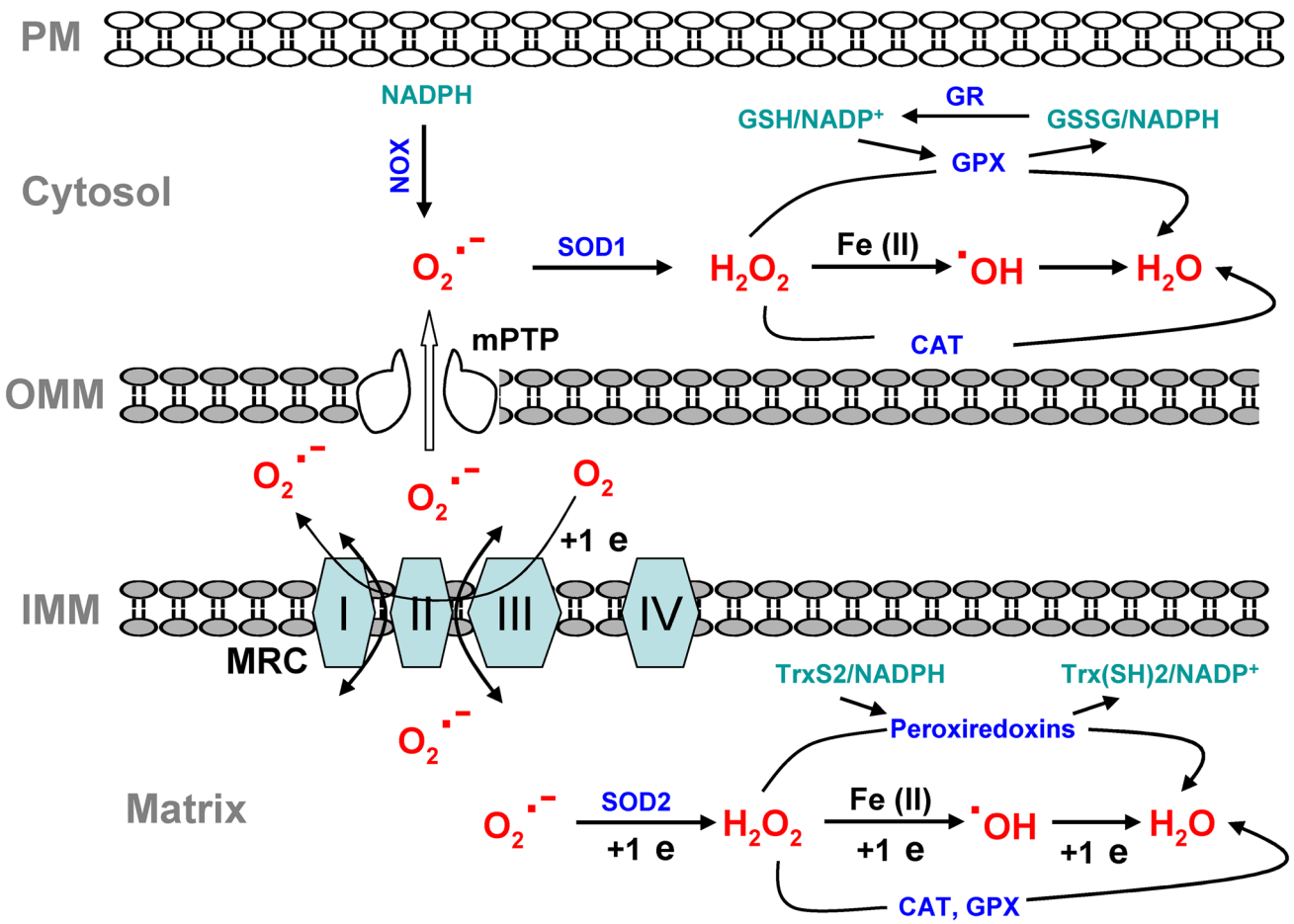


Fig. 2 Sources of endogenous ROS in cells. The major site of reactive oxygen species (ROS) production is the inner mitochondrial membrane (IMM). During respiration electrons are transited to oxygen from mitochondrial respiratory complex I and III (MRC I and III), which generate superoxide ($O_2{\cdot}^-$). The majority of $O_2{\cdot}^-$ is transferred to the matrix. And then the superoxide dismutase (SOD2) dismutated $O_2{\cdot}^-$ to hydrogen peroxide (H_2O_2). The part of $O_2{\cdot}^-$ is transferred to the cytosol. Additionally, in cytosol, activated NADPH oxidase (NOX) can also oxidize NADPH to generate $O_2{\cdot}^-$. And then $O_2{\cdot}^-$ is dismutated to H_2O_2 by the cytosolic SOD1. In cytosol and mitochondrial matrix, H_2O_2 can be eliminated by catalase, glutathione, peroxidase (GPX) and peroxiredoxins (PRX). GPX and PRX utilize NADPH-induced glutathione (GSH) and thioredoxins (Trx) respectively

apoptotic and autophagic signaling pathways through interaction with critical signaling molecules (Fig. 4). ASK1 is an upstream MAPKKK that regulates the JNK pathways leading to apoptosis through phosphorylation of JNK. Thioredoxin (Trx) is shown to directly inhibit ASK1 kinase activity by constitutively interacting with ASK1 [20]. ROS oxidize Trx and induce disulfide bond formation between Cys-32 and Cys-35 in Trx, and resulting in subsequent Trx to be separated from ASK1 [20]. And then ASK1 undergoes complete homo-oligomerization and subsequent autophosphorylation and is activated. ASK1-acitvated JNK can phosphorylate Bim and Bmf. The phosphorylated Bim and Bmf further activate Bax and Bak to initiate apoptosis respectively [21]. Ataxia-telangiectasia mutated (ATM) has been shown to serve as a sensor of oxidative stress. One side, ATM mediates

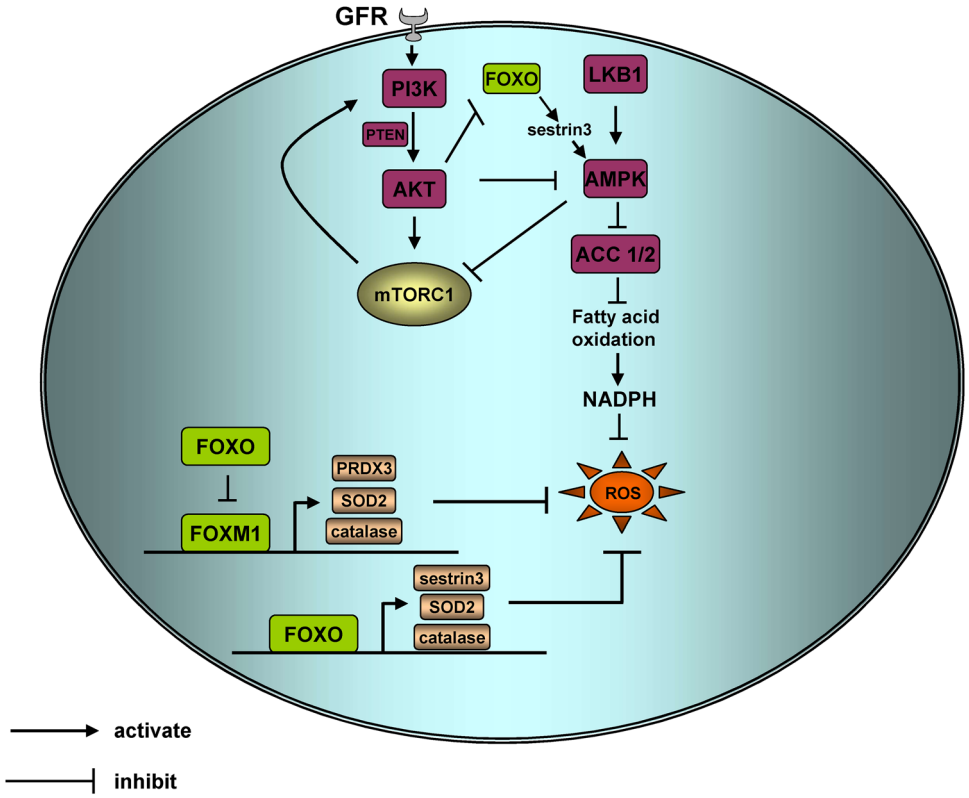


Fig. 3 Oncogenic signaling regulation of ROS in cancer cells. AKT is activated by extracellular growth factor receptor (GFR) signals via PI3K. The tumor suppressor PTEN, a phospho-lipid phosphatase, inhibits the activity of PI3K. Activated AKT promotes mTOR complex 1 (mTORC1) activity and inhibits the FOXO and AMPK activities. AKT is also activated by mTORC1 in a negative feedback signaling. FOXO as transcription factor induces the expression of anti-oxidants genes, such as SOD2, sestrin3 and catalase, which attenuate ROS levels. In addition, another transcription factor FOXM1 also can depress ROS levels by inducing anti-oxidants genes, such as SOD2, PRDX3 and catalase. Notably, FOXO can inhibit the transcription activity of FOXM1. Under energy stress conditions, AMPK is activated by LKB1. Activated AMPK phosphorylates and inhibits the acetyl-coA carboxylase 1/2 (ACC1/2), and increases NADPH production via fatty acid oxidation. NADPH production prevents oxidative stress by attenuating ROS levels

oxidative DNA damage-induced cells apoptosis through ATM-Chk2-p53 pathway [22]. Besides, studies show autophosphorylated cytoplasmic ATM by H_2O_2 activates LKB1 via phosphorylation of Thr-366 [23]. Activated LKB1 activates AMPK through Thr-172 phosphorylation. Activated AMPK in turn inhibits mTORC1 by phosphorylating the tuberous sclerosis complex 2 (TSC2) tumor suppressor protein at Thr-1271 and Ser-1387 [24]. The inhibition of mTORC1 induces autophagy. Above studies show ATM mediated ROS-induced apoptosis and autophagy.

Cancer cell death types induced by ROS

As has been mentioned earlier, high levels of ROS play an important role in cancer therapy by activating different cell death types including apoptosis, autophagy, ferroptosis and necrotic cell death [4].

