Molecular Mechanism of Oxidative Stress in Cancer and Its Therapeutics

170

Ashif Iqubal and Syed Ehtaishamul Haque

Contents

Abstract

Cancer is one of the major causes of death worldwide, and persistent oxidative stress is considered as a proactive contributor to its pathogenesis. Oxidative stress is mainly identified by an increased level of reactive oxygen species (ROS) and reduced antioxidant defense system. Various exogenous and endogenous factors and altered metabolic processes produce different types of ROS that cause DNA damage, mutation, production of pro-carcinogens, and induced programmed cell death. These ROS alter various signaling pathways such as NF-kB, Nrf2, Akt/PI3K/mTOR, MAPK, p53, etc. which further regulate other downstream signaling molecules such as CREB, c-Myc, c-Jun, c-fos, etc., leading to initiation

A. Iqubal · S. E. Haque (✉)
Department of Pharmacology, School of Pharmaceutical Education and Research, Jamia Hamdard, New Delhi, India

S. Chakraborti (ed.), *Handbook of Oxidative Stress in Cancer: Therapeutic Aspects*,
https://doi.org/10.1007/978-981-16-5422-0_150

and progression of tumorigenesis. ROS also regulate the mechanism of angiogenesis and ensure the growth, survival, as well as invasion of tumor cells. Apart from cross talk between different signaling molecules, ROS and miRNAs work in close proximity and participate in tumorigenesis. Looking into the pro-carcinogenic role of ROS, many natural and synthetic antioxidants have been explored for their anticancer effect. These antioxidants not only abrogate the progression, proliferation, and invasion of tumors but also protect the healthy cells from the deleterious effect of anticancer drugs. However, another hypothesis justifies the anticancer effect of ROS and its need to increase the sensitivity of tumor cells toward the anticancer drug. Thus, it can be said that ROS act as a double-edged sword, and a fine line or boundary exists between the use of antioxidant drug and the need of ROS for management and treatment of cancer.

Keywords

Oxidative stress · Signaling pathways · Oncogene · miRNA · Metastasis and Antioxidants

Introduction

Oxidative stress refers to an imbalance between the production of reactive oxygen species (ROS) and the level of the antioxidant defense system, triggered by various environmental factors, toxins, UV radiation, or physical-mechanical injury (Prasad and Srivastava 2020). Any imbalance between endogenous antioxidants and ROS leads to various disorders like neurological, cardiovascular, and cancer. Common ROS includes hydrogen peroxide (H_2O_2), hydroxyl free radical (OH^-), and oxygen free radicals (O_2^-). ROS, along with the reactive nitrogen species (RNS) such as nitric oxide (NO^-), are produced during various metabolic processes and react further to produce NOO^- (Chierto et al. 2020). These NOO^- are then converted into peroxynitrous acid followed by conversion into OH^- and NO_2^-. Together, these mediators induce significant oxidative stress and carry out the pathogenesis of cancer via modulating various signaling molecules (Chierto et al. 2020; Prasad and Srivastava 2020). ROS regulate the inflammatory pathways; damage DNA, RNA, lipids, and proteins; and evade DNA repair mechanisms. Additionally, it is well-known that plasma membrane contains an abundance of polyunsaturated fatty acids which becomes target of ROS. ROS acts on these fatty acids and liberate malonaldehyde (MDA), conjugated dienes, and peroxide radicals that further trigger various pathological pathways. However, to make sure that level of ROS falls under the normal level, the body's endogenous antioxidant defense systems such as superoxide dismutase (SOD), catalase (CAT), glutathione (GSH), glutathione peroxidase (GPx), and thioredoxin (TRX) together keep a check on the concentration of ROS (Chierto et al. 2020; Zhang et al. 2020). Under normal physiological condition, SOD coverts superoxide anion to H_2O_2 which is further converted into H_2O and O_2 by GPx and CAT. Additionally, for the maintenance of

