



ROS and Oxidative Stress in Cancer: Recent Advances

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Abstract

Reactive oxygen species (ROS) have long been considered as one of the major regulatory factors for the intracellular and intercellular signaling cascades. The sensitive redox balance that is controlled through an improved antioxidant system along with the enzymatic and non-enzymatic ROS production pathways sustains physiological functions in the healthy cells. During the course of cancer, a progressive deterioration of the redox balance can be followed via the overproduction of ROS, and results in the formation of malignant phenotype through induction of cancer hallmarks, including death evasion, uncontrolled proliferation, deregulating the cellular energetics, evading the immune response, provoking inflammation, inducing genome instability and mutations, developing drug resistance, angiogenesis, invasiveness, and metastasis. Apart from the carcinogenic roles of ROS, they have been employed as a target, mediator, and weapon in cancer treatment modalities because of the characteristic features considered as a double-edged sword. This chapter has consequently purposed to indicate the sophisticated roles, contributions, activities, and importance of ROS in the progression of cancer and cancer treatment strategies, and drawn the attention of scientists more to enhance the research on the complicated and versatile relationship between ROS and cancer.

Keywords

Reactive oxygen species · Cancer hallmarks · Carcinogenesis · Cell death pathways · Drug resistance

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6.1 Introduction

Although a mass effort of scientists and the considerable research budgets have been drained to find the convenient cure, drugs, and treatment strategies for cancer diseases, cancer is still ranked as the second leading cause of death and considered as a major public health problem worldwide [1]. The most recent global cancer statistics showed that there was an estimated 18.1 million new cancer cases and 9.6 million cancer deaths in 2018, and this bitter truth reminds us once again the importance of the scientific research on the mechanisms of carcinogenesis, cancer treatment strategies, and drugs [2]. It is well known that carcinogenesis is a prolonged, complicated, and multi-stage process that can be induced by the harmful environmental factors along with the genetic predispositions [3–5]. Because of the stress occurring in microenvironment, carcinogenesis begins with malignant transformation of some cells in the organism, and the malign transformation of these cells is followed by hyperproliferation, insensitivity to the growth suppressing factors (evasion), resistance to the programmed cell death (apoptosis), invasiveness, production of angiogenic factors to induce the formation of new capillary vessels from the existing ones (angiogenesis), and finally gaining metastatic ability, which is defined as the ability to reach different parts of the organism through veins [6, 7]. Additionally, the irregularities in the cellular energetics and the escape from the immune system are also considered as the substantial parts of carcinogenesis [6, 8]. Although the process of carcinogenesis is common for almost all cancer diseases, it has been demonstrated that there are more than 200 types of cancer, and a tumor tissue exhibits a morphologically and functionally heterogeneous structure that consists of various cancer cells with different physiological characters, mutations, and epigenetic profiles [9–12]. Thus, understanding the underlying mechanisms of cancer hallmarks has a great importance to find the convenient cure, drugs, and treatment strategies for cancer diseases. It is well known that the cellular levels of oxygen molecules, reactive oxygen species (ROS), and antioxidants have a balance in the healthy cells located in a homeostatic microenvironment [13–15]. However, this balance observed in the homeostatic microenvironment can be disrupted in the progress of some diseases such as cancer, diabetes, neurodegenerative diseases, premature aging, and obesity [16–19]. The disruption of the balance between ROS and antioxidant molecules is considered as a leading factor for many intracellular and intercellular problems such as the disruption of mitochondrial metabolism and cellular energetics, the occurrence of the unstable and hypoxic microenvironment, and the alteration of molecular pathways [20–22]. Moreover, it is well known that reactive oxygen species and oxidative stress play key roles in the progress of carcinogenesis and effect the all cancer hallmarks [23]. Therefore, understanding the complicated and sophisticated roles of oxidative stress and reactive oxygen species has an exclusive place in cancer biology and anticancer therapy [24–26].

6.2 Cellular Sources and Regulation of ROS

Although all living aerobic organisms need molecular oxygen vitally for their cellular respiration as a central molecule, the oxygen-containing free radicals were determined as toxic compounds for aerobic organisms by Gerschman and coworkers [27, 28]. It is well known today that the increased levels of reactive oxygen species (ROS) take disruptive effects on the function, homeostasis, and structure of cells by inducing oxidative stress and lead to the development of various pathologies such as inflammatory, cardiovascular, and neurodegenerative diseases, age-related disorders, and cancer [22, 29]. Cancer cells are characterized by the overproduction of ROS both in the various cellular compartments and in the cancer cell microenvironment, and this overproduction can alter the genetic stability of cells along with many cellular processes [30–32]. Although there is a certain balance between ROS and antioxidant factors in the healthy cells, the balance can be disrupted by endogenous and exogenous ROS generators leading to the excessive ROS production or the antioxidant defenses limitation [33, 34]. The endogenous ROS generators can be listed as mitochondria, peroxisomes, endoplasmic reticulum, transition metal ions, lipoxygenases, cytochrome P450, and NADPH oxidase, though the exogenous ROS generators are ionizing radiation, ultraviolet rays, chemotherapeutics, environmental toxins, and inflammatory cytokines [29, 35]. Reactive oxygen species can be observed as radicals that have at least one unpaired electron, and chemically reactive non-radical species without unpaired electron [36]. The non-radical species such as singlet oxygen ($^1\text{O}_2$), ozone (O_3), hydrogen peroxide (H_2O_2), hypochlorous acid (HOCl) can be converted to radical ones, though the short-lived and highly electrophilic radicals such as hydroxyl (OH^\bullet), superoxide ($\text{O}_2^{\bullet-}$), and peroxy (RO_2^\bullet) molecules show substantial cytotoxic activity by oxidizing proteins, lipids, nucleic acids, and other cellular molecules [37–39]. The generation of ROS in biological systems can be eventuated by enzymatic and non-enzymatic reactions, and the enzymatic generation of ROS can be achieved by the contribution of the cytochrome P450 enzymes, arachidonic acid, cyclooxygenase (COX), lipoxygenase (LOX), xanthine oxidase (XO), uncoupled endothelial nitric oxide synthase (eNOS), and NADPH oxidases (NOXs) [39–41]. Superoxide anion radicals that are considered as the primary reactive oxygen species are formed by transferring one electron to the molecular oxygen (O_2), and so the further interaction to generate other reactive oxygen species can be occurred, such as the formation of hydrogen peroxide (H_2O_2), which can be generated spontaneously or by the effect of the superoxide dismutase enzyme (SOD). Moreover, hydrogen peroxide can be converted to the highly toxic hydroxyl radicals (OH^\bullet) through the iron-catalyzed Fenton reaction, and leads to the cellular damage and genomic instability due to the formation of oxidized proteins, lipids, and nucleic acids [37, 42]. Apart from the enzymatic ROS generation, ROS can be non-enzymatically generated by the mitochondrial respiratory chain [43]. During the aerobic respiration, the oxygen molecules are reduced to water in the electron transport chain by cytochrome-c oxidase, though approximately 1–2% of the oxygen molecules are reduced to superoxide ($\text{O}_2^{\bullet-}$) because of the electron leakage from the electron transport steps of ATP production [29]. Then, the formed

