




# Implications of reactive oxygen species in lung cancer and exploiting it for therapeutic interventions

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Received: 17 October 2022 / Accepted: 15 November 2022 / Published online: 6 December 2022  
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## Abstract

Lung cancer is the second (11.4%) most commonly diagnosed cancer and the first (18%) to cause cancer-related deaths worldwide. The incidence of lung cancer varies significantly among men, women, and high and low-middle-income countries. Air pollution, inhalable agents, and tobacco smoking are a few of the critical factors that determine lung cancer incidence and mortality worldwide. Reactive oxygen species are known factors of lung carcinogenesis resulting from the xenobiotics and their mechanistic paths are under critical investigation. Reactive oxygen species exhibit dual roles in cells, as a tumorigenic and anti-proliferative factor, depending on spatiotemporal context. During the precancerous state, ROS promotes cancer origination through oxidative stress and base-pair substitution mutations in pro-oncogenes and tumor suppressor genes. At later stages of tumor progression, they help the cancer cells in invasion, and metastases by activating the NF-κB and MAPK pathways. However, at advanced stages, when ROS exceeds the threshold, it promotes cell cycle arrest and induces apoptosis in cancer cells. ROS activates extrinsic apoptosis through death receptors and intrinsic apoptosis through mitochondrial pathways. Moreover, ROS upregulates the expression of beclin-1 which is a critical component to initiate autophagy, another form of programmed cell death. ROS is additionally involved in an intermediary step in necroptosis, which catalyzes and accelerates this form of cell death. Various therapeutic interventions have been attempted to exploit this cytotoxic potential of ROS to treat different cancers. Growing body of evidence suggests that ROS is also associated with chemoresistance and cancer cell immunity. Considering the multiple roles of ROS, this review highlights the exploitation of ROS for various therapeutic interventions. However, there are still gaps in the literature on the dual roles of ROS and the involvement of ROS in cancer cell immunity and therapy resistance.

**Keywords** Reactive oxygen species · Oxidative stress · Cancer therapy · Lung cancer · Programmed cell death

## Introduction

The inability of the cells to abide by contact inhibition paving its way toward uncontrolled proliferation causes cancer. Cancer cells can bypass all the checkpoints that are otherwise present in a normal mitotic division and thereby disrupt the functioning of the body. The cancer is named based on

the site of origin; thus, lung cancer originates in the cells of the respiratory system. Lung cancer is among the most common ones with an estimation of 2.20 million new cases per year. About 27% of all cancer-related deaths are attributed to lung cancer making it responsible for being the leading cause of cancer-related deaths worldwide. The prognosis for lung cancer remains the poorest of all tumor types owing to the 5-year survival rate of an average of 10–20%. However, survival is particularly determined by the stage at which the diagnosis is made, the earliest stages have a 5-year survival of 92%, whereas the last stages have nil. There are two broad classifications in lung cancer namely—Non-Small Cell Lung Cancer (NSCLC) and Small Cell Lung Cancer (SCLC); the former accounts for 85% and the latter for about 15% of the total diagnoses [1, 2]. Lungs are also a primary metastatic site for cancer of other origins like the breast, skin, pancreas,

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and liver. 75% of lung cancer is endemic to smoking and the remaining is due to occupational exposure and air pollution [3].

Reactive oxygen species (ROS) are a regular ramification of cellular metabolism and the altered redox state of the cell due to these results in cancer. These are molecules with an extra unpaired electron, superoxide ( $O_2^{\cdot-}$ ) and hydroxyl ( $OH^{\cdot}$ ), and as the name suggests it is unstable and highly reactive. Superoxides are processed by superoxide dismutase (SODs) to hydrogen peroxide ( $H_2O_2$ ) and they further produce hydroxyls either via the Fenton reaction or the Haber–Weiss reaction. Hydrogen peroxide also gets converted to other damaging oxidants like hypochlorous acid and hypobromous acid. ROS can be generated exogenously and endogenously. The exogenous trigger is via exposure to environmental gases like  $NO_2$ ,  $SO_2$ , CO, and particulate matter in the air like from cigarette smoke, while mitochondria and membrane-bound NADPH oxidases are the main intracellular sources of ROS involved in the signaling cascade. Cancer cells fine-tune the ROS signaling and its scavenging to their comfort. The expected scenario in the cell upon increased ROS production would be to induce oxidative stress and eventual cell death; however, in the cancer niche detoxification of ROS via scavengers like NRF2 (nuclear factor-erythroid 2-related factor 2), PRXs (peroxiredoxins), GPXs (glutathione peroxidases), SODs (superoxide dismutases), and CAT (catalase) help maintain the required ROS for pro-tumorigenic signaling.

Mitochondrial ROS (mROS) predominantly contributes to the cellular ROS with 1% of the total oxygen consumption being utilized in superoxide release. The cancer cells have an increased ROS production which elevates multiple signaling pathways ultimately leading to the transcription of cellular proliferation genes. The prime target of mROS is the inhibition of PTEN (phosphatase and tensin homolog), which leads to incessant activation of the PI3K/AKT pathway, finally resulting in enhanced proliferation and survival. The AKT activation further increases ROS levels promoting cancer cell proliferation and survival. ROS is also known to oxidize and inhibit other phosphatases like the MAPK phosphatases that result in growth factor receptor activation, inducing MAPK/ERK signaling, an established proliferative signaling. MAPK/ERK pathway is specifically made conducive to lung cancer by mROS regulation of *Kras*-induced anchorage-independent growth of the cells [4]. ROS manipulates tumor cell survival by activating transcription factors like NF- $\kappa$ B and nuclear factor-erythroid 2-related factor (NRF2), which stimulate the expression of antioxidants to avoid ROS-mediated cell death. NRF-2 is negatively regulated by KEAP1 which is inhibited by ROS-mediated oxidation of cysteine residues on KEAP1 protein, thus stabilizing the former transcription factor to increase the transcription of GPXs to maintain the desired