ROS-activated extrinsic and intrinsic apoptosis

Apoptosis is the most common cell death type, and includes the death receptor dependent apoptosis (known as extrinsic apoptosis) and the mitochondrial-dependent apoptosis (known as intrinsic apoptosis). Two major pathways of extrinsic apoptosis are TNFα (tumor necrosis factor α) and Fas pathway. Recently, Musarat et al. show that extracellular ROS generated by atmospheric gas plasmas activate TNFα signaling and induce extrinsic apoptosis in melanoma cancer cell [25]. It has also been proposed that TNFα-induced ROS activate ASK1 and subsequent ASK1-mediated extrinsic apoptosis-signaling cascades [26]. TRAIL (TNF-related apoptosis-inducing ligand) can induce cell apoptosis via TNF pathway. ROS have been shown to suppress c-FLIP (the endogenous TRAIL inhibitor) expression post-transcriptionally, causing the facilitation of the extrinsic apoptosis. Pretreatment with ROS scavengers NAC (*N*-acetyl-L-cysteine) effectively recovers the c-FLIP expression, and

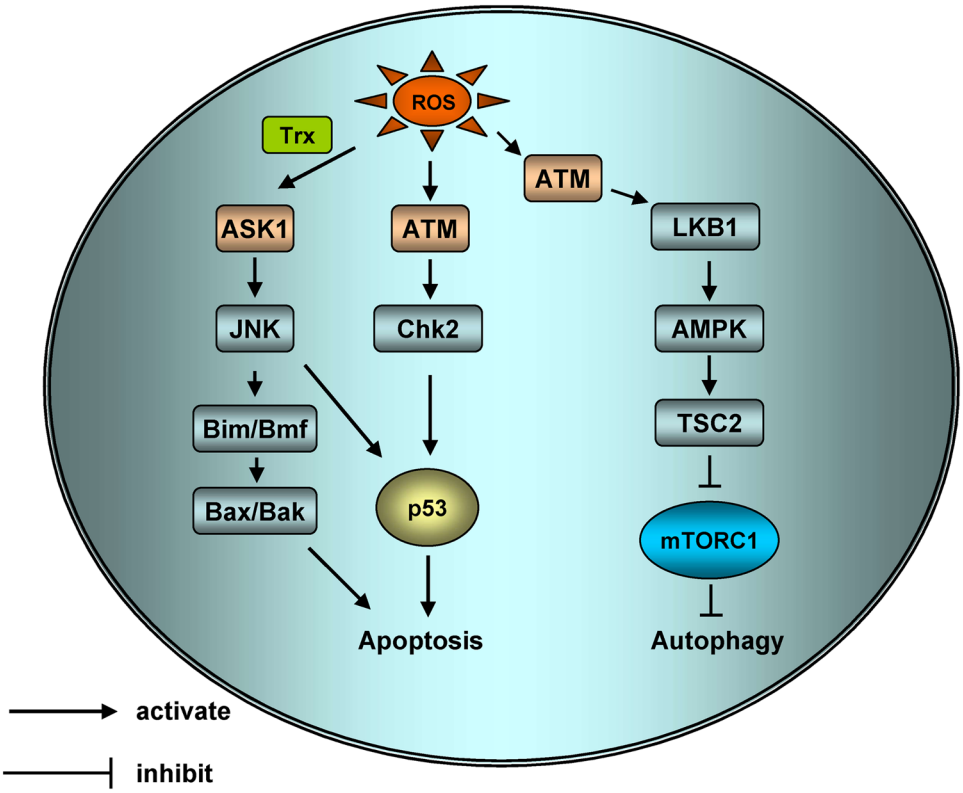


Fig. 4 Schematic of apoptotic and autophagic signaling upon reactive oxygen species. Reactive oxygen species (ROS) regulate apoptotic and autophagic signaling pathways through interaction with critical signaling molecules. ROS activate ASK1 by oxidizing thioredoxin (Trx). ASK1 is an upstream MAPKKK that regulates the JNK pathways leading to apoptosis through phosphorylation of JNK. And then JNK phosphorylated Bim and Bmf. The phosphorylated Bim and Bmf can activate Bax and/or Bak to initiate apoptosis. Additionally, JNK can increase the p53 expression and induce cancer cell apoptosis. H_2O_2 activates ATM in an MRN/Ser-1981 autophosphorylation independent manner. Activated ATM induces cell apoptosis and autophagy through Chk2/p53 and LKB1/AMPK/TSC2/mTORC1 pathway respectively

inhibits extrinsic apoptosis [27]. In primary lung epithelial cells, ROS are required for extrinsic apoptosis induced by Fas ligand (FasL) by enhancing ubiquitination-mediated FLIP degradation [28]. Furthermore, studies have identified a critical role for ROS in regulating the expression of functional FasL, which can bind the receptor Fas on tumor cell and subsequently induces tumor lysis eventually [29, 30]. Redox active substances and exogenous oxidants such as MAP kinase activators, chemotherapeutic agents and H_2O_2, could regulate the expression of FasL, suggesting that ROS control the expression of FasL [31, 32]. In addition, Liu et al. confirm that ROS mediate POX-induced extrinsic apoptotic pathway in colorectal cancer cells [33].

Most of the antitumor ROS-inducers have been shown to display their anticancer activity via a ROS-dependent mitochondrial apoptotic pathway [34]. Recently, we have demonstrated that chloroquine synergistically enhances the sensitivity of colorectal cancer cells to SN-38. The cell death induced by the two agents is involved in ROS-mediated mitochondrial apoptotic pathway [4]. In the mitochondrial apoptotic pathway, superoxide anion triggers rapid and massive Cyto-c release from mitochondrion, causing cell apoptosis via VDAC-dependent permeabilization of mitochondrial membrane [35]. In addition, oxidation of adenine nucleotide translocase (ANT) by ROS is also linked to the regulation of the mitochondrial permeability transition pore (MPTP) opening [36]. Zuo et al. have shown that oxidative modification of pro-caspase-9 at Cys403 elicits its autocleavage and activation by interacting with Apaf-1 [37]. Moreover, Katoh et al. propose an alternative mechanism of caspase-9 activation in oxidation-dependent manner. They illuminate that H_2O_2-induced disulfide bridge leads to dimer formation of caspase-9 and subsequent its auto-proteolytic cleavage, and thus activates caspase-9 in an Apaf-1-independent manner [38]. In addition, ROS have been validated to regulate the activity of Bcl-2 family proteins, the dominant regulators of mitochondrial apoptotic pathway. The main mechanisms include: (1) H_2O_2 can directly oxidize Bcl-2 at Cys158 and Cys229 and thus abate its antiapoptotic activity [39]; (2) $O_2\cdot-$ can abrogate ubiquitination of Bax and Bad, whereas promote ubiquitination of Bcl-2 [40, 41].

Dual effect of autophagy by ROS

Autophagy is a natural, regulated self-degradative process in mammalian cells, where unnecessary or dysfunctional cytoplasmic components are degraded in the lysosome. Autophagy has been indicated to be elicited by numerous anticancer drugs [42]. Autophagy promotes or inhibits cell death by drugs depending on the context. There is growing evidence that ROS can induce autophagy by regulating various signaling pathways. Notably, redox regulation of autophagy is associated with levels and duration of ROS exposure and cell types. The autophagy generated by ROS

also exerts a dual effect on the cells. Firstly, cells can protect themselves by inducing autophagy to reduce the oxidative stress. In contrast, excess ROS can stimulate autophagic cell death [43]. Of note, Chen et al. report that ROS induces autophagic cell death only in tumor cells but not in non-transformed cells [44].

The process of autophagy is negatively regulated by mammalian target of rapamycin (mTOR), a serine/threonine kinase. H_2O_2 decreases mTOR activity and induces autophagy by activating ATM/LKB1/AMPK/TSC2 pathway in breast cancer cells [45, 46]. Additionally, in C6 glioma cells, H_2O_2-induced BNIP3 expression suppresses mTOR activity and initiates autophagy [46]. Mitochondria-derived H_2O_2 by sanguinarine treatment induces autophagic cell death in malignant glioma cells [47]. Beclin-1, an important autophagy-related protein, is involved in the initiation of autophagy. It has been shown that ROS upregulate the levels of beclin-1 [44]. Another autophagy associated protein ATG4 is also demonstrated to be a ROS target. Under starvation condition, H_2O_2 oxidizes and inactivates ATG4, and thereby promotes the lipidation of LC-3 to induce autophagy [48]. Recently, Song et al. show that H_2O_2-mediated oxidative stress increases autophagy by activating NF-κB in retinal pigmented epithelial cell [49].

ROS-mediated necrotic cell death

Necrotic cell death, also known as necroptosis or necrosis, is originally deemed to be an unregulated type of cell death with organelle swelling and membrane rupture. However, accumulating evidences have shown that necrosis similar to apoptosis also involves elaborate molecular circuitry. RIP1 and RIP3 have been shown to be two critical regulators during the execution of necroptosis. Some evidences have shown that ROS as modulators of RIP1 and RIP3 participate in necroptosis. Zhang et al. show that mitochondrial ROS regulates RIP1 by promoting its autophosphorylation and causing its oxidation to form intermolecular disulfide bonds. Subsequently, phosphorylated RIP1 promotes RIP3 recruitment into necrosome to induce necroptosis [50]. In addition, mitochondrial ROS mediate calcium-induced formation of RIP1/RIP3 complex and necroptosis in human colon cancer cells [51]. Although several evidences have shown that ROS can regulate RIP1 and RIP3, the specific type of ROS involved in RIP1- or RIP3-induced necroptosis is unclear. Conversely, RIP1 and RIP3 have been considered as driving forces for ROS production. For example, Zhou et al. show that RIP1 and RIP3 contribute to production of intracellular ROS in shikonin-treated glioma cells [52]. In renal carcinoma Caki cells, artesunate-induced ROS is also dependent on RIP1 [53]. However, the mechanism of ROS induced by RIP1 or RIP3 remains to be determined. A possible mechanism is that RIP1 and RIP3 increase the activity of several metabolic enzymes including glutamate-ammonia ligase, glutamate dehydrogenase 1 and glycogen phosphorylase by directly binding to these proteins. These metabolic enzymes catalyze mitochondrial ROS generation [54, 55].