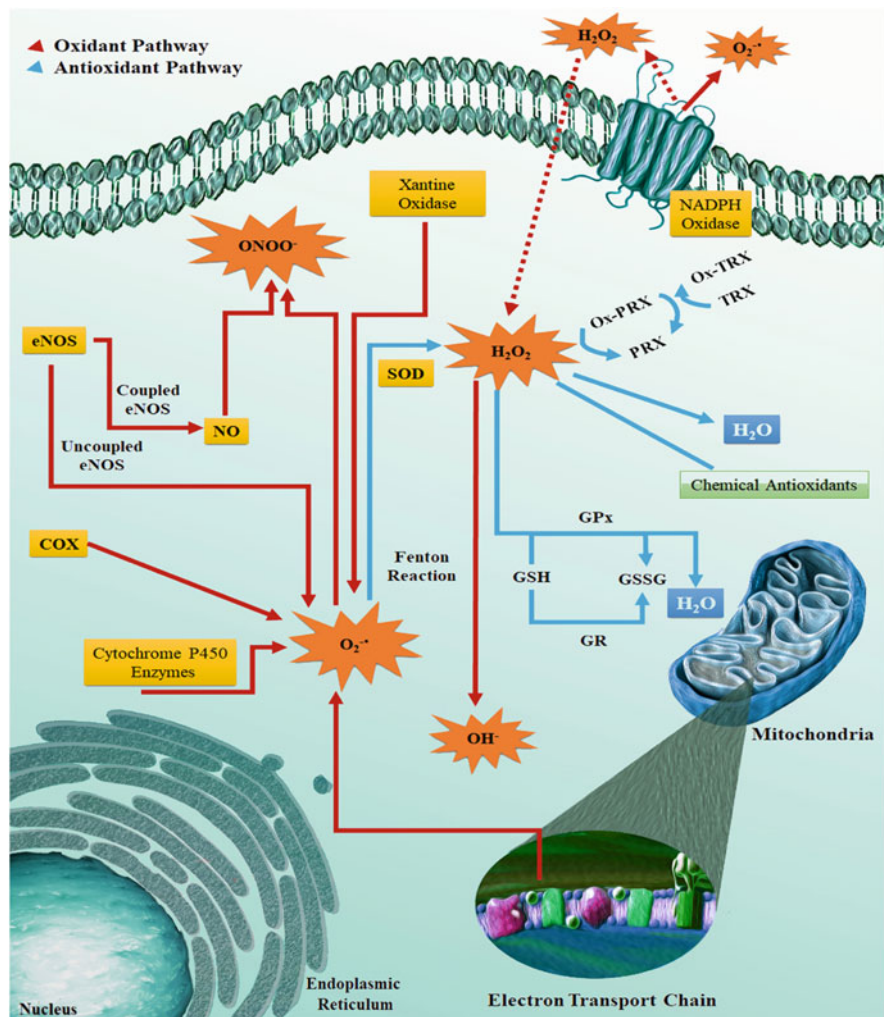


Fig. 6.1 Oxidant and antioxidant pathways for ROS homeostasis. $O_2^{\bullet -}$ (superoxide radical), OH^{\bullet} (hydroxyl radical), $ONOO^{\bullet -}$ (peroxynitrite), H_2O_2 (hydrogen peroxide), H_2O (water), COX (cyclooxygenase), eNOS (endothelial nitric oxide synthase), NO (nitric oxide), SOD (superoxide dismutase), PRX (peroxiredoxins), TRX (thioredoxin reductase), and GPx (glutathione peroxidases)

superoxide radicals can be converted by the effect of SOD enzymes to the hydrogen peroxide molecules, which can be further converted to the hydroxyl radicals (OH^{\bullet}) through the Fe^{2+} or Cu^{2+} ions-catalyzed Fenton reactions (Fig. 6.1) [31, 36, 44]. Although mitochondria is widely considered as the major source of ROS, mitochondria-generated ROS production may have been overestimated due to the generation of functional damages during the mitochondrial isolation procedures, and

performing the new techniques showed that there are much lesser amounts of mitochondria-generated ROS than the previously estimated amounts [36, 45].

The membrane-bound NADPH oxidases (NOXs) are also considered as another major source of superoxide radicals [46, 47]. NOXs can be found on the membranes of plasma, nucleus, mitochondria, and endoplasmic reticulum, and so NOXs-catalyzed reduction of oxygen molecules into superoxide radicals can be observed where these membranes are located, and NOX-derived superoxide radical that could not diffuse across membranes can be further converted by SODs into hydrogen peroxides, which have an ability to diffuse across membranes as redox signaling molecules (Fig. 6.1) [31, 37, 48]. Peroxisome organelles are recognized as another prominent source of ROS by generating superoxide radical, hydrogen peroxide, hydroxyl radical, nitric oxide (NO^{\bullet}), and peroxynitrite (ONOO^-) through the reduced catalase (CAT) activity, which has been reported in many cancers such as hepatocellular carcinoma, prostate, lung, colon, and kidney cancers [29]. Another organelle, which is ROS source, is endoplasmic reticulum that has many cellular functions including calcium storage, lipid metabolism and the synthesis, folding, posttranslational modifications, and transport of proteins [49]. Along with the NOX-derived ROS production over the membrane of the endoplasmic reticulum, the accumulation of unfolded and misfolded proteins in the lumen of endoplasmic reticulum due to the alterations in the protein folding pathways may lead to endoplasmic reticulum stress that triggers the ROS production, and the increased levels of ROS in endoplasmic reticulum induce endoplasmic reticulum stress [50, 51]. Although the endogenous and exogenous ROS generators increase the ROS levels in cells, the antioxidant defense factors maintain the ROS homeostasis [25]. The antioxidant defense components include antioxidant enzymes (e.g., catalase (CAT), glutathione peroxidases (GPXs), glutathione reductase (Gr), peroxiredoxins (PRXs), superoxide dismutase (SOD), and thioredoxin reductase (TRX)), antioxidant molecules (e.g., alpha-lipoic acid, bilirubin, coenzyme Q, ferritin, glutathione, l-carnitine, metallothionein, melatonin, and uric acid), dietary natural products (e.g., ascorbic acid, β -carotene, polyphenol metabolites, selenium, and tocopherol), and synthetic products (e.g., butylated hydroxytoluene, N-acetyl cysteine (NAC), and tiron) [36].

6.3 ROS in Cancer Cell Proliferation and Survival

It has been well established that ROS play a key role in mitogenic signaling cascades by prolonging activation of growth factors and boosting levels of cellular signaling factors [52–54]. The proliferation of many cancers such as lung, liver, and breast cancers can be enhanced by the increased ROS level though the proliferation of these cancers can be alleviated by the administration of antioxidants [55]. The metabolism of cancer cells is commonly very active because of the oncogenic signals such as Bcr-Abl, c-Myc, and Ras oncogenes-related signals, and these oncogenic signals can also increase endogenous ROS generation without the induction of apoptosis [26, 56, 57]. Oncogenic Ras mutations, for instance, induce ROS generation through