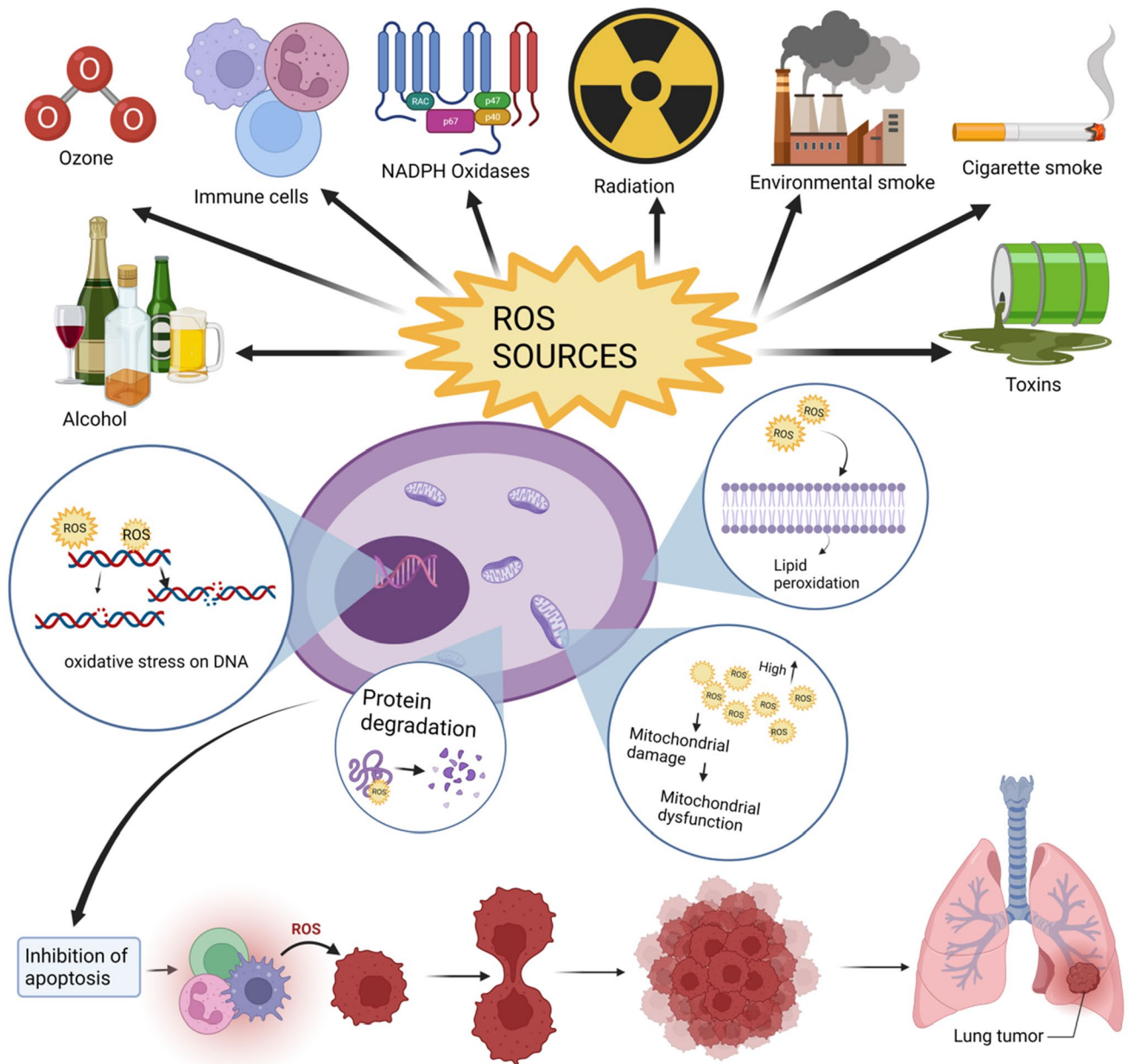
ROS balance in cancer cells. It is apparent that the annihilation of the mitochondrial respiratory chain reduces ROS production diminishing tumorigenesis and the knockout of NRF-2 promotes oxidative stress, thus killing the tumor. The cells in the cancer microenvironment thrive on hypoxia, hypoxia-induced angiogenesis, and glycolysis, wherein the role of mROS pitches in by activating and stabilizing HIF-1 $\alpha$  an oxygen-sensitive subunit of HIF-1 protein via inhibition of PHD2 activity. In normoxia condition, PHD-2 degrades the HIF-1 $\alpha$ , but under hypoxia, HIF-1 $\alpha$  dimerizes with HIF-1 $\beta$  and localize to the nucleus to transcribe pro-angiogenic genes like VEGF1, and genes implicated in glycolysis, cell survival, and mobility. Hypoxia upregulates the Serine hydroxy methyltransferase 2 (SHMT2) enzyme, which increases the catabolism of mitochondrial serine to compensate for NADPH. Matrix metalloproteases that degrade the extracellular matrix are upregulated by mROS, and additionally, it also inhibits the activity of TIMP (tissue inhibitor of metalloproteinase). It is also known that mROS specifically targets Src Homology region 2 (SH2)-containing protein tyrosine Phosphatase 2 (SHP-2) and Focal adhesion kinase (FAK), enabling the cell to migrate and prevent cell death by anoikis, matrix detachment-induced apoptosis. Mitochondrial DNA is 5 times more susceptible to mutations via oxidative damage than nuclear DNA owing to its locality in the vicinity of Electron transport chain (ETC) and lack of histone proteins and DNA repair mechanisms. A cytochrome b mutation in mitochondrial complex II of ETC induces metastasis in cancer cells and mROS is comprehensive to all factors required for tumorigenesis from fabricating a tumor favorable microenvironment to metastasis [5, 6].

## External factors regulating ROS in the lungs

Lungs are the main organ for gaseous exchange in the body, which makes them more vulnerable to ROS exposures. Factors like lifestyle, disease conditions, and medications are known to influence the ROS levels in the lungs. Lifestyle factors regulate ROS levels in different ways, for example, cigarette smoking and occupational hazards introduce exogenous ROS into the body, while healthy eating habits and supplements help scavenge ROS. Cigarette smoke enters the lungs in two phases: gaseous and tar, containing over 4000 chemical substances, including free radicals and other oxidants. The gaseous phase contains nitric oxide, an oxidant that readily reacts with superoxide ( $O_2^{\cdot-}$ ) to form peroxynitrite ( $ONOO^-$ ). The tar consists of more steady free radicals like the semiquinone radical, which readily reacts with oxygen to form  $O_2^{\cdot-}$  and  $H_2O_2$ . Individuals exposed to occupational and environmental hazards, including xenobiotics (pesticides and metals), physical factors (radiation, heat, noise), and biological agents, resulting in the production or exposure of

ROS leading to formation of tumors, are depicted in Fig. 1. The occupational situations that are reported to be involved in oxidative stress-related toxicity are textile, metal, cement, pesticide industries, and radiology centers. The prolonged exposure to pollutants and allergens stimulates ROS generation and result in inflammation in the windpipe and asthma [6]. During infection, excessive amounts of ROS are generated in the respiratory tract to fight the invasion of virus and bacteria, but the downside of this defense mechanism is that

these ROS needs to be scavenged adequately to avoid tissue damage. Indeed, recent reports suggest that COVID-19 infection and lung cancer are regulated through the ROS/NRF2-mediated mechanism. Medications used for various diseases may also result in ROS generation during the course of their metabolism, which may adversely affect the host cell physiology (Fig. 1). For example, the long-acting beta agonists asthma drug, indacaterol, enhances H<sub>2</sub>O<sub>2</sub>-induced ROS generation in THP1 cells. Doxorubicin (Dox) is an example



**Fig. 1** An overview of various sources and ill effects of ROS: Exogenous sources of ROS include radiation, environmental smoke, industrial toxins, alcohol, ozone, heavy metals, and cigarette smoke, as well as endogenous sources such as mitochondria, phagocytotic cells,

lipids, protein, -oxidation of fatty acids in peroxisomes, cancer cells, and enzymes such as cytochrome p-450 and thiol oxidase in the endoplasmic reticulum [8]

of a drug generating ROS as an intermediate metabolite in the host system. Dox can be readily reduced to semiquinone by the mitochondrial enzymes, which in turn generate superoxide anion and  $H_2O_2$ . It is noteworthy that the ability of the drugs to induce ROS in host cells was exploited for anticancer and other treatment strategies. Another important lifestyle factor in modern-day life is physical exercise. Reports suggest that heavy physical workouts augment free radical (ROS) generation through the mitochondrial metabolism of skeletal muscles and other tissues, causing oxidative injury in the corresponding tissues. Certain diets and supplements can also operate the ROS levels in the human system. Vitamins E, C, and beta-carotene are considered major choices of antioxidants that are supplemented via diet in addition to the endogenous antioxidants. Antioxidants, in general, tend to diminish ROS and protect the host cells from oxidative injury and other adverse conditions [7].

### **ROS/oxidative stress leads to cancer, metastasis, and invasion**

ROS can readily react with DNA bases to form DNA adducts, which leads to miscoding during replication. This may lead to several irreversible mutations in the genome, especially when they occur in oncogenes or tumor suppressor genes; it leads to cancerous state [9]. The oncogenes, K-Ras, Jun, and Myc and the tumor suppressor genes TP53, CDKN2A, and STK11 are the most commonly reported moieties to carry mutations in cancer patients. Moreover, lipid and protein peroxidation by the oxidants can also induce more free radicals and enhance the chances of carcinogenesis through DNA mutations. Chronic pulmonary obstructive disease is an inflammatory disease caused by free radicals released from tobacco smoking and has more possibilities for genotoxic changes and adds a 4.5-fold risk of developing lung cancer [1, 10]. When the cells have sufficient irreversible damage in their crucial genes, they attain a survival advantage state, which helps them to proliferate continuously, evading programmed cell death. These specific events are collectively known as cancer initiation and promotion [9, 11, 12]. ROS has been implicated in the regulation of integrins and other extracellular matrix proteins, which in turn regulate cellular migration and adhesion. This property of ROS has been investigated by several researchers with an interest in cancer cell migration and invasion. Although the exact mechanism of regulation remains to be elucidated, there is a growing body of evidence to suggest that integrin, an important cell anchoring protein, when interacting with antibodies or extracellular matrix proteins, produces cellular ROS through mitochondrial metabolism. Furthermore, it activates several oxidases, including NOX, 5-LOX, and COX-2; and Rac-1, a rho GTPase protein, which

is involved in ROS-mediated actin cytoskeleton rearrangement required for cell migration. Moreover, ROS regulates stabilization and destabilization of VE-cadherin junctions, which in turn regulate cell–cell adhesion, leading to permeability change, migration, and proliferation. Breaking of extracellular matrix (ECM) by the cancer cells is termed as invasion, which is crucial for metastasis [13–15]. There are several proteases involved in degradation of ECM, of which matrix metalloproteinases, cathepsins, and urokinase plasminogen activator are relevant in cancer context. ROS has been associated with abnormal upregulation of these inhibitors through NF- $\kappa$ B and MAPK pathways. ROS can trigger SNAIL and promote epithelial-to-mesenchymal transition (EMT), and SNAIL has also been shown to increase ROS levels in prostate cancer cells [18, 20]. Interestingly, ROS and TGF- $\beta$  signaling are interconnected during EMT. When TGF- $\beta$  is stimulated, ROS increases the phosphorylation of Smad2 and p38, which upregulates  $\alpha$ -SMA and fibronectin, respectively, and ERK1/2 activation leads to E-cadherin repression [16, 17]. Together, it is apparent that ROS regulates cancer cell metastasis and invasion through various signaling molecules.