adequate level of antioxidant defense systems, nuclear factor erythroid 2-related factor 2 (Nrf2) plays a critical role. Nrf2 belongs to the Cap'n'Collar (CNC) subfamily and is composed of seven functional domains Neh (Paunkov et al. 2019). Out of the seven functional domains, Neh2 domain contains two functionally important motifs, DLG and ETGE, keap Nrf2 in association with its negative downregulator, Keap1, in the cytoplasm. Keap1 is considered as the substrate adaptor for cullin-based E3 ubiquitin ligase and renders the transcriptional activity of Nrf2 via ubiquitination and proteasomal degradation during the normal physiological condition (Paunkov et al. 2019). Under the stressful condition, Nrf2 dissociates from Keap1 by phosphorylation followed by thiol modification, and the dissociated Nrf2 then translocates into the nucleus. This leads to the formation of a heterodimer with Maf protein, activates antioxidant response element (ARE), and causes transcription of phase II detoxifying enzymes and antioxidant proteins like GSH, SOD, HO-1, etc. (Iqubal et al. 2019b). Thus, increased oxidative stress is a key for multifactorial pathogenesis, whereas Nrf2 regulates the level of ROS via promoting transcription of various antioxidant enzymes.

Production of ROS and Status of Endogenous Antioxidant in Tumorigenesis

During the initial stage of tumor initiations, several factors come into play causing oxidative stress that leads to activation of pro-oncogenes and inhibition of tumor suppressor genes. Tumor necrosis factor-alpha (TNF-α), transforming growth factor-beta (TGF-β), epidermal growth factor (EGF), and platelet-derived growth factor (PDGF) have been well documented to inhibit the nuclear translocation of Nrf2 and initiate the production of ROS and oxidative stress (Kashyap et al. 2019; Paunkov et al. 2019). Apart from the aforementioned sources of oxidative stress, K-ras and Rac-1 (downstream signaling molecule for growth factor receptors) are other contributors to the production of ROS. Among various ROS, superoxide anions are produced in maximum amount as a by-product during oxidative phosphorylation, and mitochondrial complex I and III are the site of their production (Kashyap et al. 2019). Under normal condition, a small amount of O_2^- is produced and released into the mitochondrial matrix, where it gets dismutized by SOD. However, during tumorigenesis, elevated level of TNF-α, TGF-β, EGF, and PDGF causes mitochondrial dysfunction leading to release of an abundance of O_2^- into the cytoplasm, and reduces the level of SOD, thus ultimately increasing oxidative stress (Iqubal et al. 2019c). Increased oxidative stress also leads to DNA mutation and other carcinogenic events, which is taken care of by endogenous antioxidants, as shown in Fig. 1. Among various antioxidants, SOD is present in association with metallic cofactors (Mn-SOD, Zn/Fe/Cu-SOD) and play a pivotal role in cellular defense (Storz 2013). These isoforms of SOD are present in the different cellular compartments, as Mn is present in the mitochondrial matrix, Zn/Cu-SOD is present in the cytoplasm, whereas CAT (convert H_2O_2 into H_2O and O_2) is present in the cytoplasm and peroxisome (Storz 2013). GSH is another important antioxidant defense system that