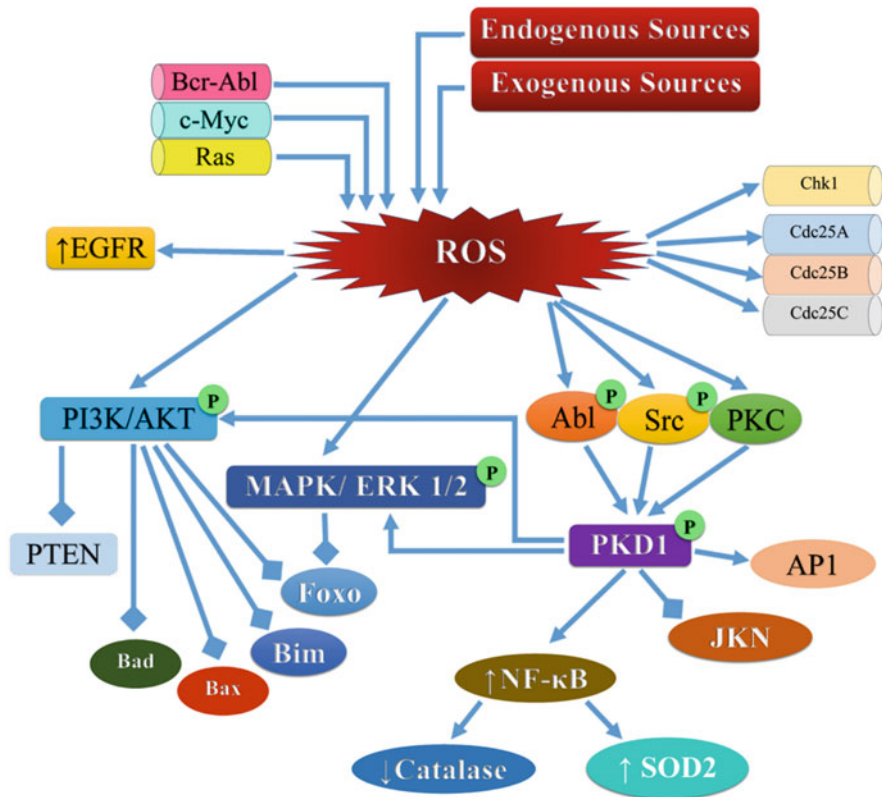


Fig. 6.2 ROS-mediated cell proliferation and cell cycle arrest signaling in cancer

NOX isoform (NOX4) that improves cell proliferation, and K-Ras oncoprotein upregulates the pro-proliferative signal epidermal growth factor receptor (EGFR) by elevating mitochondrial ROS production [31, 58]. Thus, it is widely considered that the oncogene-induced ROS generation positively regulates cancer cell proliferation by promoting mitogenic signaling cascades such as protein kinase D (PKD), mitogen activated-protein kinase/extracellular-regulated kinase 1/2 (MAPK/ERK 1/2), and phosphoinositide-3-kinase/protein kinase B (PI3K/Akt) signaling pathways (Fig. 6.2) [52]. For example, increased ROS level inhibits MAPK by oxidation of cysteine residues in the active site and the degradation of MAPK phosphatase 3 (MPK3) prominently reduces ERK 1/2 activity [59]. Similar to the inhibition of MAPK phosphatases, the protein tyrosine phosphatase 1B (PTP1B), phosphatase and tensin homolog (PTEN) protein, and ubiquitin ligase are negatively regulated by the increased level of ROS via oxidation of cysteine residues in the active sites of these proteins [53, 60, 61].

Moreover, the elevated level of ROS activates the cell proliferation-related transcription factors such as nuclear factor- κ B (NF- κ B) and activator protein-1

(AP1) that upregulate the cancer cell proliferation (Fig. 6.2) [53]. Interestingly, mitochondria-generated ROS can induce both cell proliferation and cell quiescence by playing a dual role in cell cycle. The increased level of mitochondria-generated ROS that mostly formed by superoxide ($O_2^{\cdot-}$) induces cell proliferation as well as superoxide dismutase (SOD) antioxidant defense system (Fig. 6.1), which converts the superoxide to the hydrogen peroxide (H_2O_2), and the increased hydrogen peroxide drives proliferating cells into quiescence [52, 62, 63]. Moreover, it is well known that ROS can induce DNA damage such as double-strand breaks and the DNA damage results in cell cycle arrest thanks to the cell cycle checkpoints (Fig. 6.2) [64]. For example, it has been reported that the increased ROS can result in a p53 independent G2/M arrest in colorectal cancer cells by activation of checkpoint kinase 1 (Chk1) [65]. Additionally, the phosphatase inhibition activity of ROS also induces cell cycle arrest by effecting on the cell division cycle 25 (Cdc25) protein phosphatase family consisted of Cdc25A, Cdc25B, and Cdc25C proteins that have substantial roles in the progression of the various cell cycle stages such as synthesis (S) and mitosis (M) phases [66]. For example, it has been reported that the ROS-decreased Cdc25C level leads to G2/M cell cycle arrest and the elevated ROS dramatically decrease Cdc25A level and its phosphatase activity [64, 67–69]. On the other hand, ROS accumulation can also predictably take an important role in cancer cell survival as well as cell proliferation and cell cycle arrest because of the common signaling factors such as PTEN, PI3K, PKD, Akt, ERK 1/2, and NF- κ B (Fig. 6.2) [26, 31, 52]. For example, increased generation of hydrogen peroxide leads to the oxidation of cysteine thiol groups of PTEN, PTP1B, and PP2 (protein phosphatase 2) and inactivation of these phosphatases promote cell survival by negatively regulation of PI3K/Akt signaling [31, 70, 71]. It has been reported that this kind of phosphatases' inactivation can be observed in many types of cancer such as breast, prostate, ovarian and endometrial cancers, glioblastomas, and melanomas [72, 73]. Ras activation along with growth factor signaling can be also induced by hydrogen peroxide, and this activation leads to blocking the PTEN signaling cascades and induction of PI3K/Akt/mTOR and MAPK/ERK 1/2 cell survival pathways [31, 58]. Moreover, these cell survival pathways can be regulated by ROS-induced inactivation of their downstream pro-apoptotic targets such as Bad, Bax, Bim, Foxo [52, 74–76]. Apart from the Ras oncogene, the cell survival can be also regulated by the other oncogenes such as c-Myc oncogene that induce hMre11 signals and improve the cell survival in many cancers such as cervical carcinoma, colon cancer, leukemia, lymphoma, and testicular cancer (Fig. 6.2) [77, 78].

6.4 ROS and Endogenous Signaling Molecules

It is widely known that there is a tight relationship between the ROS generation and the endogenous signaling molecules such as the growth factors and cytokines, which regulate the molecular mechanisms of many cellular phenomena such as proliferation, growth, invasion, healing, differentiation, metastasis, etc., by involving the intracellular and intercellular signaling pathways [53, 79]. Although the ROS

production can be induced by the intracellular growth factors and cytokines such as epidermal growth factor (EGF), endothelial cell growth factor (ECGF), transforming growth factor beta 1 (TGF- β 1), and hepatocyte growth factor (HGF), the elevated ROS in turn can stimulate the multiple growth factors and cytokines that play crucial roles in carcinogenesis by binding to the cell membrane receptors such as receptor serine/threonine kinases, G protein-coupled receptors, receptor tyrosine kinases, and cytokine receptors [32, 73, 79–81]. This phenomenon between the endogenous signaling molecules and ROS display the existence of a positive feedback loop [32]. For example, it has been reported that ROS production in several culture systems may be elevated by TGF- β 1, which plays substantial roles in growth regulation and tumor cell progression as a multipotent cytokine [80–82]. Similarly, the tight relationship has been shown between ROS production and HGF, which is known as a prognostic marker for hepatocellular carcinoma, ROS can mediate the HGF receptor and c-met signaling [83–85]. Moreover, the superoxide level in a cell can be elevated by the stimulation of angiotensin, epidermal growth factor (EGF), lysophosphatidic acid, platelet-derived growth factor (PDGF), and tumor necrosis factor- α (TNF- α) though the oncogenic mutation of RhoGTPase K-ras has been reported to be related with the elevation of superoxide level and the incidences of several cancers [30, 53, 86–91]. As well as the oncogenic mutation of RhoGTPase K-ras, the major ones of growth factors and cytokines including HGF, PDGF, vascular endothelial growth factor (VEGF), and TNF- α increase the production of ROS through NADPH oxidases or mitochondrial electron transport chain system depending on the cellular environment [32, 92, 93]. Although the structures of NADPH oxidases are similar to each other, their regulatory subunits and activation mechanisms are different from each other. For example, p22phox is a necessity for the activation of NOX4 though the other NADPH oxidases do not need it [94, 95]. Moreover, NOX4 can be activated by the influences of various growth factors and receptors such as TGF, bone morphogenetic protein-2 (BMP-2), insulin like growth factor-I (IGF-I) and toll like receptor 4 (TLR4), and the activated NOX4 plays role in the ROS generation [96–98]. As the effects of NOX-generated ROS production, the relationship between endogenous signaling molecules and ROS generation generally affects the fate of cancer hallmark such as cancer cell proliferation and cell survival, angiogenesis, invasion, metastasis, and increased genomic instability by the altering and blocking of related signaling cascades [52, 99].