Ferroptosis: cell death by lipid ROS

Ferroptosis, also known as ferroptotic cell death, a novel type of cell death, is dependent on intracellular iron [56]. Ferroptosis is involved in iron-dependent ROS formation and lipid oxidation [56]. Some compounds and antioxidants trigger cell ferroptosis by suppression of glutathione (GSH)-dependent antioxidant defenses [57]. As shown in Fig. 5, system x^-_c is a heterodimeric amino acid antiporter on cell surface. Cystine import via system x^-_c is necessary for glutathione synthesis. Glutathione activates GPX4 to prevent the production of lipid ROS [58]. Sorafenib, sulfasalazine and erastin induce ferroptosis by directly inhibiting system x^-_c to block cystine uptake. Glutamine is transported into the cell via SLC1A5 and metabolized to α-ketoglutarate. And then α-ketoglutarate contributes to the formation of oxidizable membrane lipids and subsequent lipid ROS [59]. Accumulating evidences have indicated that many anti-cancer drugs kill cancer cell by utilizing ferrous iron to generate radicals and lead to ferroptosis [60]. For instance, the antimalarial drug artesunate has been repurposed as an inducer of ferroptosis to exert anticancer effects [61, 62]. Salinomycin, the most promising agent against cancer stem cell, has been shown to suppress iron translocation, elicit an iron-depletion response and induce ferroptosis [63]. Recently, Guo et al. show that cisplatin induces ferroptosis in lung cance cells [64].

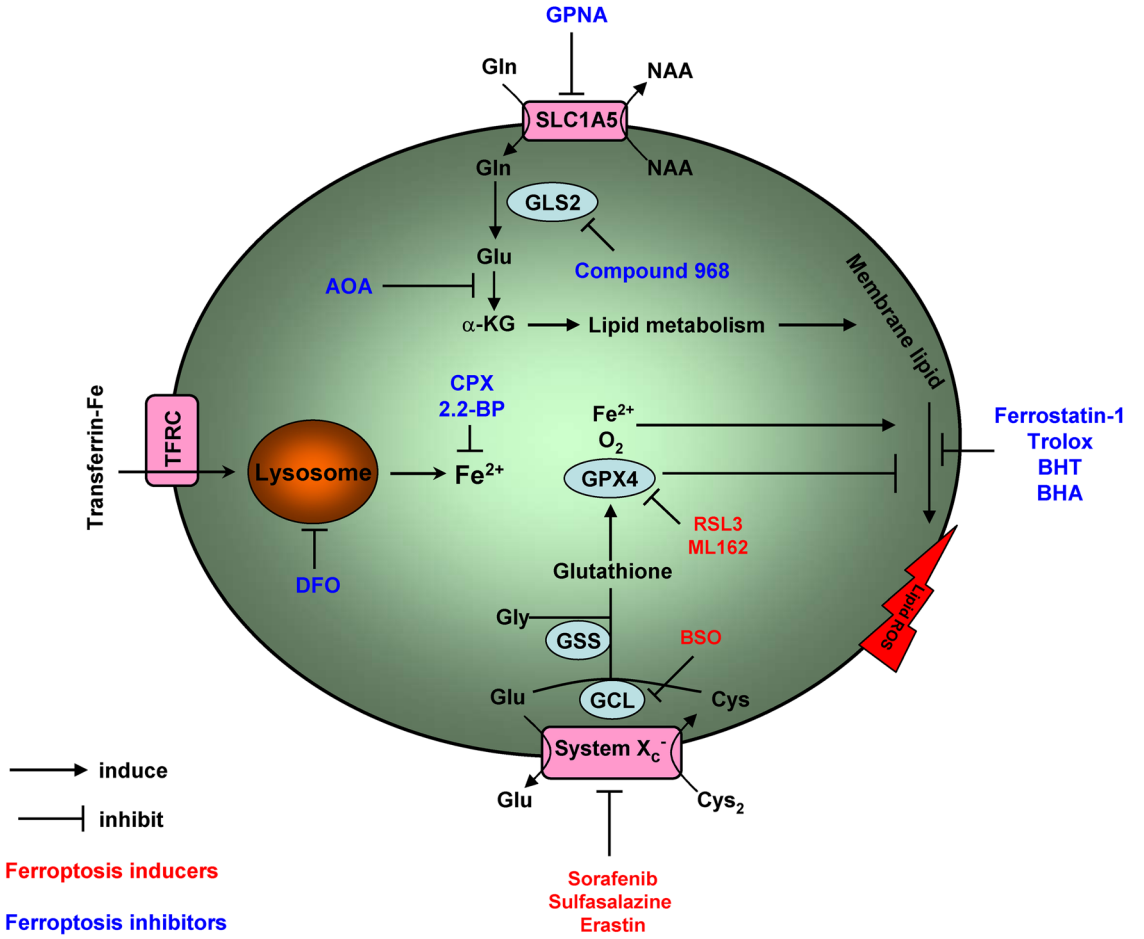
Role of ROS in cancer therapies

ROS-mediated effects of molecular targeted therapies

The current trend in cancer treatment is personalized medicine. Although marked progress has been made in the targeted treatment of cancer, the anticancer effect of molecular targeted therapy is still unsatisfactory. Moreover, prolonged use with the targeted drugs is often limited due to the acquisition of resistance. Recently, the role of ROS in tumor targeted therapy has attracted more and more attention. By increasing ROS levels induced by the molecular targeted drugs will bring a novel therapeutic regimen for treating cancer patients with drug resistance.

The roles of ROS in targeted tyrosine kinase therapies

The drugs of targeted tyrosine kinase in cancer therapies mostly include monoclonal antibodies and small-molecule inhibitors. The monoclonal antibodies include trastuzumab,



GPX4: glutathione peroxidase 4
GLS2: glutaminase 2
GCL: glutamate-cysteine ligase
GSS: glutathione synthetase
Cys: cysteine
Glu: glutamate
TFRC: transferrin receptor

Gly: glycine
Gln: glutamine
α-KG: alpha-ketoglutarate
GPNA: L-g-glutamyl-pnitroanilide
AOA: amino oxyacetate
BHA: butylated hydroxyanisole
NAA: neutral amino acid

BHT: butylated hydroxytoluene
DFO: deferoxamine
2,2-BP: 2,2-bipyridyl
CPX: ciclopirox
BSO: buthionine-(S,R)-sulfoximine

Fig. 5 The mechanisms of ferroptosis regulation. Ferroptosis is induced by iron-dependent ROS and lipid ROS. System X^-_c plays an important role in mediating ferroptotic cell death. System X^-_c promotes the cystine uptake and glutamate efflux. Cysteine (Cys), glutamate (Glu) and glycine (Gly) are converted to glutathione (GSH) by glutamate-cysteine ligase (GCL) and glutathione synthetase (GSS). GPX4 is a glutathione (GSH)-dependent enzyme that degrades lipid hydroperoxides to lipid alcohols. SLC1A5 promotes the glutamine uptake and neutral amino acid (NAA) efflux. Glutamine is converted to alpha-ketoglutarate via glutamate, and then alpha-ketoglutarate is converted to membrane lipid by lipid metabolism. In addition, the presence of divalent iron, greatly accelerates lipid peroxidation of unsaturated fatty acids on the cell membrane. Iron binds to transferrin and forms transferrin-Fe. And then transferrin-Fe is uptaked by transferrin receptor 1 (TFRC1)

pertuzumab, cetuximab and bevacizumab. The small-molecule inhibitors include axitinib, pazopanib, sorafenib, afatinib, erlotinib and gefitinib. Many studies show that these monoclonal antibodies and small-molecule inhibitors manifest ROS-mediated anticancer effects. Yang et al. show that trastuzumab, a monoclonal antibody against HER2, can induce ROS generation to contribute breast cancer cell death [65]. Cetuximab, a recombinant humanized monoclonal antibody specific to EGFR, is applied in colorectal cancer without activating mutant Ras protein. Previous study shows that cetuximab promotes ROS production by decreasing the amount of glutathione [66]. In addition, cetuximab combined with oridonine suppresses phosphorylated EGFR, and induces ROS-mediated apoptosis in laryngeal carcinoma

cells [67]. Bevacizumab, a recombinant monoclonal antibody that specific binding VEGF, has been approved for the treatment of human CRC and NSCLC. Bevacizumab has been reported to increase oxidative stress by reducing L-cysteine and GSH levels [68]. Moreover, bevacizumab combined with autophagy inhibitor induces apoptosis by enhancing ROS levels [69].

Sunitinib is one of the most commonly used tyrosine kinase inhibitors (TKIs) in the clinic. The main mechanism of anticancer action of sunitinib is mediated by effective suppressing of VEGFRs, PDGFR-α and PDGFR-β. Although sunitinib can increase ROS levels in lung cancer cells, there is no direct connection between ROS and antitumor effects of the drug [70]. However, sunitinib combined with chloroquine can obviously increase ROS levels to induce cell apoptosis [71]. Gefitinib and erlotinib are EGFR inhibitors and approved for the treatment of NSCLC with activated mutant EGFR in the first and subsequent lines of therapy. Gefitinib and erlotinib synergize with vorinostat upregulate KEAP1 and downregulate NRF2 to increase ROS levels in NSCLC cells. Induction of ROS by vorinostat plus gefitinib or erlotinib enhances mitochondria-dependent apoptosis [72]. Our recent studies also show that gefitinib and salinomycin act together to induce ROS-mediated loss of mitochondrial membrane potential and lysosomal membrane potential and apoptosis [10]. Nevertheless, Okon et al. find that chronic gefitinib treatment instigates ROS production that enhances lung cancer cells resistance to gefitinib [73]. In line with results concerning gefitinib, erlotinib is also reported to elicit cytotoxicity via ROS generation in NSCLC cells, head and neck squamous cell carcinoma cells [74, 75]. Our previous study finds that resveratrol synergistically promotes erlotinib-mediated NSCLC cell apoptosis in which ROS production is involved [76]. Notably, in all above studies, the ROS levels are detected by using the fluorogenic probes DCFH. Bonini et al. and Wardman et al. have indicated DCFH is not actually a useful specific probe for the H_2O_2 and $O_2{\cdot}-$ [77, 78]. Therefore, we could not conclude that whcih kind of ROS plays a main role in tumor targeted therapies.