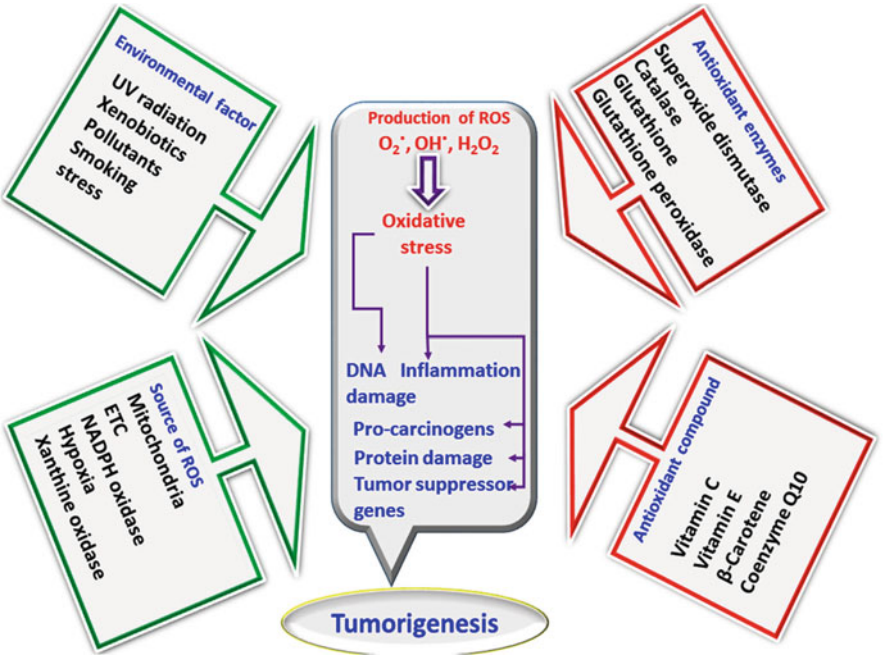

Fig. 1 Various environmental and endogenous sources of ROS (depicted in the green box) and its involvement in tumorigenesis. Various antioxidant enzymes and antioxidant compounds that inhibit or scavenge these ROS and act as anticancer agents are depicted in red box

protects the cellular component from increased ROS. It gets oxidized to GSSG (oxidized glutathione), which is further upon by glutathione reductase and reconverted into GSH (Kashyap et al. 2019). Thus, dysregulation of these antioxidant defense systems directly increases the level of ROS and aggravates the event of tumorigenesis, as shown in Fig. 1.

Association Between Oxidative Stress and Carcinogenesis

ROS has been reported to regulate and promote the level of several oncogenes and downregulate the level of tumor suppressor genes. Produced ROS and various by-products cause damage to DNA, induce mutation, and initiate the cascade of tumorigenesis. Mechanistically, ROS causes DNA oxidation and renders its repair mechanism leading to the faulty insertion or modification of nucleotides and the production of pro-carcinogens (Kashyap et al. 2019). Additionally, it has been observed that extensive production of ROS inhibits the methylation of DNA and further contributes to the pathogenesis of cancer. Published evidence has also shown the role of cellular proliferation, migration, and invasion of tumors via modulating various signaling pathways. Some of them are mitogen-activated protein kinase

(MAPK), phosphoinositide 3-kinases/protein kinase B (PI3K/Akt), mammalian target of rapamycin (mTOR), matrix metalloproteinases (MMPs), hypoxia-inducible factor 1-alpha (HIF-α), Wnt, nuclear Factor kappa-light-chain-enhancer of activated B cells (NF-kB), and vascular endothelial growth factor (VEGF) (Liou and Storz 2010). It is well-known that for the survival of cancerous cells, regular blood supply and adduct of oxygen supply are needed, and these attributes are manifested by dysregulated HIF-alpha and VEGF (Fang et al. 2007). Increased ROS has been reported to cause activation of VGEF and inhibition of HIF-alpha along with the elevation in pro-oncogenes (Fang et al. 2007). Pyruvate kinase M2 is one of the decisive modulators of the glycolytic pathway in cancer cells (Yang and Lu 2013). Its optimum level provides clearance of ROS from cancerous cells, whereas during stressed condition, it gets deactivated and deviates the glycolytic pathway toward the pentose phosphate pathway, needed for the survival of cancerous cells (Yang and Lu 2013). Further, it is established that ROS regulates cell proliferation machinery via activation of MAPK signaling pathway through stimulation of different factor receptors and inactivation of MAPK phosphatase (Kashyap et al. 2019). This activated MAPK further activates the c-Jun N-terminal kinase (JNK) signaling pathways that also contribute to tumor initiation, progression, and proliferation (Storz 2013). ROS not only regulate the proliferative phase but also positively regulate the tumor invasion, migration, and metastasis via modulation of mTOR, NF-kB, JAK-STAT, and PI3K/Akt pathways and worsen the clinical outcomes (Storz 2013).