6.5 ROS and Emerging Hallmarks of Cancer

As widely known, Hanahan and Weinberg published an influential paper in the year 2000 that describes the hallmark of cancer, including six major traits, and they updated the described cancer hallmarks in 2011 by adding two emerging and two enabling traits of cancer [6, 7]. The emerging hallmarks of cancer have been described as deregulating the cellular energetics and evading the immune response [6]. It is not surprising that there is a strict relationship between the emerging hallmarks of cancer and the intracellular accumulation of ROS, and the elevated

metabolic activity, oncogenic signals, and genetic changes in cancer cells induce an increased ROS production along with the adaptation to the antioxidant system and the compensation for the oxidative damages [31, 100]. Thus, alterations occur in the redox homeostasis and cellular signaling pathways, and cancer cell metabolism is reprogrammed [101]. Cancer cells acquire adaptations to survive under hypoxic conditions and utilize alternative metabolic pathways because of their higher metabolism than the normal cells [23, 101]. This alteration in the energy metabolism of cancer cells was firstly discovered in 1924 by Otto Warburg, who reported that cancer cells convert glucose to lactate using glycolytic pathway instead of pyruvate regardless of the presence of oxygen [102]. This phenomenon has been known as Warburg effect, which contributes an aggressive cancer phenotype because a prolonged survive under hypoxic condition leads to a series of alterations in genetic stability, metabolic pathways, organelles, etc., though the hypoxic condition leads to cell death in normal cells [52, 103]. It can be clearly seen that there is a reciprocal crosstalk between the redox balance and metabolic pathways such as glycolysis, the pentose phosphate pathway, one-carbon metabolism, fatty acid oxidation, and glutaminolysis [31, 104]. For example, redox homeostasis can be regulated by glycolysis through shuttling of the pentose phosphate pathway-generated intermediate nicotinamide adenine dinucleotide phosphate (NADPH) and glutaminolysis-generated intermediate glutathione (GSH) [52]. Although it is expected that the glucose-deprivation causes cell death by the accumulation of hydrogen peroxide, the Warburg effect provides the cancer cells to acquire adaptation of the glucose-deprivation by exaggeratedly using glycolysis pathway to prevent hydrogen peroxide-induced cell death [105, 106]. Targeting glycolysis and lactate dehydrogenase enzyme is therefore considered a successful strategy to prevent the cancer cell progression by inducing oxidative stress and decreasing the production of the intracellular ATP [31, 107–109]. For example, let-7a that is an early-discovered microRNA was used as a therapeutic enhancer because let-7a elevates the ROS generation and downregulates some enzymes involved in glycolysis such as glucose 6-phosphate dehydrogenase (G6PD) and inosine monophosphate dehydrogenase (IMPDH) [110]. Pyruvate kinase muscle isoenzyme 2 (PKM2), the isoenzyme of the rate-limiting glycolysis enzyme named pyruvate kinase, plays a crucial role in reprogramming cancer metabolism, but the ectopic expression of microRNA-1 and microRNA-133b inhibits PKM2 through silencing polypyrimidine tract-binding protein 1 (PTBP1), which can convert the active PKM2 to the inactive PKM1 [111, 112]. On the other hand, it is well documented that cancer cells produce an elevated level of ribose 5-phosphate by employing pentose phosphate pathway that is considered a key feature for many cancers, and regulate the ROS homeostasis through NOXs and replenishing the decreased GSH and TRX [31, 113]. As previously mentioned, mitochondria is considered as one of the major sources of ROS because they are inevitably generated in oxidative phosphorylation as the byproducts [114]. The elevated ROS accumulation because of hypoxia causes oxidative stress and consequently results in damages of organelles and the other cellular components such as lipids, proteins, metabolites, etc. [115, 116]. Moreover, the structure, morphology, and dynamics of mitochondria are considered linking with the

accumulated amounts of ROS, i.e., there is a mutual interaction between mitochondria and ROS [117]. The overproduction of ROS induces mitochondrial damages and these damages result in the elevated ROS production, so this phenomenon is called as ROS-induced ROS release [115, 118, 119]. For example, mitochondria induce elevated production of ROS under hypoxic condition and the hypoxia-induced ROS production can cause the mitochondrial fragmentation though the mitochondrial fusion is considered as a cellular adaptation process for the alterations in the surrounding environment, and which can prevent the elevated production of ROS [120, 121]. One of the most prominent transcription factors for the cellular adaptation to the hypoxic conditions is hypoxia inducible factor-1 (HIF-1) that is a heterodimer consisted of two subunits HIF-1 α and HIF-1 β , and it is well known that ROS play a key role in the accumulation of HIF-1 [52, 122, 123]. The increased levels of antioxidants reduce the accumulation of HIF-1 though the increased levels of hydrogen peroxide and superoxide elevate the accumulation of HIF-1 [63, 124, 125]. Apart from the endogenous ROS, the exogenous ROS can alter mitochondrial dynamics by inhibiting mitofusin-1 (Mfn1) and mitofusin-2 (Mfn2) and inducing the depolarization of mitochondrial membrane potential, which trigger the mitochondrial fission along with the overproduction of ROS [115, 126]. Moreover, the oxidative stress-induced mitochondrial fission and fusion influence on the mitochondrial metabolism and function because of the dramatic changes in mitochondrial DNA (mtDNA), ribosomes, proteins, metabolites, etc., that lead to many diseases including cancers, cardiometabolic diseases, neuropathies, and neurodegenerative diseases [127–129].