### **Effect of ROS on cancer stem cells**

Cancer stem cells (CSCs) can self-renew and differentiate into many lineages, like normal stem cells. This helps in accelerating tumor growth and heterogeneity. It is also possible for CSC to develop from differentiated cancer cells as a result of adaptation to the microenvironment and therapeutic pressures; such factors are responsible for their heterogeneity. Because of their high resistance, CSCs are resistant to conventional cancer treatments, which lead to metastasis and recurrence of cancer as well as the possibility of carcinogenesis due to EMT malfunction. This epithelial–mesenchymal transition (EMT) is important for embryonic development and the formation of various tissues or organs [18]. Based on their respective biological functions, EMTs can be divided into three distinct classes. Type 1 refers to Epithelial–Mesenchymal transition associated with embryo implantation, formation and development of the organ; type 2 corresponds to tissue regeneration, organ fibrosis and wound healing and type 3 tell how EMTs occur within tumor cells that have already undergone genetic and epigenetic changes. These types of EMT are essential for the progression of cancer and cancer stem cells.

The tumor microenvironment is typically rich in proteins like the growth factor TGF- $\beta$  and cytokines that activate the pathway that affects the longevity of CSCs [19]. Based on this analysis, the researchers concluded that cytokines and growth factors increased cancer stem cells' flexibility and EMT properties. Apart from the role of the tumor niche,

the researchers have also observed lower levels of ROS in cancer stem cells, this made the CSCs more sensitive to radiotherapy and minimized their ability to clone themselves. The excess level of ROS has increased oxidative stress that triggers cancer cell death and also damages protein, lipids, and DNA, which are regarded as oncogenic since they result in instability of the genome [20]. Regulation of ROS can be understood through the following example: in gastric cancer, a variant of CD44 (adhesion molecule) can protect CSCs in the membrane-proximal region by using insertion in alternative splicing; in the human liver, CD13 negatively regulates ROS that increases the ability of stem cells in it. And it has been observed that ROS has a specific effect on Cav-1 expression, motility of cells and also it helps in the migratory process with the help of Akt signaling in the non-small-cell lung cancer cell line [21].

Reports suggest that low concentration of ROS is necessary for CSCs, whereas a higher concentration of ROS promotes tumor growth. Research has shown that when transplanting CD24 and CD49 in mouse mammary CSCs, ROS levels were low and also, the CSCs were able to adhere and develop a novel tumor and it was demonstrated in head and neck tumors as well [22]. Together, for maintaining the prominent features of CSCs, ROS has a crucial role in redox control, cell signaling, also in therapeutic targets.

In human lung cancer A549 cells, Tan-IIA induces the ROS and decreases the mitochondrial membrane potential. Hence, it is mitochondrial dependent to increase apoptosis in Tan-IIA pathways in human A549. As TanIIA increases the production of ROS, it results in generation of cytochrome c that triggers apoptosis [22, 23]. In cancer cells, to increase the expression of CSCs and EMT, activation of the PI3K/AKT/mTOR pathway is needed as it triggers glycolysis to increase ROS levels. Since mTOR is needed for tumorigenic properties in ovarian CSCs. AKT also regulates ROS levels, Cellular longevity, and metastasis through signaling via the FOXO family of transcription factors. The production of manganese superoxide dismutase/superoxide dismutase-2 (Mn SOD/SOD2) also plays an important role for this process. Metastatic progression and invasion of markers are thought to catalase SOD2 in cancer such as colon, lung squamous, and prostate carcinoma. In addition, over expression of SOD2 induced mitochondrial ROS and HIF2 activity, as well as CSC formation, resulting in increased tumor invasiveness and poor prognosis for lung cancer patients. Activation and expression of Notch pathway components are related to insufficient outcomes and resistance to radiation or chemotherapy. The signaling cascade improves CSC's drug resistance and proliferation by stimulating angiogenesis and EMT. Notch signaling also regulates CSCs by interfering with signaling protein as demonstrated by HER2 that promotes CSCs multiplication and proliferation

in lung cancer. By upregulating AKT, Notch activates AKT in a manner that is independent of transcriptional regulation. Notch also increases ROS scavenging enzyme expression, resulting in low ROS levels [22, 24].

## ROS in cancer tumor microenvironment

It is generally known that the tumor microenvironment (TME), which is made up of a wide variety of cell types, including numerous immune cells, cancer cells, and fibroblasts linked with cancer, produces a significant amount of ROS [11]. In lung tissues, benzo(a)pyrene (BaP), which is widely regarded as the substance that causes cancer at a higher rate than any other substance, triggers a cascade of chronic oxidative stress and inflammation and also increases the likelihood of mutation in certain genes, which may eventually result in the development of cancer. NF- $\kappa$ B, a transcriptional factor that is activated by oxidative stress, is implicated in triggering lung cancer through stimulation of the inflammatory cascade. It is reported that the inflammasome NOD-like receptor protein 3 (NLRP3), a type of intracellular multiprotein complex found in the microenvironment of a tumor, has been linked to the development of chronic inflammation in patients with lung cancer induced by benzo(a)pyrene (BaP) [25]. The formation of NLRP3 is responsible for the maturation and release of inflammatory cytokines in the microenvironment of a tumor. These cytokines, which promote cancer progression and are also responsible for immunological tolerance, are accountable for both of these processes [26]. One of the main factors contributing to the TME's resistance to immunotherapy, particularly immune checkpoint blockades, is the high levels of ROS [27, 28]. The CD4 + Foxp3 + regulatory T (Treg) cells are enrolled into the tumor microenvironment and act as potent immune suppressors, according to recent findings [29, 30]. Treg cells that have migrated into tumors experience apoptosis due to the elevated ROS levels in the TME. Notably, tumor-infiltrating apoptotic Treg cells reduce the ATP to adenosine, to suppress the immunity of programmed death ligand 1-blockade-mediated anti-tumor T-cells. These cells are extremely sensitive to ROS because of their weak NRF2 linked with the antioxidant defense system. It is significant to observe that dead Treg cells have a stronger immunosuppressive effect than living Treg cells [28]. This suppression of ROS can result in improved cancer immunotherapies. In fact, a new nanoscavenger that was anchored to the extracellular matrix prevented the apoptosis of suppressive immune cells by removing ROS [31].