The roles of ROS in targeted other molecular therapies

Bortezomib, the first proteasome inhibitor, has been approved for use in clinic for the treatment of the relapsed multiple myeloma. Hui et al. find that bortezomib synergistically the histone deacetylase (HDAC) inhibitor SAHA promotes ROS production to further induce caspase-dependent apoptosis in nasopharyngeal carcinoma [79]. Yin et al. show that inhibition of MUC1-C enhances bortezomib-induced ROS production to increase myeloma cell death [80]. Recent study reports that bortezomib sensitizes human osteosarcoma cells to adriamycin by increasing ROS levels [81]. Another proteasome inhibitor MG132 has been shown to increase intracellular ROS burst and mitochondrial-mediated apoptosis [82]. In a study by Park et al. MG132 induces cleavage of HSP90 and caspase-10-mediated apoptosis by promoting ROS levels in human leukemic cells [83].

In addition, HDAC inhibitor (HDACi) has been approved for the therapy in cancer. Some studies show ROS mediated cancer cell death induced by HDACi [84, 85]. For example, Cornago et al. show that the HDACi suberanilohydroxamic acid (SAHA) and valproic acid (VPA) induce the production of ROS and cell apoptosis, and reduce cell viability, proliferation and clonogenicity in glioma [86]. Moreover, Sholler et al. report a novel HDACi abexinostat induces ROS-dependent apoptosis in neuroblastoma and lymphoma [89, 90]. They also show the proteasome inhibitor bortezomib synergistically enhances abexinostat-induced ROS production and cell death [89, 90]. Similarly, in another study, the combination of HDACi romidepsin and bortezomib significantly promotes generation of ROS and ROS-mediated extrinsic apoptotic pathway in NSCLC cells [87]. A recent study shows HDACi panobinostat and vorinostat inhibit rhabdomyosarcoma by ROS-dependent suppression of transcription factor c-myc rather than inhibition of histone acetylation. Blocking NF-κB sensitizes non-small cell lung cancer cells to HDACi-induced extrinsic apoptosis through generation of ROS [88].

In addition to proteasome inhibitors and HDACi, STAT3 inhibitors have been found to induce ROS-dependent endoplasmic reticulum stress and further cell apoptosis in human pancreatic cancer cells and gliomablastoma cells [89, 90]. When cancer cells undergo energetic stress, activated AMPK can combat oxidative stress and protect cells from ROS-induced damage [95]. Therefore, inhibition of AMPK could be an attractive strategy for ROS-mediated cancer therapy [91]. Indeed, exogenous ROS inducers and AMPK inhibitors in combination exerts significant synergistic anticancer effects.

ROS as main effectors in photodynamic and sonodynamic therapy for cancer

Photodynamic therapy (PDT) is a method for the treatment of neoplastic diseases with photosensitizing drugs and light activation [92]. As shown in Fig. 6, three elements are required in PDT process: photosensitizer (PS), oxygen and light [93]. When the PS is exposed to light with specific wavelengths, it changes from ground state to excited state. The excited PS can undergo two kinds of reactions. In a type 1 reaction, PS reacts directly with substrate molecules in cells, and transfers protons or electrons to produce radical anions or radical cations, respectively. Then, these radicals can further react with oxygen to form oxidizing free radicals and singlet oxygen. In a type 2 reaction, PS in excited state

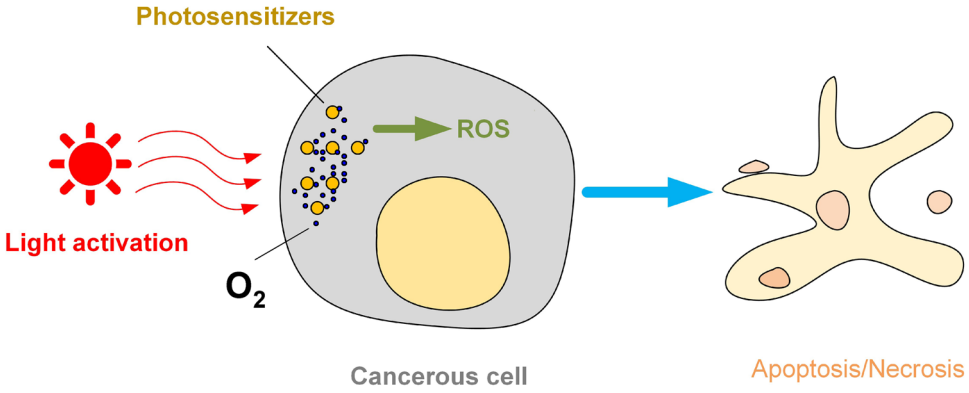


Fig. 6 The mechanism of photodynamic therapy. Photodynamic therapy (PDT) contains three basin elements: light, photosensitizer and oxygen. When the photosensitizer (PS) enters into tumor and then is exposed to specific wavelengths of light, it changes from a ground to an excited state. As excited PS returns to the ground state, it releases energy. The energy is transferred to oxygen to generate ROS. These ROS such as singlet oxygen and free radicals induces cancer cells apoptosis or necrosis

is restored to the ground state, and then releases energy and induces oxygen to convert to excited state singlet oxygen [94].

Irradiation of the tumor can selectively activate the photosensitive drugs that gather in the tumor tissue and trigger photochemical reaction to destroy the tumor. It has been shown that there are three different mechanisms associated with tumor shrinkage following PDT treatment. The three mechanisms are as following: (1) PDT-induced oxidizing free radicals and singlet oxygen can directly kill cancer cells by inducing cell apoptosis or necrosis; (2) PDT-induced oxidizing free radicals and singlet oxygen also damages tumor-related vascular tissue, leading to hemorrhage or thrombosis in tumor blood vessels, which ultimately results in hypoxia and loss of nutrition leading to tumor cell death; (3) the release of cytokines and acute inflammation induced by PDT in the tumor tissue promote invasion of immune cells into tumor to destroy tumor cells [95]. In addition, PDT can also induce cell autophagy, and this process is also closely related to the content of singlet oxygen [96]. Although induction of ROS has been known to effectively kill tumors, the mechanism of ROS generation by PDT has not been fully elucidated, thus limiting the development of PDT in cancer. Additionally, the limited penetration depth of light restricts the effect of conventional PDT on deep tumors. Of note, oxygen dependence in the type 2 reaction of PDT limits the therapeutic effects of PDT in hypoxic tumors. Moreover the rapid consumption of oxygen at the irradiation site impedes the type 2 reaction of PDT, and thus also decreases the therapeutic effect of PDT [96].

Sonodynamic therapy (SDT) developed on the basis of PDT, is a novel noninvasive approach of solid tumor with low-intensity ultrasound and sonosensitizers (sensitizing drugs) [97]. ROS are found to be the most important factors in the tumor toxicity effects induced by SDT [98, 99]. Recent studies show SDT induces the loss of mitochondrial membrane potential and activates intrinsic apoptotic pathway by generating too much ROS in cancer cells [100, 101]. Moreover, SDT promotes cancer cell death by ROS-induced the downregulation of Bcl-2 family proteins [102]. In addition, SDT can strengthen the body antitumor immunity by generating ROS to promote the M2 macrophage inside the tumor to turn into M1 macrophage [103].

ROS and tumor immune therapy

Dysregulation or disruption of immune system functions can lead to diseases including neoplasia and cancer. ROS as byproducts of intracellular oxygen metabolism or cellular response to stress stimuli, participate in cellular immune response. Several studies have pointed to a role for mitochondrial ROS induced by TCR stimulation in influencing T cell activation, proliferation, and effector functions [104, 105]. Previous study has demonstrated that ROS is induced within 15 min after TCR cross-linking [111]. In addition, treatment of primary T cells and mice with antioxidants reduces T cell proliferation and production of IL-2, suggesting that ROS is required during initial stages of T cell activation [106, 107]. Moreover, Sena et al. report that ROS derived from mitochondrial complex III is required for antigen-specific $CD4^+$ T cell expansion in vivo [104]. Mitochondrial complex I-derived ROS is required for $CD8^+$ T cell function [105]. During T cell activation, mitochondria translocate to the immunological synapse [108]. This localization will allow for the maximal efficiency of short-lived ROS signaling that are sustained targeted for neutralization by antioxidant molecules, such as catalases, superoxide dismutases, peroxiredoxins, glutathione, among others. An important implication of those is that antioxidant therapy may be detrimental because ROS is the necessity for T cell

activation. The tumor antigen is restricted expression in normal cells but frequently occurs in cancer cells. The discovery of tumor antigen offers a hope for immunotherapeutic approaches. The survival of host often depends on initiating immune response, even before reaching the optimum antigen level. Hehner et al. have shown that exposure of T cells to macrophage-produced H_2O_2 allows the activation of response even at low concentration of antigens [109]. ROS can activate signaling intermediates of the MAPK, JNK, p38 and transcription factor NF-kB and NF-AT to regulate the T cell activity (e.g. the secretion of IL-2) [110]. Thus, the generation of ROS and their subsequent effect on T cells may allow a specific and robust immune response even at low concentration of tumor antigens.

In addition, programmed death-1 (PD-1) as an inhibitory receptor expressed on activated T cells could suppress antitumor immunity. Recently, studies have shown that the expression level of PD-1 is associated with the amount of cellular ROS and subsequent oxidative metabolism [111]. It suggests there may be a potential strategy to combine ROS scavenger with PD-1 blockade for more effective treatment of cancer. Additionally, ROS can regulate the phosphorylation of proline-rich tyrosine kinase 2 (Pyk2) to amplify tyrosine phosphorylation signaling events in cytotoxic T lymphocytes [112]. ROS generated by extra-mitochondria participate in the process of granzyme B-induced cancer cell death. Granzyme B secreted by cytotoxic T lymphocytes activates apoptotic pathway and finally induces cancer cell death [113].