As previously mentioned, the second emerging hallmark has been described as evading the immune response, and ROS have been identified as immunosuppressive factors in the cancer microenvironment to facilitate the other cancer hallmarks such as growth, invasion, and metastasis [6, 31, 130]. Apart from the pathological conditions such as cancer, ROS play key roles in the regulation of immune responses and serve as central mediators of immune cells [131]. For example, dendritic cells (DC) that have a substantial role in antigen specific immune response as the major antigen-presenting cells (APC) are activated by hydrogen peroxide, which can be produced in a large quantity by phagocytic cells [132]. On the other hand, the pathological conditions such as cancer or chronic inflammatory diseases can alleviate the function of natural killer cells (NK cells) and the effector T cells depending on the macrophages- and granulocytes-generated ROS levels [133–135]. Although NK cells increase the ROS production in the early stage of the encounter with cancer cells to mediate cytolysis, it has been reported that monocyte-generated ROS production in cancer patients inhibits the interferon gamma (IFN- γ) production, proliferation, activation, and cytotoxicity of NK cells along with the induction of NK cells' apoptosis [136, 137]. The differences of NK cells responses to the ROS generation are regulated by the CD56^{bright} and CD56^{dim} that are the NK cells antigen subsets [138]. Monocyte-derived ROS direct CD56^{dim} NK cells to apoptosis though the CD56^{bright} NK cells display a significant resistance to the ROS-induced functional inhibition and apoptosis because of their stronger antioxidant capacity than CD56^{dim} NK cells [139–141]. Thus, the resistance of CD56^{bright} NK cells provides

the cancer cells to evade immune system because the ROS-sensitive CD56^{dim} NK cells have higher cytotoxic activity than the ROS-resistant CD56^{bright} NK cells [136]. Similarly, the oxidative stress in the cancer microenvironment provides cancer cells to evade immune system by regulating the accumulation of different subsets of T cells because the conventional T cells are more sensitive to the hydrogen peroxide-induced cell death than the regulatory T cells (T_{regs}) that have an ability to inhibit the functions of other infiltrating immune cells [142–144]. Moreover, either the functions of T cells can be suppressed or the apoptosis of T cells can be induced via Jak3/STAT5 signaling pathway, which is regulated through the inducible nitric oxide synthase (iNOS)-generated nitric oxide (NO) production by myeloid-derived suppressor cells (MDSCs) [136, 145–147].

6.6 ROS and Enabling Hallmarks of Cancer

In the paper published by Hanahan and Weinberg [6], two enabling hallmarks of cancer have been added to the previously described six hallmarks along with the two emerging hallmarks, and these two enabling hallmarks have been described as the tumor-promoting inflammation, and the genome instability and mutation [6, 7]. Actually, the relationship between inflammation and carcinogenesis has been known far before the paper of Hanahan and Weinberg [6]. In 1863, Rudolf Virchow reported that the “lymphoreticular infiltrate” reflected the origin of cancer at the locations of chronic inflammation by observing white blood cells or leukocytes in neoplastic tissues [23, 148–150]. The currently known data obtained from the numerous studies performed after the Virchow’s hypothesis clearly indicate that there is a tight and intricate relationship between the cancer progression and the promotion of inflammation coordinated by the level of inflammatory cytokines (TNF, interleukin-1 (IL-1), and IL-6), chemokines (CXC chemokine receptor 4 (CXCR4) and IL-8), and inflammation-related factors, especially located in the tumor microenvironment [151–153]. Moreover, it is well known that ROS predictably take important roles in the regulation of the sophisticated interaction between the course of cancer and the promotion of inflammation by effecting the presences, levels, and types of the inflammatory cytokines, chemokines, and inflammation-modulating factors such as activator protein 1 (AP-1), HIF-1 α , specificity protein 1 (Sp1), β -catenin, wingless-type MMTV integration site family (Wnt), HIF-1 α , NF- κ B, peroxisome proliferator-activated receptors-gamma (PPAR- γ), p53, signal transducer and activator of transcription 1 (STAT1)/STAT3, and nuclear factor erythroid 2-related factor 2 (Nrf2) [154–157]. A series of signal transduction cascades can be activated by the accumulation of inflammatory cells in the tumor microenvironment, which provokes the further recruiting of inflammatory cells by producing cytokines and chemokines, and a massive ROS production is therefore occurred by the activation of the oxidation-related enzymes such as iNOS, NOX, XO, and myeloperoxidase (MPO), and the upregulation of the expression of COX2 and LOXs [156, 158]. The massively produced ROS leads to significant oxidative damages in genetic materials, macromolecules, and organelles, which support the progression of carcinogenesis

and overpowered production of supplemental ROS, and this excessively produced ROS activate again the inflammatory cytokines, chemokines, and inflammation-modulating factors. This phenomenon may be named as “inflammatory response-mediated ROS-induced ROS release” by considering its similarity with “ROS-induced ROS release” because ROS-induced inflammatory responses release excessive ROS that induce again inflammatory responses [154, 155, 159, 160].

The other enabling hallmark of cancer has been described as the genome instability and mutation, the main cause of the genetic diversity in many cancers and the cancer cell heterogeneity within the tumor tissue, and the overproduced ROS are known as the prominent factors leading the oxidative DNA damages, including base damages and modifications, deletions and insertions in DNA sequence, DNA miscoding lesions, DNA single-strand and double-strand breaks, gene amplification, and the activation of oncogenes, which contribute in cancer initiation and progression [26, 31, 161–164]. It can be possible to say that there is a cycle between the overproduction of ROS and the oxidative DNA damages. As previously mentioned, the elevated ROS level can activate the oncogenes such as Bcr-Abl, c-Myc, and Ras, which can play substantial roles in the regulation of tumor suppressor genes, cancer cell proliferation, mitochondrial dysfunction, angiogenesis, and metastasis, and the oncogene activation is known as the main cause of the overproduction of ROS that leads to the formation of replication stress [165–167]. For example, it has been reported that the replication fork velocity can be reduced because the polymerase activity is affected by the occurrences of ROS-oxidized deoxyribonucleotide triphosphates (dNTPs) [168, 169]. The progression of replication fork can be regulated by ROS by dissociation of peroxiredoxin2 oligomers (PRDX2), and the fork accelerator named TIMELESS can be inhibited by a replisome associated ROS sensor formed PRDX2 [170]. Thus, the replication fork speed can be reduced through the dissociation of PRDX2 and TIMELESS, which is regulated by the overproduced ROS [170]. Moreover, the replication forks can be prevented physically due to the occurrences of oxidized bases, and this phenomenon can cause the breakdown of replication forks at fragile sites across the genetic material along with the under-replicated or over-replicated DNA [64, 171]. On the other hand, the highly accumulated ROS may directly effect on DNA through reacting with purines, pyrimidines, and chromatin proteins, and causing the DNA single-strand and double-strand breaks [172, 173]. A point mutation, for example, can be formed because of the production of 8-hydroxy-2'-deoxyadenosine (8-OH-dAdo) or 8-hydroxy-2'-deoxyguanosine (8-OHdG) that are the widely known oxidative DNA damage markers, which can be occurred through the reaction of hydroxyl radicals with adenine or guanine nucleotides, respectively [173–176]. Additionally, it should be noted that quinine is considered as the most sensitive nucleobase to oxidation than other nucleotides, and so 8-OHdG emerges as the most common oxidized nucleobase [177]. Apart from the 8-OHdG, there are some other oxidative DNA damage markers such as 8-oxo-7,8-dihydroguanine (8-oxoGua), 8-oxo-7,8-dihydroadenine (8-oxoAde), 8-oxo-7,8-dihydro-2-deoxyguanosine (8-oxodG), 5,6-dihydroxy-5,6-dihydrothymine, 2,6-diamino-4-hydroxy-5-formamido-

pyrimidine, and 4,6-diamino-5-formamido-pyrimidine [29, 172, 178]. ROS-induced production of oxidized nucleobases generally leads to further mutations and DNA damages along with the accumulation on some specific location such as telomere sites, which are less efficiently repaired than the other genomic sites [179]. 8-oxoGua can be, for example, accumulated on the telomere sites, and behaves as a blockage for telomerase activity through reducing the binding potential of telomeric proteins, disrupting telomere length, and precluding of chromosomal-end capping, and this phenomenon can result in cell death, aging, carcinogenesis, chromosome instability, and genotoxic formations such as nuclear buds (NBUDs), nucleoplasmic bridges (NPBs), and micronuclei (MN) [179–181].