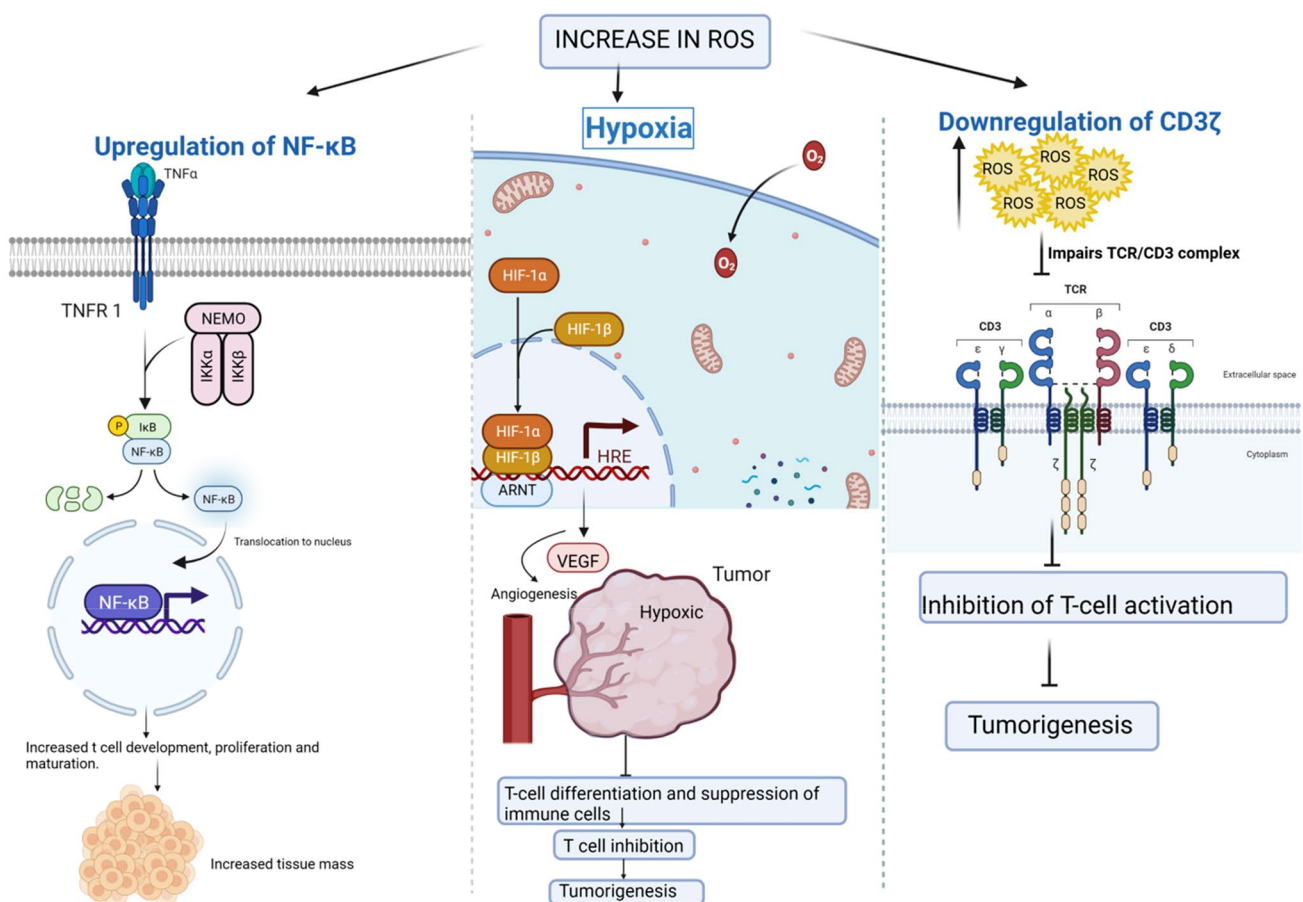
## Dual roles of ROS

ROS levels in noncancerous cells are closely regulated by balance between ROS production and scavenging, which is mainly caused by cellular respiration, and antioxidant levels, which are predominantly maintained by intracellular pools of glutathione and NADPH [32].

ROS act as signaling molecules/secondary messengers at low levels, regulating cellular and differentiation proliferation, inflammation associated with tissue maintenance, adaptive and innate immunity and aging, among other biological processes crucial for sustaining physiologic function (15) (Fig. 2). ROS have high reactivity and so play an essential function in a cell's redox balance. Increased amounts of ROS, on the other hand, can cause oxidative stress, resulting in damage to particular biomolecules like lipids, nucleic acids, and proteins and eventually death [33]. Apart from inducing cancer, ROS is also held responsible for the inhibition of cancer. This occurs due to extremely high levels of ROS accumulation above a

cytotoxic threshold, which triggers the apoptotic pathways, ultimately leading to cancer cell senescence.

As reported, overexpression of ROS causes normal cells to turn into cancerous cells, which activates oncogenes to promote tumorigenesis. ROS oxidizes various entities of the biomolecules, in this case, the bases of the nucleotide. This leads to the formation of various kinds of oxidation products which facilitate damage of the biomolecules via mutation and bond breaking. The ROS oxidizes guanine to form 7,8-dihydro-8-oxo-2-deoxyguanosine, causing G to mispair with T instead of C, while deoxyguanosine triphosphate is also oxidized to form 8-oxo-deoxyguanosine as a substrate, which causes A to mispair with C instead of T. When ROS oxidizes with adenine, there are two possible substrates: (i) 2-hydroxy-2-deoxyadenosine, which mispairs A with C, A with G, and A with T, and (ii) 7,8-dihydro-8-oxo-2-deoxyadenosine, which mispairs A with G, and A with C [34]. Exposure of purines and pyrimidines caused by hydrogen bond breakage, unfolding, double-, and single-strand breakage of DNA, facilitates ROS oxidation, thus resulting in DNA mutation and mispairing. Another way



**Fig. 2** Increased ROS causes NF- $\kappa$ B transcription to be upregulated, resulting in increased tissue mass. As a result, tissues become hypoxic, triggering angiogenesis and the release of VEGF. However, excessive levels of VEGF lead to T-cell activation and tumor development [9, 13, 15]

through which ROS damages DNA is by directly attacking and repressing the DNA repair system. This occurs when ROS attacks human 8-Oxoguanine DNA N-Glycosylase 1 (hOGG1) by oxidizing it. The hOGG1 is responsible for the elimination of 8-oxo-guanosine. So, in this case, the oxidation of hOGG1 makes 8-oxo-guanosine sticks around, which makes the turnover of epithelial cells faster. Colorectal carcinogenesis follows the very same mechanism [35].

T-cell death is induced due to high levels of ROS. This causes repression of T-cell differentiation, maturation, and activation. When ROS is increased, NF- $\kappa$ B is upregulated, causing T-cell development, maturation, and proliferation, resulting in more tissue mass. These new regions are in hypoxia conditions. This triggers HIF-1 $\alpha$  induction to stimulate the release of vascular endothelial growth factor, hence resulting in angiogenesis [36]. When VEGF is overproduced, it blocks and represses T-cell development and migration, in turn causing immunodeficiency. An increase in the ROS downregulates the CD3 expression, thereby inactivating T-cells by suppressing immune responses. The immunosuppressive cells present in the tumor regions over express ROS to inhibit T-cell activation, thus promoting tumor metastasis. The myeloid-derived suppressor cells in the tumor region increase the count of CD8+ and CD4+ as a response to the defense mechanism of the above. This is again taken care of by ROS by releasing an apoptosis-inducing factor, ultimately leading to T-cell death.

Increased levels of ROS downregulate NK cell function by repressing CD16 $\zeta$  expression, thereby abating the cytotoxicity of NK cells. The mechanism is similar, but with a slight difference, in various forms of carcinomas. In breast cancer, high amounts of ROS reduce the release of cytotoxic factors by NK cells by downregulating the expression of NK group 2 and its ligands, hence promoting cancer cell growth and metastasis. NK cells facilitate the elimination of malignant cells [37]. In melanoma, ROS spike causes decrease in the production of IFN- $\gamma$  in the NK cells, which results in melanoma metastasis. ROS elevation also induces apoptosis leading to the death of NK cells. In chronic myelomonocytic leukemia, the cells themselves release ROS, which causes the death of NK cells, leading to the metastasis of chronic myelomonocytic leukemia. Apoptosis of NK cells in the liver occurs due to the high production of ROS in the mitochondria. This results in tumor growth leading to colorectal carcinoma.