It has been reported that physiological level ROS upregulate T cell activity [114], and the activity of T cells is downregulated when they are exposed to a high level ROS milieu [115]. In tumor microenvironment, the anti-tumor immune responses is suppressed by ROS generated from tumor cells and tumor-infiltrating leukocytes, including regulatory T cells (Treg), tumor-associated macrophages (TAM) and myeloid-derived suppressor cells (MDSC). Cemerski et al. have shown that excessive ROS produced in the tumor tissues can reach T cells and subsequently cause oxidative stress which could induce T cell hyporesponsiveness in patients [116]. Reduced ROS generation by NOX inhibitors or antioxidants also induces Treg hypoactivation in vitro [117]. TAM is a major player in the tumor microenvironment. Macrophage-derived ROS can suppress T-cell responses by inducing Treg [118]. Interestingly, upon PMA stimulation, M-CSF- and IL-10-induced macrophages show a high ROS producing capacity, whereas have a reduced T cell stimulatory capacity compared to IL-4-induced macrophages [118]. Administration of ROS inhibitor N-acetylcysteine (NAC) completely eliminates the suppressive effect of MDSCs on T cells [119]. Furthermore, $CD8^+$ T cell tolerance in tumor is associated with accumulation of MDSC containing high ROS levels [120]. Considering the ROS-mediated immunosuppressive properties in tumor microenvironment, a probable implication of therapeutic strategy targeting ROS is to employ antioxidant agents or supplements in situ tumor, which may enhance antitumor T cell responses. Moreover, increasing evidences demonstrate TAM are polarized M2 subtype of macrophage that displays pro-tumor effects and promotes tumor resistance to chemotherapy [121]. Autophagy has been shown to suppress M2 macrophage polarization by isoprenaline via the ROS/MAPK and mTOR signaling pathway [122].

Role of ROS in cancer chemotherapy

Although chemotherapy has strong side effects, it is still the most common treatment in cancer. Chemotherapeutic drugs exert anticancer effects through a variety of mechanisms. Many chemotherapeutic drugs can directly cause DNA damage and thus induce cancer cell death. Of note, some chemotherapeutic drugs induce ROS-involved tumor impairment [1]. The redox balance system regulates rates of ROS generation and elimination that determines the overall ROS levels. Different chemotherapeutic agents can induce obvious accumulation of ROS via accelerating ROS generation or suppressing ROS elimination in cancer cells [123]. Intracellular ROS can activate several signaling pathways involved in cell death. For example, in p53 wild-type cancer cells, DNA damage induced by ROS promotes p53 accumulation and activates p53/bax signaling pathway, resulting in cancer cell apoptosis in mitochondrial-dependent manner [124]. Recently, our study shows that chemotherapy agent SN-38 induces ROS production in colorectal cancer cells. Moreover, the autophagy inhibitor chloroquine synergistically sensitizes the anticancer effects of SN-38 through lysosomal and mitochondrial apoptotic pathway via p53-ROS cross-talk [4]. Excessive ROS accumulation induced by chemotherapeutic agents leads to tumor cytotoxic effects by direct damage of cell membranes or DNA. For example, ROS burst induced by the combination of cisplatin and FK228 directly induces DNA damage and breast cancer cell death [125].

Chemotherapy resistance is the major obstacle for successful cancer treatment in patients treated with long-term chemotherapeutic drugs [126, 127]. Although treatment methods for chemotherapy resistance have been continuously improved, one of the major concerns in tumor therapeutics is the development of multidrug resistance to various commercially used agents. As mentioned earlier, ATP-binding cassette (ABC) transporter protein, known as P-glycoprotein (P-gp) or multidrug resistance protein 1 (MRP1), is a multidrug resistance protein. MRP1 plays an important role in the efflux of drugs from the cells, and therefore reducing drugs-induced cell death [128]. Inhibition of MRP1 as a strategy to circumvent multidrug resistance to anticancer

therapeutic agents has been applied to in clinic. For chemoresistance in cancer, regulatory function of ROS is complex and paradoxical. Accumulating evidences suggest induction of ROS can depress MRP1 expression, leading to chemosensitization. By contrast, ledoux et al. report that ROS has been shown to promote MRP1 expression in cancer cells [129]. This raises the possibility that regulation of MRP1 by ROS is involved in ROS concentration. Indeed, low concentration of H_2O_2 increases MRP1 expression in human colon cancer cells Caco-2. However, MRP1 expression is decreased by high concentrations of ROS [130]. Consistent with this report, high levels of H_2O_2 induced by inhibition of catalase remarkably decrease MRP1 expression in HepG2 cells [131]. Similarly, Pandey et al. find that hyperglycemia reduces ROS-dependent MRP1 expression and enhances the toxicity of carboplatin and 5-fluorouracil in MCF-7 cells [132]. Altogether, ROS not only induces tumor resistance to chemotherapy, but also promotes tumor chemosensitivity by regulating MRP1 expression. However, the exact molecular mechanism linked to ROS-mediated regulation of MRP1 and other multi-drug resistant proteins remains unclear. Further detailed researches are required to identify the mechanism.

ROS in radiotherapy

Radiotherapy using ionizing radiation is one of the most reliable and widely used treatments for solid tumor. Ionizing radiation induces cancer cell death by damaging the cellular DNA. Moreover, ionizing radiation also can generate ROS through water radiolysis react with oxygen. ROS induced by water radiolysis during radiotherapy mainly includes hydroxyl radicals and other radicals. High level of hydroxyl radicals induced by ionizing radiation increases oxidative stress to destabilize cancer cells integrity and DNA damage by form stable DNA peroxides, and subsequently results in cell death. However, not all tumor cells are sensitive to radiotherapy. In general, rapidly dividing cells are more sensitive to radiotherapy than those with slowly dividing cells [133]. In addition, cells in M phase of mitosis and G2 phase are most sensitive to radiotherapy-induced hydroxyl radicals compared with S phase [134]. Above results suggest the dividing cancer cells sensitivity to radiotherapy is associated with hydroxyl radicals induced by ionizing radiation. Due to that ROS production contributes to cancer cell death induced by radiotherapy, increasing ROS production will be a feasible strategy for mitigating cancer cells resistance to radiotherapy. In many recent studies, certain ROS-inducers have been shown to promote the ROS-mediated susceptibility of cancer cells to radiation. For example, coroglaucigenin promotes the production of hydroxyl radicals by inhibiting the expression of antioxidant molecules, thereby enhancing the sensitivity of human lung cancer cells to radiation therapy [135]. Recently, Wang et al. show Auranofin enhances radiosensitivity of breast cancer cells through suppressing thioredoxin reductase and therefore resulting in burst of hydroxyl radicals. In nasopharyngeal carcinoma cells, salinomycin sensitizes cancer cells to raditherapy by inactivation of Nrf2 and increase of hydroxyl radicals generation [136].

Prospect

Convincing evidence suggests that the levels of ROS in cancer cells are markedly increased relative to normal cells due to a variety of complex factors including tumor microenvironment and active energy metabolism of cancer cells. Therefore, further increase of ROS levels by using ROS stimulators can effectively kill cancer cells, whereas may be inadequate to trigger apoptosis in normal cells with low level of intrinsic ROS. This provides a biochemical basis for developing a novel therapeutic strategy. Increasing intracellular ROS levels may involve the use of drugs that directly increase ROS production, or drugs that inhibit antioxidant defense, or the two types of agents in combination. Consequently, it is necessary to develop some agents that can trigger the ROS-mediated damage and ultimately cause cancer cell death. It is worth noting that most of studies fail to identify the specific kind of ROS molecules induced by those ROS-induced drugs. It is also not clear which kind of ROS plays a main role in cancer cell death induced by ROS-induced agents. It requires further study to elucidate the mechanism. Additionally, accumulating studies show that ROS is a double-edged sword displaying both tumor promoting and suppressing functions. Generally, low levels of ROS promote the tumor progression, whereas high levels of ROS hinder tumor progression. Thereby, it is pivotal and necessary to carefully control the amount of ROS induced by drugs in the treatment of cancer.

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Compliance with ethical standards

Conflict of interest The authors declare no competing financial interest.

References

1. Trachootham D, Alexandre J, Huang P (2009) Targeting cancer cells by ROS-mediated mechanisms: a radical therapeutic approach? Nat Rev Drug Discov 8:579–591
2. Idelchik M, Begley U, Begley TJ, Melendez JA (2017) Mitochondrial ROS control of cancer. Semin Cancer Biol. doi:10.1016/S1044-579X(17)30098-6