6.7 ROS and Angiogenesis

Angiogenesis can be considered as one of the most important cancer hallmarks because cancer cells rapidly proliferate to form and expand the tumor tissue, but the tumor tissue expansion increases the distance between cells and capillary vessels [12]. However, the appropriate distance between the cells and capillary vessels is restricted to 100–200 μm to maintain the balanced composition of oxygen, carbon dioxide, nutrient substances, and metabolic wastes [182, 183]. Additionally, the tumor tissue enlargement provides a hypoxic, hypoglycemic, hypoferric, and acidified microenvironment along with the occurrence of an intolerable mechanical stress on the cancer cells, and so the cancer cells are driven to migrate, invade, and metastasize [12]. The cancer cells induce therefore angiogenesis to form new capillary vessels originated from the existing vessels, run away from the stressed microenvironment by participating in the circulatory system, and sustain the course of carcinogenesis [184]. Angiogenesis is regulated via an angiogenic switch, which can be opened and closed by variation of the balance between angiogenesis promoting (angiogenic) and suppression (anti-angiogenic) factors [185]. The formation of ROS and the occurrence of oxidative stress within the cells and microenvironment predictably regulate the direction of the angiogenic switch along with the activation of angiogenic or anti-angiogenic factors through the regulation of transcriptional factors, releasing of some growth factors, and alteration of the cellular signaling cascades (Fig. 6.3) [186, 187]. For example, the cancer cells in a hypoxic microenvironment can induce the releasing of proangiogenic growth factors such as vascular endothelial growth factor (VEGF), epidermal growth factor (EGF), fibroblast growth factor (FGF), hepatocyte growth factor (HGF), and platelet-derived growth factor (PDGFB) and the increased production of the other angiogenic proteins such as angiopoietin-1, leptin, endoglin, prominin-1, transforming growth factor beta (TGF-beta), integrins, and matrix metalloproteinase (MMP) enzymes [12, 188–190]. After the opening of angiogenic switch and the formation of new capillaries surrounding the tumor tissue, the tumor cells and their microenvironments are re-oxygenated. Contrary to the expectations, the tumor-induced angiogenesis and re-oxygenation of tumor cells lead to larger problems instead of solving the problem of cancer cells [187].

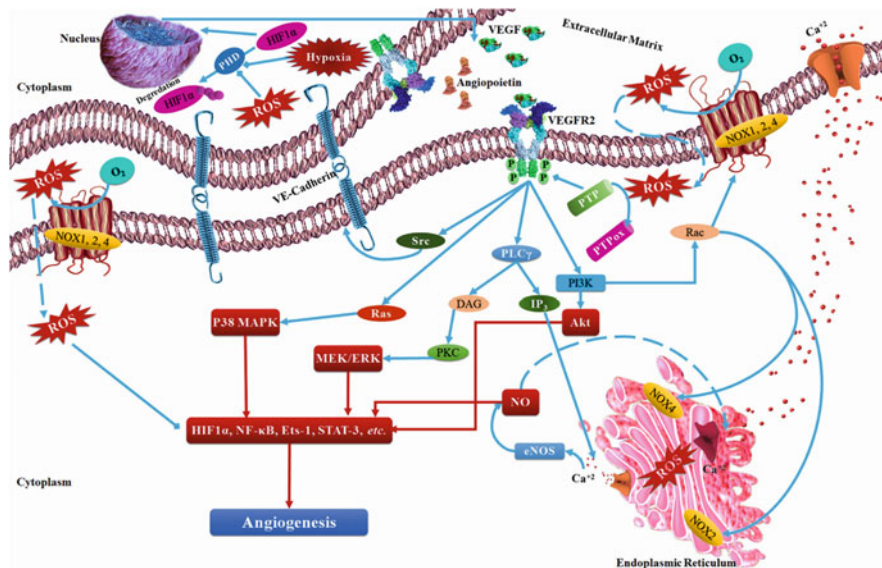


Fig. 6.3 ROS and VEGF-mediated angiogenesis. Hypoxic environment and elevated ROS increase the production of angiogenic factors such as VEGF and angiopoietin, and activate a series of signaling cascades to regulate angiogenesis

Although the hypoxic microenvironment induces an overproduction of ROS by disrupting the mitochondrial metabolism and the electron transport system, an oxygen abundance occurs because of the angiogenesis-motivated re-oxygenation phase, which results in 100 times higher ROS production than hypoxic state [191–193]. This phenomenon is named as “cyclic hypoxia” because hypoxia-induced angiogenesis leads to an excessive production of ROS and these ROS induce again angiogenesis through the direct activation of HIF-1 α , VEGF, and VEGFR2, and the oxidation of lipids that stimulate NF- κ B pathway-mediated angiogenesis [187]. ROS-induced equilibrium corruptions in the angiogenic switch can lead to many structural and functional abnormalities within the newly formed capillaries surrounding tumor tissue, and these abnormalities result in hyperpermeability, hypoglycemia, hypoxia, abnormal blood flow, and increased pressure, which also increase the ROS production [187, 194]. Additionally, ROS such as superoxide anion and hydrogen peroxide molecules have a special importance for the vascular cells because they can regulate the fate of these cells depending on the concentrations [195]. For example, the low concentrations of hydrogen peroxide such as 0.1–10 μ M induce the capillary tube-like formation of endothelial cells though its high concentrations (>125 μ M) induce lethal damages [187, 195]. Consequently, ROS have a substantial role in the regulation of angiogenesis though the tumor-induced angiogenesis is one of the major causes of the excessive ROS production, and it is widely considered that the main source of ROS caused by tumor-induced angiogenesis is mitochondria and electron transport system because

of the hypoxic condition and the cyclic hypoxia-induced oxygen abundance [12, 187].

6.8 ROS in Cancer Cell Invasion and Metastasis

Cancer cell invasion and metastasis are commonly considered as the carcinogenesis processes that can be possible depending on the formation of angiogenesis though the metastasis can be observed in many solid tumors regardless of the early or late stages of the carcinogenesis [6, 187, 196]. Besides the similarities of the underlying reasons of the tumor-induced angiogenesis and metastasis, the close interaction between angiogenesis and metastasis has been known since the first observations of Judah Folkman (1971) and Pietro Gullino (1978) [189, 197–199]. As previously mentioned, the uncontrolled expansion of tumor tissue results in an unsuited microenvironment qualified with the hypoxic, hypoglycemic, hypoferric, and acidified features along with the mechanical stress, and so the cells forming tumor tissue would like to escape from this microenvironment by inducing angiogenesis and operating the complex processes of metastasis [12, 187]. Metastasis can be occurred employing a series of cellular phenomena, including the degradation of extracellular matrix (ECM), losing the cellular polarity and detaching from the ECM, cancer cell invasion along with the amoeboid or mesenchymal migration, accessing to the capillary vessels, intravasation, sustaining the anchorage-independent growth and survival by evading anoikis (anchorage-dependent apoptosis), bypassing the immune surveillance, extravasation, adhesion, proliferation, and colonization within the secondary tumor site [200–203]. Numerous papers have revealed that ROS have substantial regulative roles in the complex processes of metastasis as well as in the angiogenesis, and many clinical and experimental data have suggested that the level of ROS is changed during the metastasis [204]. For example, several studies reported that the overproduced ROS induce the epithelial to mesenchymal transition (EMT), a biological phenomenon that acts on the metastasis-related cellular functions such as the cell–cell and cell–matrix interactions along with the cellular motility and migration, and EMT can be regulated by various cytokines such as TGF- β 1 and EGF, transcription factors including Twist, Snai1, Slug and ZEB1/2 (zinc-finger E-box-binding homeobox), and signaling pathways such as the inhibitory kappa B kinase (IKK)/NF- κ B, MAPK, Notch, PI3K/Akt, TGF- β /Smad, and Wnt/ β -catenin signaling pathways [205–208]. Although the cell invasion is facilitated by the elevated expression of urokinase plasminogen activator (uPA) and matrix metalloproteinases (MMPs), the cell–cell and cell–matrix adhesions are emaciated by decreasing the epithelial markers and tight junction proteins such as occludin, claudin, and e-cadherin, and increasing the mesenchymal markers such as fibronectin, vimentin, and n-cadherin [205, 208]. The overproduced ROS are commonly considered as the prominent regulator for the processes of EMT and metastasis, and ROS-induced cancer cell metastasis by affecting the molecular pathways, transcription factor, cytokines, and growth factor have been extensively reviewed by many scientists [204, 205, 209–212]. For example, one of the prominent inducer of EMT named