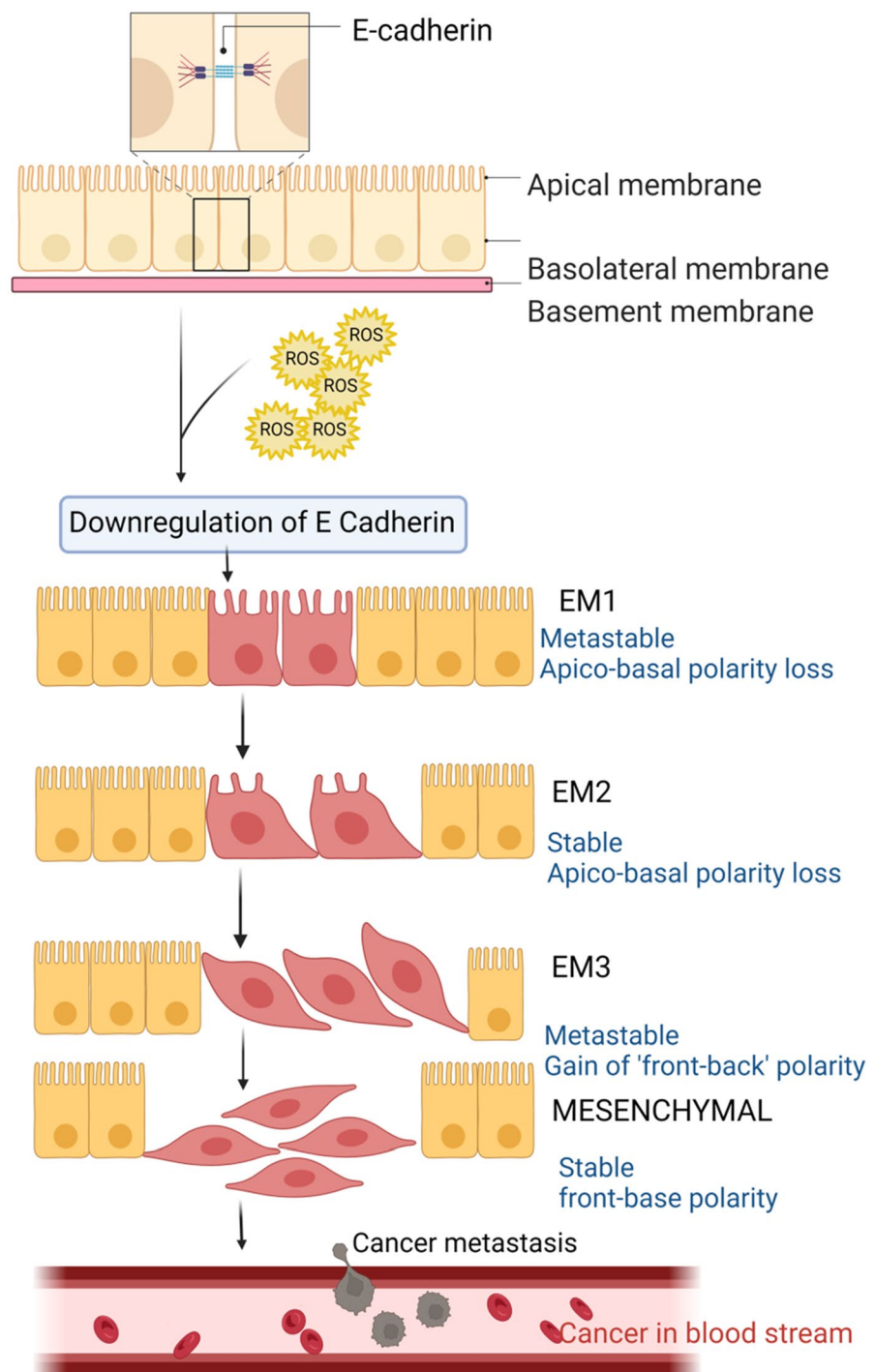
Higher levels of ROS cause an increase in the production of NF- $\kappa$ B. This downregulates E-cadherins causing dissociation of the cell–cell junctions. This initiates endothelial–mesenchymal transition (EMT), which facilitates cancer cell invasion and metastasis (Fig. 3). Apart from NF- $\kappa$ B, ROS can also interact with TGF- $\beta$ , PI3K/Akt, NRF2, HIF-1 $\alpha$  signaling pathways to cause cell–cell disruption resulting in EMT [10].

ROS inhibits the proliferation signaling pathway. An increase in the ROS production downregulates epithelial growth factor and its expression. This inhibits the phosphorylation of extracellular signal-regulated kinase, which leads to the decreased production of EGF and EGFR, ultimately inhibiting cancer cell growth. Ascorbic acid (sourced from vitamin C) and koumine are known to promote ROS production. They are also known to inhibit ERK phosphorylation, suppressing cancer cell carcinoma [38, 39]. Non-small cell lung cancer cells proliferation is inhibited when ROS blocks the PI3K/Akt/NF- $\kappa$ B signaling pathway [10, 40]. ROS buildup downregulates the production of cyclins and cyclin-dependent kinases and stops cancer cell proliferation, since CDKs and cyclins are responsible for the promotion of mitotic cell cycle of somatic cells. In multiple myeloma, ROS downregulates JK1 and JK2 to block cyclin D, B1, E, CDK2, and CDK4 to stop the cancer cell cycle and growth [9, 18]. If ROS is overexpressed in human non-small cancer cells, CDK1 is phosphorylated and cyclin B1 and CDK1 expression is suppressed. This causes arresting of G2/M phase in cell cycle [41]. Researchers found that Physalin A (PA) inhibits G2/M cell cycle progression in A549 cells by causing ROS to accumulate excessively in their cells. This effect was attributed to Physalin A's inhibition of the p38 MAPK/ROS pathway [25].

ROS induces tumor cell apoptosis. ROS promotes the release of Ca<sup>2+</sup> ions from the lumen of the endoplasmic reticulum, which causes defects such as protein misfolding and/or unfolding. Overproduction of ROS causes phosphorylation of eIF2 $\alpha$ . This activates transcription factor IV, inducing C/EBP homologous protein which leads to apoptosis. High levels of ROS accumulation cause it to oxidize cardiolipin (phospholipid), which causes cytochrome c (Cyt c) to detach from the outer surface of the mitochondrial membrane. The freely suspended Cyt c will now interact with the apoptotic protease activating factor 1 and forms an apoptosome. This in turn activates caspase-3 and caspase-9 to initiate apoptosis. In gastric cancer cells,  $\alpha$ -heredin is known to trigger overproduction of ROS. In the A549 cell line, Atractylodin (ATR) which is known for its antitumor activities-induced apoptosis by upregulating ROS production by regulating STAT, MAPK, and NF- $\kappa$ B pathways and inhibits the G2/M phase of the cell cycle by mediating the AKT signaling pathway [42].

ROS enhances the transcription effects of P53 and then translocate it to the mitochondria. ROS accumulation can also damage cancer cell DNA leading to apoptosis. Mutations of P53 cause cancer. ROS accumulation can reverse and restore the normal functions of the mutated P53 in cancer cells. Occurrence of lung cancer is associated with various kinds of cell death, one of which is ferroptosis [43]. In ferroptosis, cell death occurs with the help of iron where the ROS is of lipid type. Ferroptosis significantly targets only

**Fig. 3** Epithelial–mesenchymal transition (EMT) in cancer metastasis: Down-regulation of E-cadherin results in loss of adherence. This results in a loss of apical-basal polarity and mesenchymal transition; thus, motility is increased and cancer metastasis is facilitated [18, 20, 22]



cancerous cells due to its iron dependency. High amounts of ROS are accumulated due to faulty metabolism of iron along with the lack of natural antioxidants in the cells. This leads to lipid peroxidation followed by ferroptosis [44]. In a recent

study, it was discovered that hemin promotes the growth of noncancerous lung cells while increasing the production of reactive oxygen species (ROS) that cause lipid peroxidation and ferroptosis in lung cancer cells [45].



## ROS balance and homeostasis in cancer cells

Cancer cells have developed a reliable ROS detoxification system. As a result, cancer cells' reliance on antioxidant systems provides a unique vulnerability which must be targeted to cause targeted cell death. This is achieved by elevating oxidative stress level above the toxicity threshold, sparing the normal cells, which are distinguished by lower intracellular ROS levels. ROS serve as both 'good' and 'bad' molecules, regulating cellular physiology or causing cytotoxicity, relying on the duration, quantity, and location of their formation. The methods of ROS detoxification can be directly supported by malignant cells [46]. High doses of vitamin C (antioxidant) cause colon cancer cell death by increasing ROS levels [39]. Lung cancer progresses swifter when antioxidants like N-acetylcysteine are consumed and increasing Nrf2 gene expression causes accelerated lung tumor growth [47]. The methods of ROS detoxification can be directly supported by malignant cells.

In glycolysis, the pro-glycolytic shift caused by the activation of oncogenes and the loss of tumor suppressors gives tumors a selective advantage by giving them the building blocks they need to make the macromolecules that keep them growing and spreading. Since glycolytic intermediates can be transported into the metabolic pathways that directly or indirectly produce reducing equivalents, primarily PPP-derived NADPH or glutaminolysis-derived GSH, glucose metabolism is crucial in the regulation of redox homeostasis in malignancies. In order to reduce the load of ROS and avoid cell death by hydroperoxide, cancer cells enhance their glucose metabolism. By lowering ATP at intracellular levels and inhibiting the lactate dehydrogenase-A by the small drug FX11 prevents the growth of cancer cells.

In cancer cells, activation of pentose phosphate pathway constitutes a major feature, as this pathway synthesizes nucleotides, which is required during cell reproduction. PPP-dependent production of NADPH is enhanced by the regulation of glucose-6-phosphate dehydrogenase (G6PD). The availability of glucose may influence G6PD regulation: glucose funneling into the oxidative branch of PPP directly regulates the redox balance of human renal cell carcinoma cells (Fig. 4). The serine-glycine one-carbon metabolism (SGOC) is a network of biochemical reactions in which amino acids and their derivatives are converted into multiple outputs as carbon units that serve various cellular functions. These carbon units from glycine and serine depend on three different pathways: the folate cycle, the methionine cycle, and the trans-sulfuration pathway [20]. Recent evidence indicates that this pathway also plays a crucial role for redox balance [48].