Signaling Pathways Involved in ROS-Mediated Tumorigenesis

It seems much clear that increased oxidative stress and reduced antioxidants lead to tumorigenesis via regulating multiple signaling pathways such as MAPK/ERK1/2, Akt/PI3K, and NK-kB. These pathways further regulate their downstream signaling molecules leading to tumor initiation, progression, proliferation, and invasion.

ROS- and NF-kB-Mediated Tumorigenesis

NF-kB, a transcription factor, plays an important role in tumor initiation, progression, proliferation and invasion. NF-kB, in its normal condition, is attached with the nuclear factor of kappa light polypeptide gene enhancer in B-cell inhibitor (IkB) that restricts its entry into the nucleus (Iqubal et al. 2019b). For the nuclear translocation of NF-kB, IkB needs to be phosphorylated by IkB kinase (IKK) (Dolcet et al. 2005). ROS has been reported to activate IKK which in turn phosphorylate IkB and stimulate nuclear translocation of NF-kB. Additionally, a negative correlation was found between reduced Nrf2 and increased nuclear translocation of NF-kB (Dolcet et al. 2005). Once NF-kB is translocated, it increases the transcription of various inflammatory cytokines such as TNF-α, ILs, and ROS and participates in the formation of NLRP3 inflammasome (Nguyen et al. 2019; Teng et al. 2020). These

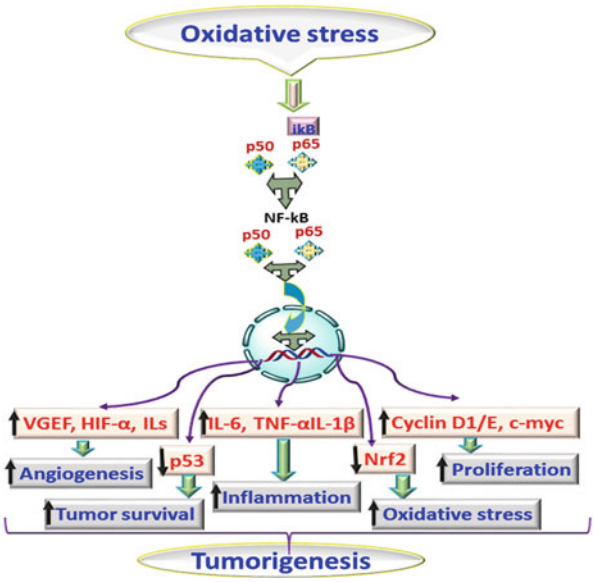

Fig. 2 The role NF-kB in tumorigenesis under the influence of ROS. Under normal physiological conditions, NF-kB remains in the cytoplasm in association with IkB. However, increased oxidative stress phosphorylates IkB and enables nuclear translocation of NF-kB. This leads to the increased level of VGEF, HIF-α, ILs, TNF-α, cyclin D1/E, and c-Myc and reduced level of antioxidant transcription factor (Nrf2) and tumor suppressor gene (p53), leading to angiogenesis, inflammation, proliferation, and survival of tumor cells

attributes together participate in the further production of ROS, cellular proliferation, and endothelial cell (EC) migration, degradation, angiogenesis, and tumorigenesis, as shown in Fig. 2.