TGF- β 1 can be regulated by ROS-dependent pathway; the Rac1-NOXs-ROS-dependent activation of NF- κ B pathway mediates the TGF- β 1-regulated uPA and MMP9 activities on cell migration and invasion [210, 213]. Additionally, the Rac-dependent ROS production has been suggested to be related to the activities of MMPs (e.g., MMP2, MMP3, and MMP9) and the transduction of mechanical perturbations into a pro-invasive gene expression [209, 214–216]. Moreover, the loss of TGF- β 1-activated kinase 1 (TAK1) can lead to the integrin-Ras-induced ROS production that activates the EMT signaling cascade [217]. Pelicano and coworkers reported that mitochondria-derived ROS production leads to the AP-1 signaling pathway-mediated upregulation of C-X-C motif chemokine 14 (CXCL14) expression and the boost in cell motility by increasing the amount of cytosolic Ca²⁺ levels [218]. On the other hand, the evading anoikis (anchorage-dependent apoptosis), which is the most important part of metastasis is succeeded by ROS-dependent mechanisms. Anoikis resistance of cancer cells can be conferred through NOX4-induced ROS-activated the epidermal growth factor receptors (EGFR) and angiopoietin-like 4 (ANGPTL4)-integrin complex-induced ROS-activated PI3K/Akt and ERK pathway [219–221].

6.9 ROS and Cancer Cell Death Pathways

Although the overproduced ROS are well known as a key factor in the initiation and development of cancer through the disrupting effects on the genetic materials, cellular macromolecules, organelles, signaling cascades, components, and homeostatic balances along with the significant contribution in the cancer cell survival, the disproportionately increased ROS emerge as a substantial approach for the cancer treatment strategies because of the cell death provoking activity [23, 52]. Apart from the non-inflammatory, caspase-independent, and ROS-sensitive special cell death pathway named “oxeiptosis,” there are well-described ROS-induced cell death pathways such as caspase-dependent apoptosis, caspase-independent ferroptosis, and necroptosis, inflammasome-driven pyroptosis, and autophagic cell death (Fig. 6.4) [222–227].

Kelch-like ECH-associated protein 1 (Keap1) is known as a main sensor to monitor oxidative and electrophilic stress, and regulates the expression of cytoprotective molecules by ubiquitination and degradation of Nrf2 under the physiological conditions, though the overproduced ROS-oxidized Keap1 leads to insufficient expression of cytoprotective molecules and highly expressed antioxidant factors, viz., NAD(P)H quinone dehydrogenase 1 (NQO1), homeobox protein 1 (Hox1), and Thioredoxin (Txn) because of the highly accumulated Nrf2 [227, 228]. Besides the accumulation and translocation of Nrf2, the oxidized Keap1 could not interact with the phosphoglycerate mutase 5 (PGAM5) that is known as a common factor for many caspase-independent cell death pathways, and so the released PGAM5 dephosphorylates the apoptosis inducing factor mitochondria associated 1 (AIFM1) at Ser116 [227, 229]. Thus, AIFM1-deficient cells undergo to the oxeiptosis by ROS-induced cell death pathway that includes KEAP1, PGAM5 and AIFM1 [227].

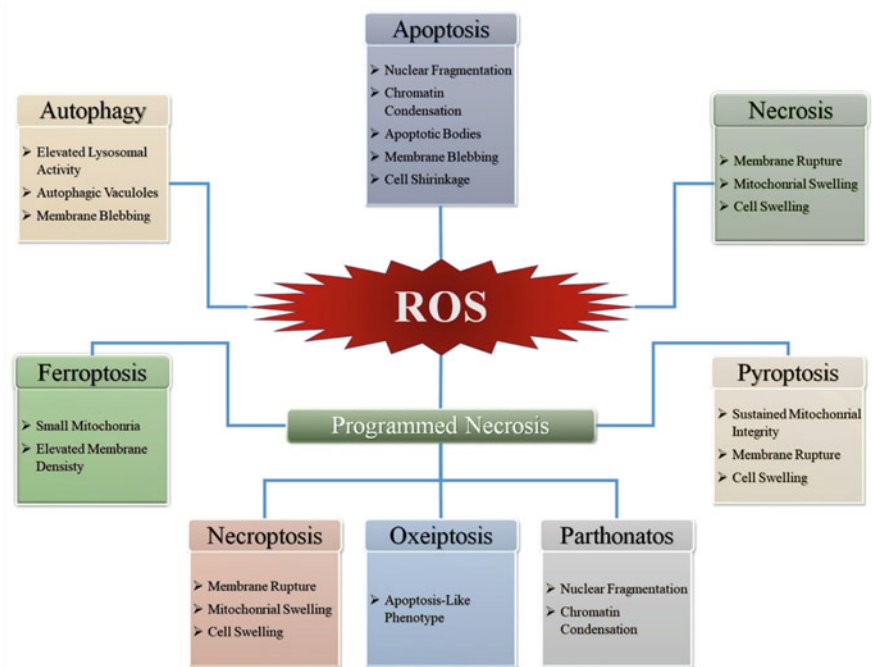


Fig. 6.4 ROS-induced cell death pathways

On the other hand, the ROS-induced caspase-dependent cell death pathways are well known and the cellular pathways have been broadly described in many papers [222–227]. For example, cytochrome-c is released from mitochondria because of the ROS-induced mitochondrial abnormalities and dysfunctions, and so apoptosome complex can be formed by the incorporation of the released cytochrome-c, Apaf-1 (apoptotic peptidase activating factor 1), and procaspase-9 to activate effector caspases, e.g., caspase-3, which leads to the cleavage of cellular proteins and apoptosis [23, 230]. Additionally, the intracellular accumulation of ROS regulates the expression of the pro-apoptotic (Bad, Bak, Bax, Bid, and Bim) and anti-apoptotic (Bcl-2, Bcl-w, and Bcl-xL) members of the Bcl-2 family via their phosphorylation and ubiquitination, and the Bcl-2 family proteins play key roles in the regulation of the mitochondrial membrane permeabilization and apoptotic signaling [52, 231, 232]. The other well-known ROS-induced cell death is autophagy that can be regulated by several kinase cascades such as the most familiar mammalian target of rapamycin complex1 (mTORC1), which can be regulated by PTEN/PI3K/AKT signaling pathway [233]. The overproduced ROS-induced autophagy results in degradation of the mitochondria that excessively produce ROS, and so this kind of autophagy is called as mitophagy that leads to the reduced ROS levels as a result of the NIX/BNIP3L and PARKIN/PTEN induced putative kinase 1 (PINK1) molecular pathways [52, 234–236]. Moreover, the ROS-induced autophagy can be occurred through Nrf2/Keap1 pathway by preventing degradation of Nrf2 as well as the

ROS-induced oxeiptosis [237]. The attentions of many scientists seem to be focused on the ROS-dependent cell death pathways because there is certainly a complex relationship between the intracellular ROS and the cell death pathways, and the ROS-mediated anticancer drugs and treatment strategies are commonly considered as the beneficial treatment modalities.