## ROS-mediated cancer therapy

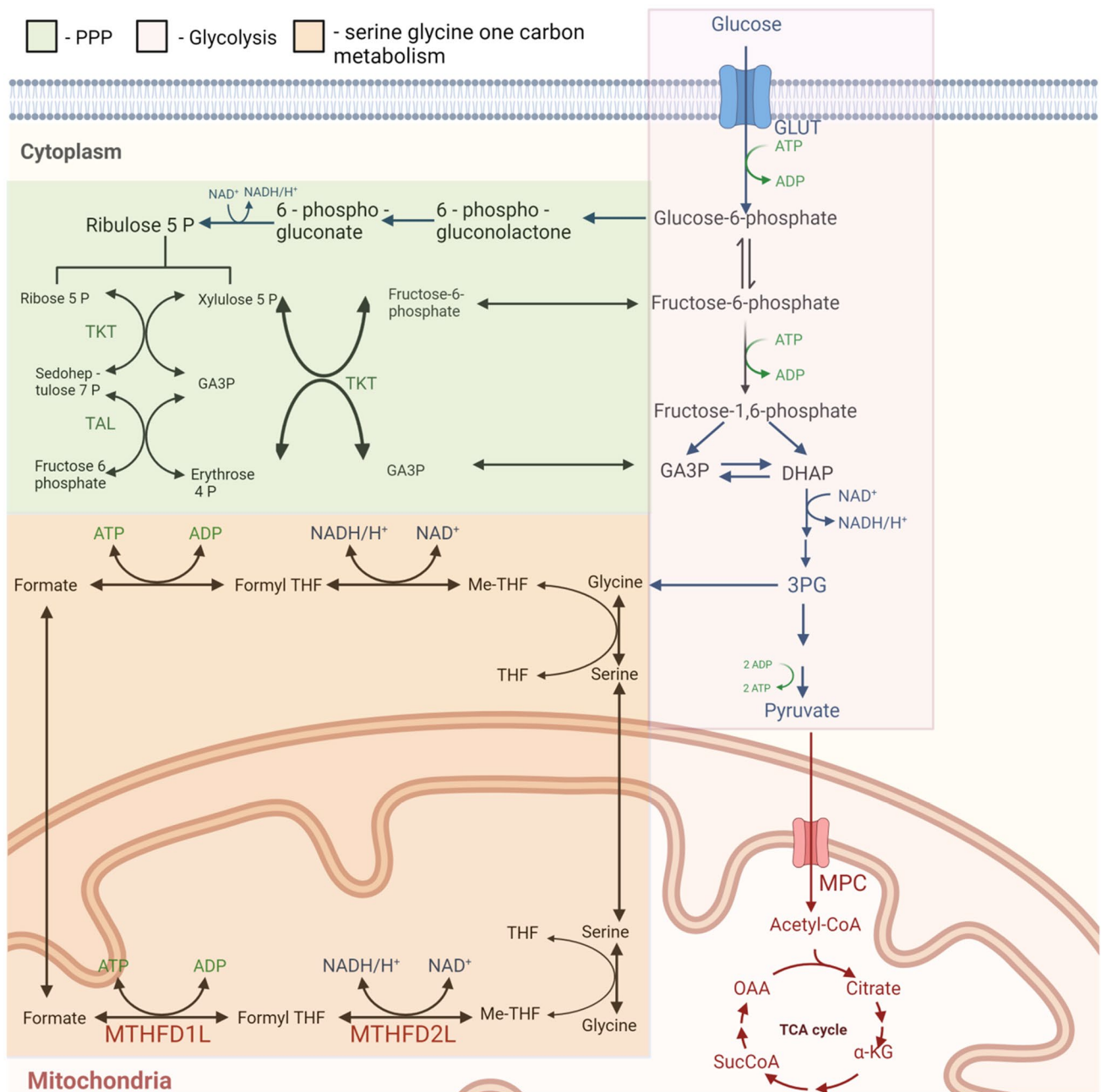
### Roles of ROS in cancer chemotherapy

To treat cancer patients in a clinical context, chemotherapy has been widely employed. A majority of chemotherapy drugs generate ROS, and several of them can change the redox balance in cancer cells [12]. The main medications that enhance ROS in cancer cells are alkylating agents, camptothecins (irinotecan and topotecan), anthracyclines (daunorubicin, epirubicin, and doxorubicin), and platinum coordination complexes (cisplatin, oxaliplatin, and carboplatin) [49]. The two main causes of the increase in ROS in response to chemotherapeutic drugs are the production of ROS by mitochondria and the suppression of the cellular antioxidant system. For instance, cisplatin, one of the most popular and successful chemotherapy drugs for treating lung cancer, causes mitochondria-dependent ROS that cause nuclear DNA damage and ultimately lead to cell death. Because of the direct impact of cisplatin on mitochondrial DNA, there is an increase in ROS production that impairs the production of proteins for the electron transport chain.

ROS are crucial in the development of multidrug resistance. One of the main causes of chemotherapy's failure in the treatment of cancer is such resistance [50]. The plasma has P-glycoprotein (P-gp) and other transporter-based efflux pumps and these pumps are closely linked to drug resistance [15]. P-gp, a member of the large ATP-binding cassette protein family, is encoded by the MDR1/ABCB1 gene. P-gp plays a defensive role for the uptake of chemotherapeutic drugs [51]. NRF2 is turned on and overexpressed in cancer cells as a defense against too much ROS, as was previously mentioned. A NRF2 target gene called MDR1/ABCB1 is involved in the upregulation of P-gp and drug resistance in cancer cells [52]. In conclusion, elevated production of ROS in response to therapy is essential for destroying the cancer cells and this also plays an important role in drug resistance.

### ROS-mediated programmed cell death pathways

As previously mentioned, cancer cells have higher ROS levels compared to non-malignant cells. Therefore, antioxidant enzyme activity increases in malignant cells to negate the detrimental effects of excess ROS production. Indeed, the disruption of this delicate balance has been of interest as potential anticancer interventions because either increasing ROS generation and/or decreasing antioxidant activity may result in activating of cell death pathways. Some of the key cell death pathways induced through the manipulation of ROS levels will be discussed below. Table 1 explains the



**Fig. 4** ROS Balance and Homeostasis. Metabolic pathways such as PPP, glycolysis, and serine-glycine one carbon involved in ROS balance and homeostasis in cancer cells [27, 48]

anticancer compounds/drugs widely used for eliminating the cancer cell via modulating ROS generation.

### ROS and apoptosis

The most common and well characterized form of cell death is apoptosis, which is also known as type I programmed cell death. Caspases, a distinct family of aspartate-directed cysteine-dependent proteases, are responsible for both

starting and finishing this process (Fig. 2). There are two major pathways for apoptosis, the extrinsic (death receptor-dependent) and the intrinsic (mitochondrial) mechanisms and ROS have been found to play a major role in both mechanisms [13].

Through the binding of death-inducing ligands like tumor necrosis factor-related apoptosis-inducing ligand (TRAIL-R1/2), Fas (FasL) and tumor necrosis factor receptor 1 to their corresponding receptors like TNF (TNFR), Fas