3. Hendrick E, Peixoto P, Blomme A, Polese C, Matheus N, Cimino J et al (2017) Metabolic inhibitors accentuate the anti-tumoral effect of HDAC5 inhibition. Oncogene 36:4859–4874
4. Chen P, Luo X, Nie P, Wu B, Xu W, Shi X et al (2017) CQ synergistically sensitizes human colorectal cancer cells to SN-38/CPT-11 through lysosomal and mitochondrial apoptotic pathway via p53-ROS cross-talk. Free Radic Biol Med 104:280–297
5. Scialo F, Mallikarjun V, Stefanatos R, Sanz A (2013) Regulation of lifespan by the mitochondrial electron transport chain: reactive oxygen species-dependent and reactive oxygen species-independent mechanisms. Antioxid Redox Signal 19:1953–1969
6. Raj L, Ide T, Gurkar AU, Foley M, Schenone M, Li X et al (2011) Selective killing of cancer cells by a small molecule targeting the stress response to ROS. Nature 475:231–234
7. Trachootham D, Zhang H, Zhang W, Feng L, Du M, Zhou Y et al (2008) Effective elimination of fludarabine-resistant CLL cells by PEITC through a redox-mediated mechanism. Blood 112:1912–1922
8. Chio I, Tuveson DA (2017) ROS in cancer: the burning question. Trends Mol Med 23:411–429
9. Nakamura S, Nakanishi A, Takazawa M, Okihiro S, Urano S, Fukui K (2016) Ionomycin-induced calcium influx induces neurite degeneration in mouse neuroblastoma cells: analysis of a time-lapse live cell imaging system. Free Radic Res 50:1214–1225
10. Zou ZZ, Nie PP, Li YW, Hou BX, Rui-Li, Shi XP et al (2017) Synergistic induction of apoptosis by salinomycin and gefitinib through lysosomal and mitochondrial dependent pathway overcomes gefitinib resistance in colorectal cancer. Oncotarget 8:22414–22432
11. Dickinson BC, Chang CJ (2011) Chemistry and biology of reactive oxygen species in signaling or stress responses. Nat Chem Biol 7:504–511
12. Sumimoto H (2008) Structure, regulation and evolution of Nox-family NADPH oxidases that produce reactive oxygen species. FEBS J 275:3249–3277
13. Schieber M, Chandel NS (2014) ROS function in redox signaling and oxidative stress. Curr Biol 24:R453-R462
14. Jeon SM, Chandel NS, Hay N (2012) AMPK regulates NADPH homeostasis to promote tumour cell survival during energy stress. Nature 485:661–665
15. Luo X, Yao J, Nie P, Yang Z, Feng H, Chen P et al (2016) FOXM1 promotes invasion and migration of colorectal cancer cells partially dependent on HSPA5 transactivation. Oncotarget 7:26480–26495
16. Zhang S, Zhao BS, Zhou A, Lin K, Zheng S, Lu Z et al (2017) m6A demethylase ALKBH5 maintains tumorigenicity of glioblastoma stem-like cells by sustaining FOXM1 expression and cell proliferation program. Cancer Cell 31:591–606
17. Park HJ, Carr JR, Wang Z, Nogueira V, Hay N, Tyner AL et al (2009) FoxM1, a critical regulator of oxidative stress during oncogenesis. EMBO J 28:2908–2918
18. Liou GY, Doppler H, DelGiorno KE, Zhang L, Leitges M, Crawford HC et al (2016) Mutant KRas-induced mitochondrial oxidative stress in acinar cells upregulates EGFR signaling to drive formation of pancreatic precancerous lesions. Cell Rep 14:2325–2336
19. Ray PD, Huang BW, Tsuji Y (2012) Reactive oxygen species (ROS) homeostasis and redox regulation in cellular signaling. Cell Signal 24:981–990
20. Tobiume K, Matsuzawa A, Takahashi T, Nishitoh H, Morita K, Takeda K et al (2001) ASK1 is required for sustained activations of JNK/p38 MAP kinases and apoptosis. EMBO Rep 2:222–228
21. Dhanasekaran DN, Reddy EP (2008) JNK signaling in apoptosis. Oncogene 27:6245–6251
22. Zhang J, Wang X, Vikash V, Ye Q, Wu D, Liu Y et al (2016) ROS and ROS-mediated cellular signaling. Oxid Med Cell Longev 2016:4350965
23. Alexander A, Cai SL, Kim J, Nanez A, Sahin M, MacLean KH et al (2010) ATM signals to TSC2 in the cytoplasm to regulate mTORC1 in response to ROS. Proc Natl Acad Sci USA 107:4153–4158
24. Potter CJ, Pedraza LG, Xu T (2002) Akt regulates growth by directly phosphorylating Tsc2. Nat Cell Biol 4:658–665
25. Ishaq M, Kumar S, Varinli H, Han ZJ, Rider AE, Evans MD et al (2014) Atmospheric gas plasma-induced ROS production activates TNF-ASK1 pathway for the induction of melanoma cancer cell apoptosis. Mol Biol Cell 25:1523–1531
26. Nadeau PJ, Charette SJ, Landry J (2009) REDOX reaction at ASK1-Cys250 is essential for activation of JNK and induction of apoptosis. Mol Biol Cell 20:3628–3637
27. Kim S, Lee TJ, Leem J, Choi KS, Park JW, Kwon TK (2008) Sanguinarine-induced apoptosis: generation of ROS, down-regulation of Bcl-2, c-FLIP, and synergy with TRAIL. J Cell Biochem 104:895–907
28. Wang L, Azad N, Kongkaneramit L, Chen F, Lu Y, Jiang BH et al (2008) The Fas death signaling pathway connecting reactive oxygen species generation and FLICE inhibitory protein down-regulation. J Immunol 180:3072–3080
29. Devadas S, Zaritskaya L, Rhee SG, Oberley L, Williams MS (2002) Discrete generation of superoxide and hydrogen peroxide by T cell receptor stimulation: selective regulation of mitogen-activated protein kinase activation and fas ligand expression. J Exp Med 195:59–70
30. Gulow K, Kaminski M, Darvas K, Suss D, Li-Weber M, Krammer PH (2005) HIV-1 trans-activator of transcription substitutes for oxidative signaling in activation-induced T cell death. J Immunol 174:5249–5260
31. Jin X, Song L, Liu X, Chen M, Li Z, Cheng L et al (2014) Protective efficacy of vitamins C and E on p,p'-DDT-induced cytotoxicity via the ROS-mediated mitochondrial pathway and NF-kappaB/FasL pathway. PLoS ONE 9:e113257
32. Faris M, Latinis KM, Kempiak SJ, Koretzky GA, Nel A (1998) Stress-induced Fas ligand expression in T cells is mediated through a MEK kinase 1-regulated response element in the Fas ligand promoter. Mol Cell Biol 18:5414–5424
33. Liu Y, Borchert GL, Surazynski A, Hu CA, Phang JM (2006) Proline oxidase activates both intrinsic and extrinsic pathways for apoptosis: the role of ROS/superoxides, NFAT and MEK/ERK signaling. Oncogene 25:5640–5647
34. Dewangan J, Tandon D, Srivastava S, Verma AK, Yapuri A, Rath SK (2017) Novel combination of salinomycin and resveratrol synergistically enhances the anti-proliferative and pro-apoptotic effects on human breast cancer cells. Apoptosis. doi:10.1007/s10495-017-1394-y
35. Madesh M, Hajnoczky G (2001) VDAC-dependent permeabilization of the outer mitochondrial membrane by superoxide induces rapid and massive cytochrome c release. J Cell Biol 155:1003–1015
36. Queiroga CS, Almeida AS, Martel C, Brenner C, Alves PM, Vieira HL (2010) Glutathionylation of adenine nucleotide translocase induced by carbon monoxide prevents mitochondrial membrane permeabilization and apoptosis. J Biol Chem 285:17077–17088
37. Zuo Y, Xiang B, Yang J, Sun X, Wang Y, Cang H et al (2009) Oxidative modification of caspase-9 facilitates its activation via disulfide-mediated interaction with Apaf-1. Cell Res 19:449–457
38. Katoh I, Tomimori Y, Ikawa Y, Kurata S (2004) Dimerization and processing of procaspase-9 by redox stress in mitochondria. J Biol Chem 279:15515–15523