6.10 ROS and Anticancer Treatment Strategies

Although unexpected and long-term changes in the intracellular ROS level are considered as the main factors for the occurrence of extremely complex cellular processes that induce carcinogenesis, numerous studies have shown that cancer cells are more susceptible to the changes in intracellular ROS accumulations and more dependent on the antioxidant systems than their healthy counterparts [39]. The exogenous ROS generation is therefore considered as a promising option for the anticancer treatment strategies because the vulnerability of cancer cells towards oxidative stress provides a therapeutic selectivity in anticancer therapies [238]. ROS-dependent treatment strategies are generally based on three different approaches such as directing cancer cells to the cell death pathways by promoting an excessive ROS generation, activating ROS-dependent cancer cell death by blocking the antioxidant systems, and inhibiting carcinogenesis by reducing ROS generation via activating antioxidant systems and employing antioxidant molecules. There are many chemotherapeutic agents that increase ROS generation to selectively induce cancer cell death because of the ROS-induced irreparable damages [54]. Examples of these chemotherapeutics include, but not limited to the arsenic trioxide, anthracyclines (e.g., daunorubicin, doxorubicin, epirubicin, and idarubicin), bleomycin, β -lapachone, cisplatin, elesclomol, and sulindac [54, 238–240]. These drugs can induce ROS generation by using different cellular mechanisms. For example, doxorubicin that is a topoisomerase inhibitor, DNA intercalation agent, and also one of the most known chemotherapeutics employed in the treatment of many cancers, including bile duct, breast, endometrium, esophagus, gastric, pancreatic and liver cancers, osteosarcoma, Kaposi's sarcoma and soft tissue sarcomas, Hodgins and non-Hodgins lymphomas, induces intracellular ROS generation by reacting with flavoprotein reductases, intracellular chelation of iron, which respectively result in apoptosis and ferroptosis [52, 241, 242]. Apart from the application of chemotherapy-induced ROS generation, there are different cancer treatment strategies that induce intracellular ROS generation, such as photodynamic cancer therapy (PDT) and sonodynamic cancer therapy (SDT) [243, 244]. PDT is a non-invasive and clinically approved treatment method that induces excessive ROS generation in the presence of molecular oxygen thanks to the synergic interactions of a non-thermal light source and a nontoxic photosensitizer molecule to induce apoptosis by damaging the cellular components of target cells [244, 245]. Similar to the PDT, the ultrasound-mediated cancer therapy (SDT) induces apoptosis in the target cells through the production of ultrasonic cavitation, sonochemical bubble collapse, and finally free radicals and ROS generation

[243, 246]. The second ROS-dependent cancer treatment approach emerges as the suppressing cellular antioxidant systems (e.g., glutathione and thioredoxin) resulting in the overproduced ROS-dependent activation of cell death pathways, and examples of these chemotherapeutics include 2-methoxyestradiol, buthionine sulfoximine, phenylethyl isothiocyanate, imexon, mangafodipir, and tetrathiomolybdate [39]. For example, buthionine sulfoximine, phenylethyl isothiocyanate, and imexon lead to the increased accumulation of intracellular ROS by reducing the intracellular GSH level [247–249]. Moreover, it has been thought that the redox adaptation mechanisms can be evaded by combining the first and second ROS-dependent anticancer treatment approaches, viz., the promoting an excessive ROS generation and the suppressing cellular antioxidant systems [39]. The last one of the ROS-dependent treatment approaches is known as the targeting ROS production by using antioxidant molecules that can be employed as a cancer preventive therapy by using daily dietary compounds such as green tea-derived epigallocatechin-3-gallate (EGCG), carotenes vitamin C and vitamin D [250, 251]. However, it should be noted that there are also some reports indicating that some antioxidants such as carotene, vitamin A, and vitamin E can be effective on the elevated risk of cancer [252–254]. The substantial contributions of ROS into the drug resistance development processes and further progression of carcinogenesis should be also noted, because the overproduced ROS are well known as the prominent factors for the oncogenic signaling, genetic instability, and DNA damages along with the metabolic adaptations, enhanced proliferation, and survival [255]. Consequently, it should be thoroughly considered the advantages, disadvantages, and the exact activity mechanisms of the ROS-dependent anticancer therapies because it is clear that targeting redox homeostasis of cells may lead to the unexpected and unwanted consequences as well as the expected and wanted outcomes.

6.11 Concluding Remarks and Future Prospects

As can be clearly seen in the previous parts of the chapter, ROS play substantial roles in the regulation of physiological homeostasis such as the controlling of cellular signaling cascades via low-level productions and the provoking of cell death pathways via overproductions. Although numerous studies have shown that more than 150 human disorders are related to the disruption of redox homeostasis, the bulk of ROS-mediated intracellular signaling pathways and the consequences remain unknown. It is well known that cancer cells induce the overproduction of ROS and the elevated ROS production contributes in the progression of carcinogenesis through provoking the DNA damage and genetic instability, cancer cell proliferation and survival, metabolic adaptations, and drug resistance. Interestingly, ROS-induced cell deaths in a cancer tissue can result in a more aggressive and chemotherapy resistant cancer tissue in some cases because the elevated ROS can kill the sensitive cancer cells though the aggressive ones can cope with the same amount of ROS. Conversely, the combination therapy that is employed by using redox-active

molecules and conventional treatment strategies is considered as a rational option to overcome chemotherapy resistance. Many anticancer drugs, for example, kill the cancer cells by activation of ROS-dependent cell death pathways though the cancer cells develop resistance towards them by activating the antioxidant systems. The antioxidant system inhibitors can be therefore employed to evade the cancer cell resistance. It should be also noted that the anticancer drugs that kill the cancer cells by ROS-dependent cell death pathways are preferred more to perform a selective treatment because the cancer cells are regarded as more sensitive than health counterparts due to the lack of redox homeostasis. On the other hand, the antioxidant dietary substances are generally considered as beneficial to preclude carcinogenesis and many scientists have recommended people to include these substances in their daily diets to keep them away from the cancer risks. However, some papers have displayed the link between the increased cancer risks and some dietary antioxidants such as carotene, vitamin A, and vitamin E. Thus, the activity mechanisms of the dietary antioxidants need to be extensively investigated to understand well their benefits and harms, and give recommendations to the people who would like to keep themselves healthy. Although the consequences of the enhanced ROS production in the cells seem to be not predictable because of the dependence on many different factors, the ROS-mediated treatment strategies such as photodynamic therapy and sonodynamic therapy seem to be promising because of their non-invasive features. The relationship between microRNAs and ROS was not extensively discussed to keep concise in this chapter, but this relationship seems to be substantial for the regulation of many intracellular signals and epigenetic changes. Moreover, the extensive investigations of ROS-mediated effects of drugs on epigenetic mechanisms seem to be quite beneficial because ROS are known as the effective factors on epigenetic regulations and aberrations that play crucial roles in cancer heterogeneity and carcinogenesis. Consequently, ROS are considered as a double-edged sword and the effects of the edges should be therefore extensively investigated by developing interdisciplinary projects and collaborations to understand well and employ efficiently this sword as a weapon, target, or mediator in the cancer treatment modalities.

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