**Table 1** Various compounds exhibiting anticancer potential through ROS-mediated programmed cell death pathways

Cell death pathway induced	Stimulus	ROS type	Model system	References
Apoptosis	3,3'-OH curcumin	H <sub>2</sub> O <sub>2</sub>	HepG2	[67]
Apoptosis	6-gingerol	ROS	U937 & K562	[68]
Apoptosis	Arctigenin	H <sub>2</sub> O <sub>2</sub> & O <sub>2</sub> <sup>·-</sup>	MDA-MB-231	[69]
Apoptosis	Arsenic trioxide	O <sub>2</sub> <sup>·-</sup>	HPF	[70]
Apoptosis	Artesunate	ROS	TE671 & RD18	[5]
Apoptosis	Butein	ROS	HeLa	
Apoptosis		H <sub>2</sub> O <sub>2</sub> & O <sub>2</sub> <sup>·-</sup>	HepG2 & Hep3B	[71]
Apoptosis	Cannabidiol	ROS	Molt-4 & Jurkat	[72]
Apoptosis	Capsaicin	H <sub>2</sub> O <sub>2</sub>	NB4 & Kasumi-1	[73]
Apoptosis		ROS	HepG2	
Apoptosis		H <sub>2</sub> O <sub>2</sub> & O <sub>2</sub> <sup>·-</sup>	BxPC-3 & AsPC-1	
Apoptosis		ROS	Jurkat	
Apoptosis	Carnosic acid	ROS	IMR-32	[74]
Apoptosis		ROS	Caski	
Apoptosis		ROS	HCT116	
Apoptosis	Carnosol	ROS	HCT116	[75]
Apoptosis	CAY10598	ROS	HCT116	[76]
Apoptosis	Celastrol	ROS	B16	[77]
Apoptosis	Cepharanthine	ROS	H1299 & A549	[1]
Apoptosis	Curcumin	H <sub>2</sub> O <sub>2</sub> & O <sub>2</sub> <sup>·-</sup>	HuT-78	[8]
Apoptosis	Curcumin	O <sub>2</sub> <sup>·-</sup>	HCT-116	
Apoptosis	Curcumin	ROS	L929	
Apoptosis	FTY720	ROS	Jeko & Mino	[4]
Apoptosis	Gambogic acid	ROS	MDA-MB-231	[78]
Apoptosis	Glucocorticoid	H <sub>2</sub> O <sub>2</sub>	WEHI7.2	
Apoptosis	Gossypol	ROS	COLO205	[79]
Apoptosis	Isoliensinine	ROS	MDA-MB-231	[80]
Apoptosis	LZ-106	O <sub>2</sub> <sup>·-</sup>	H460 & A549	[81]
Apoptosis	Methylglyoxal	O <sub>2</sub> <sup>·-</sup>	Jurkat	[82]
Apoptosis	Nimbolide	H <sub>2</sub> O <sub>2</sub>	MG63	[83]
Apoptosis	Patulin	O <sub>2</sub> <sup>·-</sup>	HCT116 & HEK293	[28]
Apoptosis	Piperlongumine	H <sub>2</sub> O <sub>2</sub>	EJ & U2OS	[84]
Apoptosis	Plumbagin	H <sub>2</sub> O <sub>2</sub> & O <sub>2</sub> <sup>·-</sup>	A375.S2	[85]
Apoptosis	Plumbagin	ROS	EL4	
Apoptosis	Resveratrol	ROS	SGC7901	[86]
Apoptosis		ROS	SUDHL4 & HBL-1	
Apoptosis		O <sub>2</sub> <sup>·-</sup>	U937	
Apoptosis	Rotenone	H <sub>2</sub> O <sub>2</sub>	PC12	[87]
Apoptosis	Salinomycin	ROS	PC3	[88]
Apoptosis	Salvicine	H <sub>2</sub> O <sub>2</sub>	HeLa	
Apoptosis	Sanguinarine	H <sub>2</sub> O <sub>2</sub>	Jurkat & Molt-4	
Apoptosis	Shikonin	O <sub>2</sub> <sup>·-</sup>	HL60	[89]
Apoptosis	Sodium selenite	ROS	HepG2	[16]
Apoptosis	Sodium selenite	ROS	HCT116 & SW480	
Apoptosis	TRAIL	O <sub>2</sub> <sup>·-</sup>	Jurkat	
Apoptosis	Thymoquinone	ROS	MCF-7	[90]
Apoptosis		H <sub>2</sub> O <sub>2</sub> & O <sub>2</sub> <sup>·-</sup>	BC1	
Apoptosis	Wogonin	H <sub>2</sub> O <sub>2</sub>	Jurkat	
Apoptosis		H <sub>2</sub> O <sub>2</sub>	HepG2	[91]
Apoptosis	Zebularine	O <sub>2</sub> <sup>·-</sup>	Calu-6	[3]
Apoptosis	Zerumbone	ROS	K562	[92]

**Table 1** (continued)

Cell death pathway induced	Stimulus	ROS type	Model system	References
Autophagy/Apoptosis	Calyxin Y	H <sub>2</sub> O <sub>2</sub>	NCI-H460	[2]
Apoptosis/Autophagy	Cannabidiol	ROS	MDA-MB-231	[93]
Autophagy/Apoptosis	Carnosol	ROS	MDA-MB-231	[94]
Apoptosis/Autophagy	Celastrol	ROS	HOS & MG-63	[95]
Apoptosis/Autophagy	Colistin	ROS	N2a	[96]
Autophagy/Apoptosis	Compound K	ROS	HCT-116	[97]
Apoptosis/Autophagy	FTY720	ROS	U266	[98]
Apoptosis/Autophagy	Gambogic acid	ROS	T24	[99]
Apoptosis/Autophagy	Isoorientin	ROS	HepG2	[100]
Autophagy/Apoptosis	KIOM-C	ROS	HT1080	[101]
Apoptosis/Autophagy	MomordinIc	H <sub>2</sub> O <sub>2</sub>	HepG2	[102]
Apoptosis/Autophagy	Plumbagin	ROS	PC-3 & DU145	[103]
Apoptosis/Autophagy	Resveratrol	ROS	OVCAR-3 & Caov-3	
Apoptosis/Autophagy	Sanguinarine	H <sub>2</sub> O <sub>2</sub>	U87MG & U118MG	
Apoptosis/Autophagy	Sodium selenite	ROS	A549	[17]
Autophagy	2-methoxyestradiol	O <sub>2</sub> <sup>·-</sup>	U87MG & HEK293	[60]
Autophagy	Bufalin	ROS	HT-29 & Caco-2	[104]
Autophagy	Ciclopirox	ROS	Rh30 & RD	[105]
Autophagy	Cucurbitacin B	O <sub>2</sub> <sup>·-</sup>	HeLa	[106]
Autophagy		ROS	MCF-7	
Autophagy	Curcumin	ROS		
Autophagy	Dichloroacetate	ROS	HT29 & HCT116	[107]
Autophagy	Dihydroartemisinin	ROS	BxPC-3 & PANC-1	
Autophagy	Rotenone	O <sub>2</sub> <sup>·-</sup>	U87MG & HEK293	
Autophagy	Salinomycin	H <sub>2</sub> O <sub>2</sub>	SW620 & RKO	[108]
Autophagy	Sodium selenite	O <sub>2</sub> <sup>·-</sup>	U87MG & T98G	[109]
Autophagy	Ursolic acid	ROS	U87MG	[110]
Apoptosis/Necrosis	Auranofin	O <sub>2</sub> <sup>·-</sup>	HeLa	
Necrosis	Salinomycin	ROS	U251MG	[111]
Necrosis	Sodium selenite	H <sub>2</sub> O <sub>2</sub> & O <sub>2</sub> <sup>·-</sup>	Jurkat & J774.2	
Necroptosis	Dimethylfumarate	ROS	CT26	
Necroptosis	Lycorine	ROS	ARH-77	

(FasR) and death receptors (DR4 and DR5), ROS has been shown to initiate the extrinsic pathway of apoptosis. Adaptor proteins such as procaspases 8 and 10 and Fas-associated protein with death domain (FADD) are recruited to the cytoplasmic surface to form death-inducing signaling complexes when transmembrane death receptors are activated (DISCs). Following, caspases 8 and 10 are activated and this causes apoptosis by directly activating effector caspases [53]. Cellular FLICE-inhibitory protein (c-FLIP) is one of the proteins that helps ROS initiate the extrinsic apoptosis. Procaspases and this protein compete with each other to attach

to the adaptor protein, which prevents the creation of DISCs and induces apoptosis as a result. It has been demonstrated that ROS adversely control c-FLIP through two different ways, (i) the nitric oxide is negatively regulated by oxygen (O<sub>2</sub>), which stops S-nitrosation and subsequently enables ubiquitin-proteasomal destruction of c-FLIP [54], (ii) ROS sets c-FLIP up for ubiquitination and phosphorylation and this leads to the destruction of subsequent proteasomes.