39. Luanpitpong S, Chanvorachote P, Stehlik C, Tse W, Callery PS, Wang L et al (2013) Regulation of apoptosis by Bcl-2 cysteine oxidation in human lung epithelial cells. Mol Biol Cell 24:858–869
40. Li D, Ueta E, Kimura T, Yamamoto T, Osaki T (2004) Reactive oxygen species (ROS) control the expression of Bcl-2 family proteins by regulating their phosphorylation and ubiquitination. Cancer Sci 95:644–650
41. Luanpitpong S, Chanvorachote P, Nimmannit U, Leonard SS, Stehlik C, Wang L et al (2012) Mitochondrial superoxide mediates doxorubicin-induced keratinocyte apoptosis through oxidative modification of ERK and Bcl-2 ubiquitination. Biochem Pharmacol 83:1643–1654
42. Zou Z, Yuan Z, Zhang Q, Long Z, Chen J, Tang Z et al (2012) Aurora kinase A inhibition-induced autophagy triggers drug resistance in breast cancer cells. Autophagy 8:1798–1810
43. Zhao Y, Qu T, Wang P, Li X, Qiang J, Xia Z et al (2016) Unravelling the relationship between macroautophagy and mitochondrial ROS in cancer therapy. Apoptosis 21:517–531
44. Chen Y, McMillan-Ward E, Kong J, Israels SJ, Gibson SB (2008) Oxidative stress induces autophagic cell death independent of apoptosis in transformed and cancer cells. Cell Death Differ 15:171–182
45. Tripathi DN, Zhang J, Jing J, Dere R, Walker CL (2016) A new role for ATM in selective autophagy of peroxisomes (pexophagy). Autophagy 12:711–712
46. Byun YJ, Kim SK, Kim YM, Chae GT, Jeong SW, Lee SB (2009) Hydrogen peroxide induces autophagic cell death in C6 glioma cells via BNIP3-mediated suppression of the mTOR pathway. Neurosci Lett 461:131–135
47. Pallichankandy S, Rahman A, Thayyullathil F, Galadari S (2015) ROS-dependent activation of autophagy is a critical mechanism for the induction of anti-glioma effect of sanguinarine. Free Radic Biol Med 89:708–720
48. Scherz-Shouval R, Shvets E, Fass E, Shorer H, Gil L, Elazar Z (2007) Reactive oxygen species are essential for autophagy and specifically regulate the activity of Atg4. EMBO J 26:1749–1760
49. Song C, Mitter SK, Qi X, Beli E, Rao HV, Ding J et al (2017) Oxidative stress-mediated NFkappaB phosphorylation upregulates p62/SQSTM1 and promotes retinal pigmented epithelial cell survival through increased autophagy. PLoS ONE 12:e171940
50. Zhang Y, Su SS, Zhao S, Yang Z, Zhong CQ, Chen X et al (2017) RIP1 autophosphorylation is promoted by mitochondrial ROS and is essential for RIP3 recruitment into necrosome. Nat Commun 8:14329
51. Sun W, Wu X, Gao H, Yu J, Zhao W, Lu JJ et al (2017) Cytosolic calcium mediates RIP1/RIP3 complex-dependent necroptosis through JNK activation and mitochondrial ROS production in human colon cancer cells. Free Radic Biol Med 108:433–444
52. Zhou Z, Lu B, Wang C, Wang Z, Luo T, Piao M et al (2017) RIP1 and RIP3 contribute to shikonin-induced DNA double-strand breaks in glioma cells via increase of intracellular reactive oxygen species. Cancer Lett 390:77–90
53. Chauhan AK, Min KJ, Kwon TK (2017) RIP1-dependent reactive oxygen species production executes artesunate-induced cell death in renal carcinoma Caki cells. Mol Cell Biochem. doi:10.1007/s11010-017-3052-7
54. Dixon SJ, Stockwell BR (2014) The role of iron and reactive oxygen species in cell death. Nat Chem Biol 10:9–17
55. Zhang DW, Shao J, Lin J, Zhang N, Lu BJ, Lin SC et al (2009) RIP3, an energy metabolism regulator that switches TNF-induced cell death from apoptosis to necrosis. Science 325:332–336
56. Dixon SJ, Lemberg KM, Lamprecht MR, Skouta R, Zaitsev EM, Gleason CE et al (2012) Ferroptosis: an iron-dependent form of nonapoptotic cell death. Cell 149:1060–1072
57. Viswanathan VS, Ryan MJ, Dhruv HD, Gill S, Eichhoff OM, Seashore-Ludlow B et al (2017) Dependency of a therapy-resistant state of cancer cells on a lipid peroxidase pathway. Nature 547:453–457
58. Yang WS, SriRamaratnam R, Welsch ME, Shimada K, Skouta R, Viswanathan VS et al (2014) Regulation of ferroptotic cancer cell death by GPX4. Cell 156:317–331
59. Gao M, Monian P, Quadri N, Ramasamy R, Jiang X (2015) Glutaminolysis and transferrin regulate ferroptosis. Mol Cell 59:298–308
60. Liu L, Wei Y, Zhai S, Chen Q, Xing D (2015) Dihydroartemisinin and transferrin dual-dressed nano-graphene oxide for a pH-triggered chemotherapy. Biomaterials 62:35–46
61. Roh JL, Kim EH, Jang H, Shin D (2017) Nrf2 inhibition reverses the resistance of cisplatin-resistant head and neck cancer cells to artesunate-induced ferroptosis. Redox Biol 11:254–262
62. Mi YJ, Geng GJ, Zou ZZ, Gao J, Luo XY, Liu Y et al (2015) Dihydroartemisinin inhibits glucose uptake and cooperates with glycolysis inhibitor to induce apoptosis in non-small cell lung carcinoma cells. PLoS ONE 10:e120426
63. Hamai A, Caneque T, Muller S, Mai TT, Hienzsch A, Ginestier C et al (2017) An iron hand over cancer stem cells. Autophagy 13:1465–1466
64. Guo J, Xu B, Han Q, Zhou H, Xia Y, Gong C et al (2017) Ferroptosis: a novel anti-tumor action for cisplatin. Cancer Res Treat. doi:10.4143/crt.2016.572
65. Yang Y, Guo R, Tian X, Zhang Z, Zhang P, Li C et al (2017) Synergistic anti-tumor activity of Nimotuzumab in combination with Trastuzumab in HER2-positive breast cancer. Biochem Biophys Res Commun 489:523–527
66. Santoro V, Jia R, Thompson H, Nijhuis A, Jeffery R, Kiakos K et al (2016) Role of reactive oxygen species in the abrogation of oxaliplatin activity by cetuximab in colorectal cancer. J Natl Cancer Inst 108:v394
67. Cao S, Xia M, Mao Y, Zhang Q, Donkor PO, Qiu F et al (2016) Combined oridonin with cetuximab treatment shows synergistic anticancer effects on laryngeal squamous cell carcinoma: involvement of inhibition of EGFR and activation of reactive oxygen species-mediated JNK pathway. Int J Oncol 49:2075–2087
68. Fack F, Espedal H, Keunen O, Golebiewska A, Obad N, Harter PN et al (2015) Bevacizumab treatment induces metabolic adaptation toward anaerobic metabolism in glioblastomas. Acta Neuropathol 129:115–131
69. Guo XL, Li D, Sun K, Wang J, Liu Y, Song JR et al (2013) Inhibition of autophagy enhances anticancer effects of bevacizumab in hepatocarcinoma. J Mol Med (Berl) 91:473–483
70. Gautam J, Ku JM, Regmi SC, Jeong H, Wang Y, Banskota S et al (2017) Dual inhibition of NOX2 and receptor tyrosine kinase by BJ-1301 enhances anticancer therapy efficacy via suppression of autocrine stimulatory factors in lung cancer. Mol Cancer Ther. doi:10.1158/1535-7163
71. Abdel-Aziz AK, Shouman S, El-Demerdash E, Elgendy M, Abdel-Naim AB (2014) Chloroquine synergizes sunitinib cytotoxicity via modulating autophagic, apoptotic and angiogenic machineries. Chem Biol Interact 217:28–40
72. Leone A, Roca MS, Ciardiello C, Terranova-Barberio M, Vitagliano C, Ciliberto G et al (2015) Vorinostat synergizes with EGFR inhibitors in NSCLC cells by increasing ROS via up-regulation of the major mitochondrial porin VDAC1 and modulation of the c-Myc-NRF2-KEAP1 pathway. Free Radic Biol Med 89:287–299
73. Okon IS, Coughlan KA, Zhang M, Wang Q, Zou MH (2015) Gefitinib-mediated reactive oxygen specie (ROS) instigates mitochondrial dysfunction and drug resistance in lung cancer cells. J Biol Chem 290:9101–9110

74. Shan F, Shao Z, Jiang S, Cheng Z (2016) Erlotinib induces the human non-small-cell lung cancer cells apoptosis via activating ROS-dependent JNK pathways. Cancer Med 5:3166–3175
75. Orcutt KP, Parsons AD, Sibenaller ZA, Scarbrough PM, Zhu Y, Sobhakumari A et al (2011) Erlotinib-mediated inhibition of EGFR signaling induces metabolic oxidative stress through NOX4. Cancer Res 71:3932–3940
76. Nie P, Hu W, Zhang T, Yang Y, Hou B, Zou Z (2015) Synergistic induction of Erlotinib-mediated apoptosis by resveratrol in human non-small-cell lung cancer cells by down-regulating survivin and up-regulating PUMA. Cell Physiol Biochem 35:2255–2271
77. Wardman P (2007) Fluorescent and luminescent probes for measurement of oxidative and nitrosative species in cells and tissues: progress, pitfalls, and prospects. Free Radic Biol Med 43:995–1022
78. Bonini MG, Siraki AG, Atanassov BS, Mason RP (2007) Immunolocalization of hypochlorite-induced, catalase-bound free radical formation in mouse hepatocytes. Free Radic Biol Med 42:530–540
79. Hui KF, Lam BH, Ho DN, Tsao SW, Chiang AK (2013) Bortezomib and SAHA synergistically induce ROS-driven caspase-dependent apoptosis of nasopharyngeal carcinoma and block replication of Epstein-Barr virus. Mol Cancer Ther 12:747–758
80. Yin L, Kufe T, Avigan D, Kufe D (2014) Targeting MUC1-C is synergistic with bortezomib in downregulating TIGAR and inducing ROS-mediated myeloma cell death. Blood 123:2997–3006
81. Xian M, Cao H, Cao J, Shao X, Zhu D, Zhang N et al (2017) Bortezomib sensitizes human osteosarcoma cells to adriamycin-induced apoptosis through ROS-dependent activation of p-eIF2alpha/ATF4/CHOP axis. Int J Cancer 141:1029–1041
82. Fan WH, Hou Y, Meng FK, Wang XF, Luo YN, Ge PF (2011) Proteasome inhibitor MG-132 induces C6 glioma cell apoptosis via oxidative stress. Acta Pharmacol Sin 32:619–625
83. Park S, Park JA, Yoo H, Park HB, Lee Y (2017) Proteasome inhibitor-induced cleavage of HSP90 is mediated by ROS generation and caspase 10-activation in human leukemic cells. Redox Biol 13:470–476
84. Bhalla S, Balasubramanian S, David K, Sirisawad M, Buggy J, Mauro L et al (2009) PCI-24781 induces caspase and reactive oxygen species-dependent apoptosis through NF-kappaB mechanisms and is synergistic with bortezomib in lymphoma cells. Clin Cancer Res 15:3354–3365
85. Sholler GS, Currier EA, Dutta A, Slavik MA, Illenye SA, Mendonca MC et al (2013) PCI-24781 (abexinostat), a novel histone deacetylase inhibitor, induces reactive oxygen species-dependent apoptosis and is synergistic with bortezomib in neuroblastoma. J Cancer Ther Res 2:21
86. Cornago M, Garcia-Alberich C, Blasco-Angulo N, Vall-Llaura N, Nager M, Herreros J et al (2014) Histone deacetylase inhibitors promote glioma cell death by G2 checkpoint abrogation leading to mitotic catastrophe. Cell Death Dis 5:e1435
87. Karthik S, Sankar R, Varunkumar K, Anusha C, Ravikumar V (2015) Blocking NF-kappaB sensitizes non-small cell lung cancer cells to histone deacetylase inhibitor induced extrinsic apoptosis through generation of reactive oxygen species. Biomed Pharmacother 69:337–344
88. Hedrick E, Crose L, Linardic CM, Safe S (2015) Histone deacetylase inhibitors inhibit rhabdomyosarcoma by reactive oxygen species-dependent targeting of specificity protein transcription factors. Mol Cancer Ther 14:2143–2153
89. Miao Z, Yu F, Ren Y, Yang J (2017) D, L-Sulforaphane induces ROS-dependent apoptosis in human gliomablastoma cells by inactivating STAT3 signaling pathway. Int J Mol Sci 18
90. Hu Y, Zhao C, Zheng H, Lu K, Shi D, Liu Z et al (2017) A novel STAT3 inhibitor HO-3867 induces cell apoptosis by reactive oxygen species-dependent endoplasmic reticulum stress in human pancreatic cancer cells. Anticancer Drugs 28:392–400
91. Shackelford DB, Shaw RJ (2009) The LKB1-AMPK pathway: metabolism and growth control in tumour suppression. Nat Rev Cancer 9:563–575
92. Wu S, Xing D (2012) Mechanism of mitochondrial membrane permeabilization during apoptosis under photofrin-mediated photodynamic therapy. J Xray Sci Technol 20:363–372
93. van Straten D, Mashayekhi V, de Bruijn HS, Oliveira S, Robinson DJ (2017) Oncologic photodynamic therapy: basic principles, current clinical status and future directions. Cancers (Basel) 9(2):E19
94. Gdovin MJ, Kadri N, Rios L, Holliday S, Jordan Z (2017) Focal photodynamic intracellular acidification as a cancer therapeutic. Semin Cancer Biol 43:147–156
95. Garg AD, Dudek AM, Ferreira GB, Verfaillie T, Vandenabeele P, Krysko DV et al (2013) ROS-induced autophagy in cancer cells assists in evasion from determinants of immunogenic cell death. Autophagy 9:1292–1307
96. Wei Y, Song J, Chen Q, Xing D (2012) Enhancement of photodynamic antitumor effect with pro-oxidant ascorbate. Lasers Surg Med 44:69–75
97. McHale AP, Callan JF, Nomikou N, Fowley C, Callan B (2016) Sonodynamic therapy: concept, mechanism and application to cancer treatment. Adv Exp Med Biol 880:429–450
98. Trendowski M (2014) The promise of sonodynamic therapy. Cancer Metastasis Rev 33:143–160
99. Misik V, Riesz P (2000) Free radical intermediates in sonodynamic therapy. Ann N Y Acad Sci 899:335–348
100. Trendowski M (2015) Using the promise of sonodynamic therapy in the clinical setting against disseminated cancers. Chemother Res Pract 2015:316015
101. Chen H, Gao W, Yang Y, Guo S, Wang H, Wang W et al (2014) Inhibition of VDAC1 prevents Ca(2)(+)-mediated oxidative stress and apoptosis induced by 5-aminolevulinic acid mediated sonodynamic therapy in THP-1 macrophages. Apoptosis 19:1712–1726
102. Wang H, Yang Y, Chen H, Dan J, Cheng J, Guo S et al (2014) The predominant pathway of apoptosis in THP-1 macrophage-derived foam cells induced by 5-aminolevulinic acid-mediated sonodynamic therapy is the mitochondria-caspase pathway despite the participation of endoplasmic reticulum stress. Cell Physiol Biochem 33:1789–1801
103. Wang S, Hu Z, Wang X, Gu C, Gao Z, Cao W et al (2014) 5-Aminolevulinic acid-mediated sonodynamic therapy reverses macrophage and dendritic cell passivity in murine melanoma xenografts. Ultrasound Med Biol 40:2125–2133
104. Sena LA, Li S, Jairaman A, Prakriya M, Ezponda T, Hildeman DA et al (2013) Mitochondria are required for antigen-specific T cell activation through reactive oxygen species signaling. Immunity 38:225–236
105. Yi JS, Holbrook BC, Michalek RD, Laniewski NG, Grayson JM (2006) Electron transport complex I is required for $CD8^+$ T cell function. J Immunol 177:852–862
106. Laniewski NG, Grayson JM (2004) Antioxidant treatment reduces expansion and contraction of antigen-specific $CD8^+$ T cells during primary but not secondary viral infection. J Virol 78:11246–11257
107. Chaudhri G, Clark IA, Hunt NH, Cowden WB, Ceredig R (1986) Effect of antioxidants on primary alloantigen-induced T cell activation and proliferation. J Immunol 137:2646–2652
108. Schwindling C, Quintana A, Krause E, Hoth M (2010) Mitochondria positioning controls local calcium influx in T cells. J Immunol 184:184–190