ROS- and MAPK/ERK ½-Mediated Tumorigenesis

MAPK or ERK ½ pathway is also referred to as Ras-Raf-MEK-ERK pathways. Before going to discuss the association between ROS- and MAPK/ERK ½-mediated tumorigenesis, it is important to understand the general working principle of MAPK in tumorigenesis so that its association with ROS can be better understood. In response to the stimulus, Ras (a member of small GTPase) gets activated, which then potentiates the kinase activity of RAF or MAP3K (Small GTPase enzyme), leading to phosphorylation and activation of MEK or MAP2K (an enzyme responsible for MAPK activation) that finally activate MAPK (Je et al. 2004). Once MAPK is activated, it carries our phosphorylation and activation of many downstream signaling molecules and genes such as cAMP response element-binding protein (CREB) and c-Myc, needed for cell cycle progression and proliferation (Dunn et al. 2005; Tang et al. 2016). Cyclin D, with its two complexes (Cdk4 and Cdk6), is a critical target that gets phosphorylated by MAPK (Tang et al. 2016). Activation of Cdk4/6 causes the destabilization of retinoblastoma protein (Rb). Rb under normal condition is attached to E2F (transcription factor) and regulates the controlled transition of the G1 phase of the cell cycle to S phase. Once Rb gets destabilized by Cdk4/6 and E2F gets activated, the uncontrolled cell cycle transition

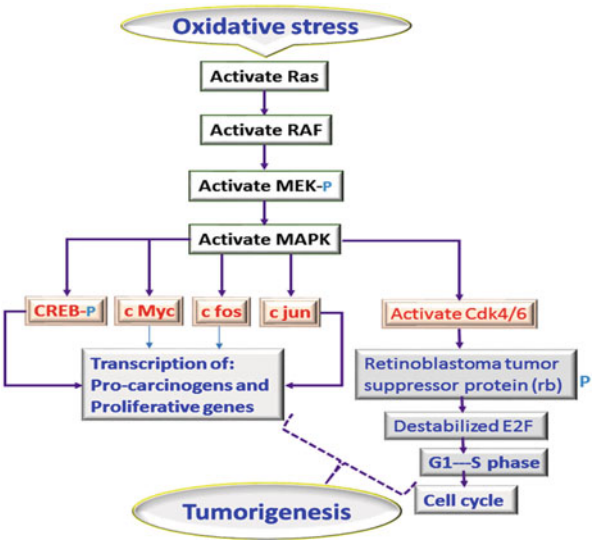

Fig. 3 The role of MAPK pathways in the pathogenesis of cancer. Increased oxidative stress activates Ras and its subsequent downstream signaling molecules such as RAF, MEK, and MAPK. Activated MAPK carry out the transcription of various pro-oncogenes such as CERB, c-Myc, c-fos, and c-Jun and activate Cdk4/6 leading to the production of carcinogenic, proliferative proteins. Activated Cdk4/6 causes phosphorylation and inactivation of Rb that destabilize E2F and allow irreversible entry of G1 to S phase leading to tumorigenesis

from G1 to S phase occurs (Li et al. 2019). Additionally, destabilized Rb and activated E2F cause recruitment of CDK2 and cyclin E, which together block the decisive mechanism of S phase to return back into G1 phase, and thus, uncontrolled cell division and proliferation continue as shown in Fig. 3 (Li et al. 2019). ROS play a critical role in all aforementioned mechanism. ROS causes activation of Ras via modification at the cytosine-118 position and inhibits the activity of negative regulator (MKP3) of MAPK, leading to a persistent increased in MAPK-mediated cell proliferation (Wang et al. 2017), as shown in Fig. 3.

ROS- and Akt/PI3K/mTOR-Mediated Tumorigenesis

Akt/PI3K/mTOR is another important signaling pathway that regulates tumor initiation, progression, proliferation, and survival. Under the influence of increased oxidative stress, PI3K causes phosphorylation, activation, and recruitment of Akt at the surface of the cell membrane (Hoxhaj and Manning 2019). Once Akt is activated, it further causes activation of CREB and mTOR and inhibition of p27 along with the recruitment of "forkhead box or FOXO" in the cytoplasm (Braglia et al. 2020; Li et al. 2015). CERB is a transcription factor that gets attached to the specific sequence of DNA, i.e., cAMP response elements (CRE), and carries out the transcription of pro-oncogenes (Daniel et al. 2017). Akt, once gets activated,