The intrinsic pathway of apoptosis is also referred to as the mitochondrial pathway. Indeed, the [54] mitochondria are a major source of ROS generation and mitochondria

contribute a major function in initiating apoptosis. Due to this, mitochondria also often considered as a target of ROS. During apoptosis, the mitochondrial membrane becomes more permeable and release pro-apoptotic factors through the mitochondrial permeability transition pore (MPTP). Apoptosis-inducing factor (AIF), second mitochondria-derived activator of caspase/direct inhibitor of apoptosis-binding protein with low pI (Smac/DIABLO), and cytochrome c (Cyt-c) are few of the substances released from the cytoplasm [9]. When cytochrome-c enters the cytosol, it forms a complex with apoptotic protease activating factor 1 (APAF1), which creates an apoptosome and activates caspase-9 [55]. A crucial component of intrinsic pathway, caspase-9 triggers the caspases 3, 6, and 7, which causes the breakdown of cellular proteins and induces apoptosis [56]. Additionally, in the mitochondria, Cardiolipin and Cyt-c form a combination that prevents Cardiolipin from being released into the cytosol.  $H_2O_2$  inhibits this complex by oxidizing cardiolipin, which lowers its affinity for Cyt-c and permits its release into the cytosol [57]. Also, the ratio of pro-apoptotic Bcl-2 proteins (such as Bax, Bid, and Bad) to anti-apoptotic Bcl-2 proteins (such as Bcl-2 itself, Mcl-1 and Bcl-xL) controls intrinsic apoptosis [55]. (Fig. 5) Once again, ROS controls this equilibrium by directly oxidizing Bcl-2 at Cys229 and Cys158 by  $H_2O_2$ , which eliminates its anti-apoptotic activity, decreases the Bax and Bad ubiquitination and increases the Bcl-2 ubiquitination by  $O_2$  [12, 24].

### ROS and autophagy

Autophagy, also referred to as type II programmed cell death, is a process of self-digestion which occurs in the lysosome and aims to recycle cytoplasmic components and damaged organelles when cells are exposed to stress [58]. In cancer cells, more recent literature has found that autophagy acts in a context-dependent manner and may function in tumor promotion and suppression [59]. Autophagy's redox regulation is, in reality, influenced by a number of variables, including the expression of ROS, time frame, and cell type. For instance, ROS affects cancer cells but does not cause autophagic cell death in untransformed cells [60]. Both the induction of autophagy by ROS generation and the decrease of ROS by autophagy show the close relationship between ROS and autophagy [61]. A key negative regulator of autophagy is mammalian target of rapamycin (mTOR), a serine/threonine kinase, and  $H_2O_2$  inhibits mTOR activity resulting in autophagy [6, 62]. Autophagy is a multi-step process and is strategically controlled by autophagy-related genes (ATGs). ROS upregulates the expression of beclin-1 an important component for initiation of autophagy. Furthermore, nutrient starvation-induced  $H_2O_2$  oxidizes

ATG4 which promotes lipidation of LC-3 and increasing autophagosomes formation [63].

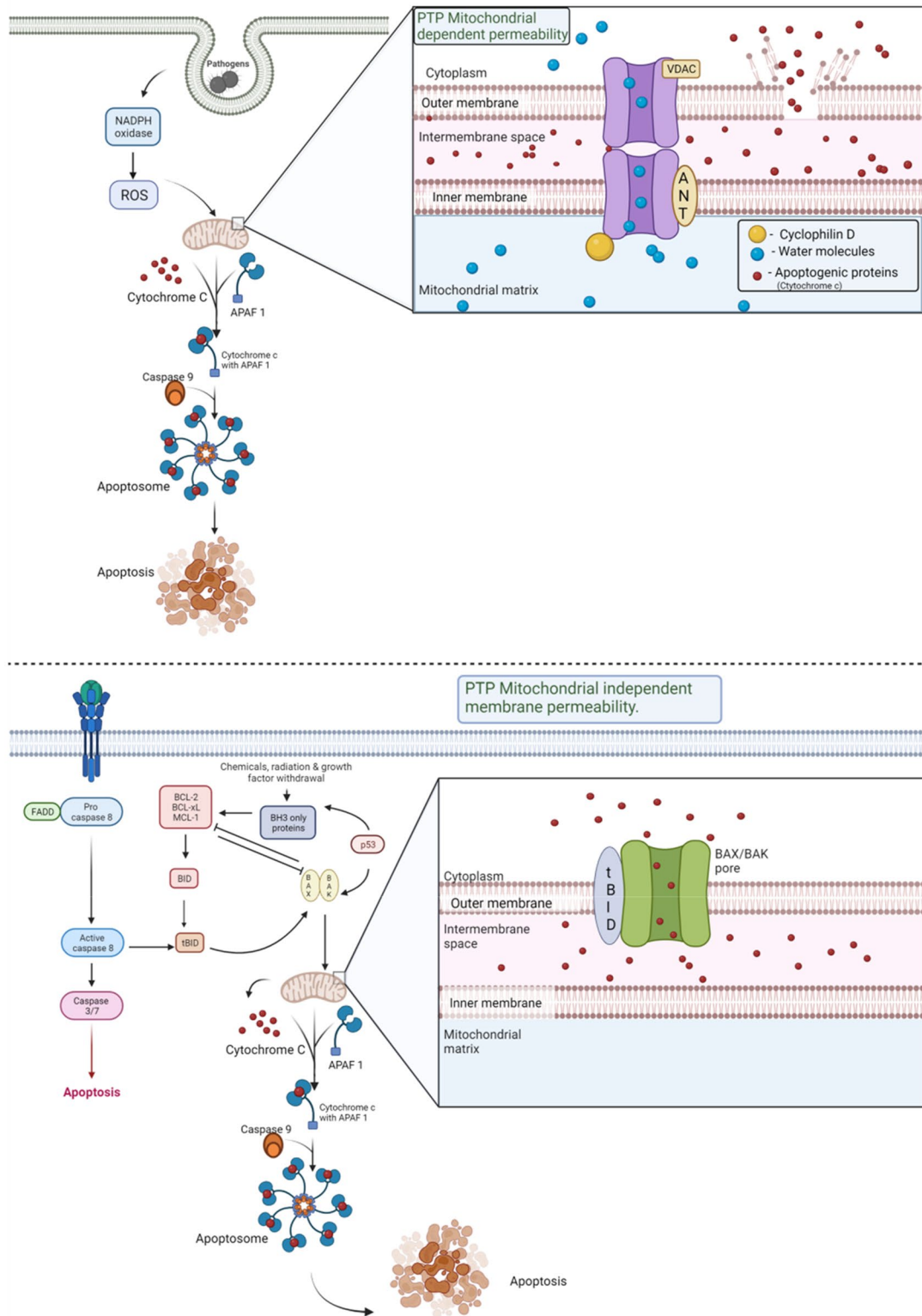
### ROS and necroptosis

Necrosis was once thought to be an uncontrolled kind of cell death, but studies now suggest that there is another form of necrosis that is available and is controlled by a protein-mediated platform. This alternative form of necrosis is known as necroptosis. Necroptosis is started by death-inducing receptors like FasR and TNFR, once they have been stimulated by their corresponding ligands, just like the extrinsic apoptotic pathway. There are two critical regulators involved in necroptosis execution such as receptor-interacting protein kinases RIP1 and RIP3 [64]. In fact, RIP3 enhances the mitochondrial catalytic activity and increases the amount of ROS production in the mitochondria, interacting directly with metabolic enzymes [14, 65]. Furthermore, RIP3 phosphorylates PGAM5, which is a subunit of mitochondrial protein phosphatase. This role results in the activation and dephosphorylation of the dynamin-related protein 1 (Drp1), which fragments the mitochondria, increases ROS production, and induces necroptosis as a result [66].

### Future perspectives

The levels of reactive oxygen species (ROS) within cells are the target of many different chemotherapeutic approaches. The non-steroidal and anti-inflammatory drug sulindac is undergoing clinical trials for the treatment of tumors at the moment. It causes lung cancer cells to undergo induced apoptosis by increasing intracellular ROS. The propensity of mtDNA to mutate as a consequence of being subjected to reactive oxygen species (ROS) is something that could potentially be utilized in the treatment of various cancer treatments. Reactive oxygen species (ROS)-based nanoparticles such as gold nanoparticles and cerium-based nanoparticles induce cancer cell death by increasing the intracellular ROS.

Using 2-deoxyglucose as a glucose analog increases oxidative stress in the cell. This leads to cancer cell death due to the inhibition of glucose metabolism as cancerous cells are more vulnerable to glucose toxicity than non-cancerous cells. One way to activate ROS-based nanoparticles is through photodynamic therapy [44], also known as PDT. In this case, light energy is used to increase ROS activity inside the cell. With its photostability and low toxicity to non-cancerous cells, graphene has recently been used in PDT for cancer treatment purposes. Thus, activating intracellular ROS by suitable targeted therapy in cancer cells holds a great potential in anticancer treatment strategies.



**Fig. 5** An overview of apoptosis: The mitochondrial-mediated apoptosis can be permeability transition pore dependent or permeability transition pore independent [1, 56]

**Acknowledgements** The authors are grateful to the SRM Institute of Science and Technology for providing support for this work. KK is supported by the SRM IST scholarship.

**Funding** The authors have not disclosed any funding.

## Declarations

**Conflict of interest** The authors declare that there are no conflicts of interest.

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