109. Hehner SP, Breitkreutz R, Shubinsky G, Unsoeld H, Schulze-Osthoff K, Schmitz ML et al (2000) Enhancement of T cell receptor signaling by a mild oxidative shift in the intracellular thiol pool. J Immunol 165:4319–4328
110. Uzhachenko R, Ivanov SV, Yarbrough WG, Shanker A, Medzhitov R, Ivanova AV (2014) Fus1/Tusc2 is a novel regulator of mitochondrial calcium handling, Ca^{2+}-coupled mitochondrial processes, and Ca^{2+}-dependent NFAT and NF-kappaB pathways in $CD4^+$ T cells. Antioxid Redox Signal 20:1533–1547
111. Tkachev V, Goodell S, Opipari AW, Hao LY, Franchi L, Glick GD et al (2015) Programmed death-1 controls T cell survival by regulating oxidative metabolism. J Immunol 194:5789–5800
112. Lysechko TL, Cheung SM, Ostergaard HL (2010) Regulation of the tyrosine kinase Pyk2 by calcium is through production of reactive oxygen species in cytotoxic T lymphocytes. J Biol Chem 285:31174–31184
113. Aguilo JI, Anel A, Catalan E, Sebastian A, Acin-Perez R, Naval J et al (2010) Granzyme B of cytotoxic T cells induces extramitochondrial reactive oxygen species production via caspase-dependent NADPH oxidase activation. Immunol Cell Biol 88:545–554
114. Murphy MP, Siegel RM (2013) Mitochondrial ROS fire up T cell activation. Immunity 38:201–202
115. Gelderman KA, Hultqvist M, Holmberg J, Olofsson P, Holmdahl R (2006) T cell surface redox levels determine T cell reactivity and arthritis susceptibility. Proc Natl Acad Sci U S A 103:12831–12836
116. Cemerski S, Cantagrel A, Van Meerwijk JP, Romagnoli P (2002) Reactive oxygen species differentially affect T cell receptor-signaling pathways. J Biol Chem 277:19585–19593
117. Efimova O, Szankasi P, Kelley TW (2011) Ncf1 (p47phox) is essential for direct regulatory T cell mediated suppression of $CD4^+$ effector T cells. PLoS One 6:e16013
118. Kraaij MD, Koekkoek KM, van der Kooij SW, Gelderman KA, van Kooten C (2013) Subsets of human type 2 macrophages show differential capacity to produce reactive oxygen species. Cell Immunol 284:1–8
119. Wei J, Zhang M, Zhou J (2015) Myeloid-derived suppressor cells in major depression patients suppress T cell responses through the production of reactive oxygen species. Psychiatry Res 228:695–701
120. Nagaraj S, Gupta K, Pisarev V, Kinarsky L, Sherman S, Kang L et al (2007) Altered recognition of antigen is a mechanism of $CD8^+$ T cell tolerance in cancer. Nat Med 13:828–835
121. Wang Q, Hu B, Hu X, Kim H, Squatrito M, Scarpace L et al (2017) Tumor evolution of glioma-intrinsic gene expression subtypes associates with immunological changes in the microenvironment. Cancer Cell 32:42–56
122. Shan M, Qin J, Jin F, Han X, Guan H, Li X et al (2017) Autophagy suppresses isoprenaline-induced M2 macrophage polarization via the ROS/ERK and mTOR signaling pathway. Free Radic Biol Med 110:432–443
123. Magda D, Miller RA (2006) Motexafin gadolinium: a novel redox active drug for cancer therapy. Semin Cancer Biol 16:466–476
124. Ray T, Chakrabarti MK, Pal A (2016) Hemagglutinin protease secreted by V. cholerae induced apoptosis in breast cancer cells by ROS mediated intrinsic pathway and regresses tumor growth in mice model. Apoptosis 21:143–154
125. Pluchino LA, Choudhary S, Wang HC (2016) Reactive oxygen species-mediated synergistic and preferential induction of cell death and reduction of clonogenic resistance in breast cancer cells by combined cisplatin and FK228. Cancer Lett 381:124–132
126. Ao X, Nie P, Wu B, Xu W, Zhang T, Wang S et al (2016) Decreased expression of microRNA-17 and microRNA-20b promotes breast cancer resistance to taxol therapy by upregulation of NCOA3. Cell Death Dis 7:e2463
127. Zhu J, Zou Z, Nie P, Kou X, Wu B, Wang S et al (2016) Downregulation of microRNA-27b-3p enhances tamoxifen resistance in breast cancer by increasing NR5A2 and CREB1 expression. Cell Death Dis 7:e2454
128. Zhang LH, Yang AJ, Wang M, Liu W, Wang CY, Xie XF et al (2016) Enhanced autophagy reveals vulnerability of P-gp mediated epirubicin resistance in triple negative breast cancer cells. Apoptosis 21:473–488
129. Ledoux S, Yang R, Friedlander G, Laouari D (2003) Glucose depletion enhances P-glycoprotein expression in hepatoma cells: role of endoplasmic reticulum stress response. Cancer Res 63:7284–7290
130. Terada Y, Ogura J, Tsujimoto T, Kuwayama K, Koizumi T, Sasaki S et al (2014) Intestinal P-glycoprotein expression is multimodally regulated by intestinal ischemia-reperfusion. J Pharm Pharm Sci 17:266–276
131. Li L, Xu J, Min T, Huang W (2006) Up-regulation of P-glycoprotein expression by catalase via JNK activation in HepG2 cells. Redox Rep 11:173–178
132. Pandey V, Chaube B, Bhat MK (2011) Hyperglycemia regulates MDR-1, drug accumulation and ROS levels causing increased toxicity of carboplatin and 5-fluorouracil in MCF-7 cells. J Cell Biochem 112:2942–2952
133. Dayal R, Singh A, Pandey A, Mishra KP (2014) Reactive oxygen species as mediator of tumor radiosensitivity. J Cancer Res Ther 10:811–818
134. Yu S, Wang L, Cao Z, Gong D, Liang Q, Chen H et al (2017) Anticancer effect of Polyphyllin Iota in colorectal cancer cells through ROS-dependent autophagy and G2/M arrest mechanisms. Nat Prod Res 16:1–4
135. Sun M, Pan D, Chen Y, Li Y, Gao K, Hu B (2017) Coroglaucigenin enhances the radiosensitivity of human lung cancer cells through Nrf2/ROS pathway. Oncotarget 8:32807–32820
136. Zhang G, Wang W, Yao C, Ren J, Zhang S, Han M (2017) Salinomycin overcomes radioresistance in nasopharyngeal carcinoma cells by inhibiting Nrf2 level and promoting ROS generation. Biomed Pharmacother 91:147–154