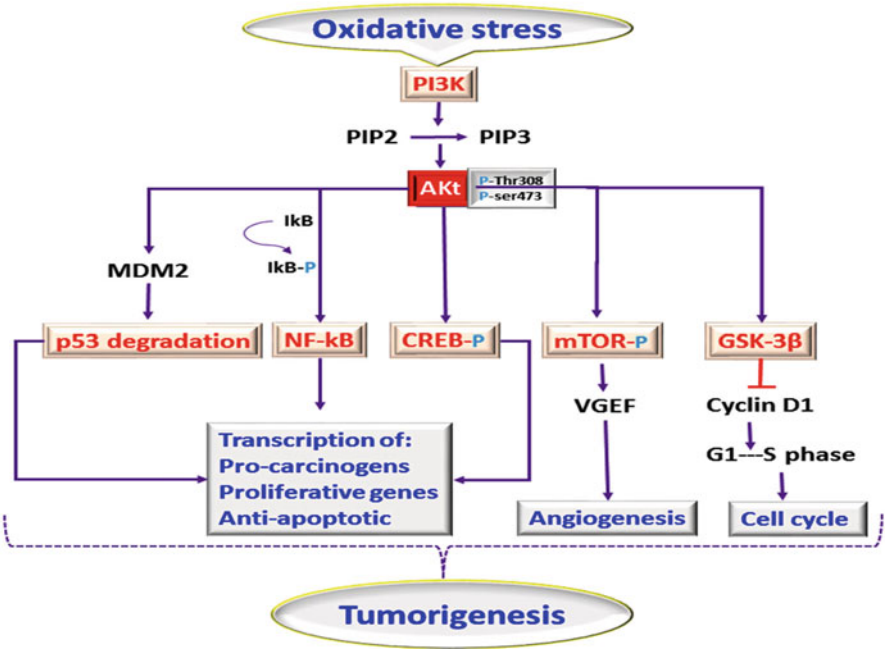

Fig. 4 The role of Akt/PI3k/mTOR pathway in tumorigenesis. Increased oxidative stress causes phosphorylation and activation of Akt at Thr-308 and Ser-473 residues by PI3K. This activation further increases the transcription and level of various pro-carcinogens responsible for proliferation and tumor survival. Activated Akt further phosphorylates GSK-3β that inhibit the proteasomal degradation of cyclin D1, leading to the transition of tumor cells from G1 to S phase

participates in tumor survival and invasion, whereas activated mTOR regulates cell proliferation, inhibits autophagy, increases the level of HIF-α, and promotes angiogenesis (Braglia et al. 2020; Fang et al. 2007). FOXO is a pro-apoptotic protein and activated Akt causes its phosphorylation and renders its apoptotic property leading to tumor survival as shown in Fig. 4 (Daniel et al. 2017; Pei et al. 2019).

ROS- and Tumor-Suppressive Genes

Tumor-suppressive genes are considered as protective genes as their elevated levels suppress the initiation and progression of cancerous cells (Hong et al. 2014). Nrf2, a well-known transcription factor for antioxidants, gets downregulated in the presence of elevated ROS (Paunkov et al. 2019). Downregulation of Nrf2 not only reduces the level of phase II antioxidants but also acts as a positive modulator of several inflammatory and immunogenic genes that participate in carcinogenesis (Paunkov et al. 2019). Breast cancer gene-1 (BRCA-1) is responsible for the dissociation of Nrf2-Keap-1 complex and its nuclear translocation, thereby increasing the level of antioxidants and reducing the level of ROS (Wang et al. 2013). However, in

case of breast cancer, downregulation and mutation of this BRCA-1 gene were found to be positively correlated with oxidative stress (Wang et al. 2013). Ras gene family, mainly k-ras, plays an important role in the event of tumorigenesis via point mutation and activation of various growth factor receptors (Shaw et al. 2011). It was found that point mutation caused by the k-ras induces severe oxidative stress that further causes DNA damage and other attributes of carcinogenesis (Shaw et al. 2011). Increased ROS has also been found to upregulate the pro-oncogenes such as RAF, MEK 1/2, cyclin D, cyclin E, etc. and downregulate tumor-suppressive genes such as p53, $p21^{CIP1}$, and PTEN (Prasad and Srivastava 2020). Sirtuin (Sirt) is a well-known protein with potent HDAC activity and positively correlated with metabolism, aging, and longevity (Chen et al. 2017). Among various Sirt, Sirt3 has been the extensively explored protein and is found to be tumor suppressive. However, the reduced level was found to be associated with diminished oxidative phosphorylation, increased level of ROS, and progression of tumorigenesis (Li et al. 2018). Therefore, it can be concluded that increased oxidative stress is directly associated with the downregulation of tumor-suppressive genes.

ROS and Angiogenesis

When tumor cell starts growing, demand for adequate energy and blood supply increases. Angiogenesis is the mechanism for the production of new blood vessels, and during tumorigenesis, multiple fold increase in angiogenesis has been reported. Accumulating shreds of evidences have shown a direct relationship between oxidative stress and increased angiogenesis (Fang et al. 2007). During tumor initiation and proliferation, metabolic rate increases and so the production of ROS increases, which then induces the transcription of various angiogenic genes and their modulators. VGEF is the primary mediator of angiogenesis. ROS generating machinery such as NADPH oxidase, mitochondrial dysfunction, downregulated Nrf2, and reduced antioxidant enzymes increase the production of ROS, leading to activation of PI3K and epidermal growth factor (EGF) and inhibition of PTEN (Carmeliet and Jain 2000). Activated PI3K further activates Akt that causes the recruitment of mTOR and HIFα, whereas activated EGF stimulates k-ras which in turn activate MAPK that also participates in the recruitment of HIF-α. HIF-α then increases the level of VGEF that binds with the VGEF receptor and carries out the event of angiogenesis (Khromova et al. 2009). Increased ROS also elevates the level of various MMPs that help in EC degradation, whereas by-products of oxidized lipid bind with Toll-like receptor, activate NF-kB, and increase the level of pro-inflammatory cytokines leading to angiogenesis (Mori et al. 2019).

ROS and Tumor Initiation and Proliferation

Increased oxidative stress or various ROS cause damage to macromolecules such as lipid, proteins, amino acids and DNA. Damage to DNA directly leads to tumor

initiation (Prasad and Srivastava 2020). Hydroxyl ion plays a critical role in tumor initiation as it is highly diffusible, easily oxidizes nucleotide base-pairs, and forms DNA adducts (Prasad and Srivastava 2020). The presence of such DNA adducts enables the cell to escape apoptotic pathways, gets proliferated, and may transform into a tumor cell. ROS-induced oxidation of proteins and amino acids has direct implications in the inactivation of tumor-suppressive genes (PTEN) and activation of pro-oncogenes (k-ras) that trigger the initiation and progression of tumorigenesis (Storz 2013). Followed by the tumor initiation, tumor cells enter into the phase of proliferation where different signaling molecules, under the influence of increased ROS, drive rapid cell division and bypass programmed cell death. Increased oxidative stress regulates proliferative pathways such as ERK ½, Akt/PI3K, as well as protein kinase C, TGF-β, E-cadherin, and MMPs leading to uncontrolled cell division (Boutwell and Sivak 1973).

ROS and Tumor Survival and Invasion

It is important for tumor cells that once it has undergone the proliferative phase, it can assure survival. Tumor survival or bypass of programmed cell death is primarily achieved via the recruitment of anti-apoptotic proteins (Feitelson et al. 2015). One of the well-studied pathways for recruitment of the anti-apoptotic gene is ROS-mediated activation and nuclear translocation of NF-kB that causes suppression of p53 and thus promotes tumor survival. Akt/PI3K pathway is another important cell survival pathway (Feitelson et al. 2015; Luo et al. 2017). Increased level of Akt participates in tumor survival via inactivation of pro-apoptotic proteins such as FOXO, Bad, and Bax. Tumor cell survival pathways and cell invasion go simultaneously, and MMPs play a critical role in cellular invasion along with other signaling molecules (Li et al. 2015; Li et al. 2018). MMPs, basically MMP-3, cause endothelial cell degradation and damage to collagen laminin and fibronectin leading to tumor invasion. Action of MMP3 is further regulated by reduced E-cadherin and this is achieved by increased ROS. Apart from the role of MMP3, Wnt, MAPKs, and PKC also play a pivotal role in epithelial-mesenchymal transition and help in tumor invasion (Huang and Xin 2018).

ROS and miRNA

miRNAs are small (22–24) nucleotides present in plants, viruses, and animals. miRNAs are noncoding RNA molecules that control and regulate various gene activities via translational repression or RNA silencing. There are more than 1000 miRNAs that exist in the human genome (Iqbal et al. 2019). These miRNAs regulate various signaling pathways and play a pivotal role in the initiation and progression of tumor cells (Iqbal et al. 2019). Studies have shown a direct relationship between oxidative stress and the functioning of miRNA that causes tumorigenesis. Significant elevation in the level of miR-135a and miR-135b was

found in the in vitro model of cancer, and treatment with antioxidants causes downregulation of these miRNAs and shows anticancer effect (Aggarwal et al. 2019; Reid et al. 2013). Exposure to oxidative stress has also been reported with increased expression of miR-200 s, miR-182, and miR-34 s (Aggarwal et al. 2019; Liu et al. 2015). However, reduced expression of miR-145, miR-128, miR-125b, and miR-199a has been observed in hepatocellular carcinoma when exposed to oxidative stress (Aggarwal et al. 2019; He et al. 2012). These miRNAs have also been reported to be the regulator of genes that control expression of various antioxidant enzymes. miR-9 was identified as a suppressor of *GOT1* gene that regulates ferroptosis and thus promotes tumor survival. miR-23b has been reported to cause downregulation of the proline oxidase gene (tumor suppressor gene) and is involved in tumorigenesis (Zhang et al. 2018).

ROS, Mitochondrial Dysfunction, and Tumorigenesis

Mitochondria is an important organelle that plays a diverse biological function in all eukaryotic cells. Under normal physiological conditions, glycolysis and Krebs's cycle that together produce 36 ATP are used for various physiological conditions. However, during tumorigenesis involving mitochondrial dysfunction, the Warburg effect is observed (Moro 2019). In Warburg effect, glycolytic pathway is diverted toward the lactate pathway that creates an acidic environment, favoring tumorigenesis. Mitochondrial dysfunction has been well-documented as an important contributor to tumorigenesis via modulation of biogenesis, mitophagy, fission-fusion dynamic, apoptosis, calcium dysregulation, and generation of ROS (Moro 2019). Peroxisome proliferator-activator receptor gamma coactivator-1 alpha (PGC-1α) is considered as the master regulator of mitochondrial biogenesis. In normal condition, PGC-1α also act as a tumor suppressor and promote apoptosis under the influence of healthy mitochondrial signals (Bost and Kaminski 2019). However, with mitochondrial dysfunctions, PGC-1α evades its apoptotic property and promotes tumor cell survival via deactivation of pro-apoptotic proteins (Bost and Kaminski 2019). Apart from downregulated PGC-1α, mitochondrial dysfunction also promotes the activation of c-Myc and mTOR that further participate in tumorigenesis. Mitophagy is a process of clearing damaged or dysfunction mitochondria but during carcinogenesis, this process gets altered (Moro 2019). PTEN-induced putative kinase-1 (PINK-1) or Parkin pathway is a major player of mitophagy, but during tumorigenesis, downregulation of PTEN has been observed under the influence of ROS-induced mitochondrial dysfunction. Downregulated PTEN further leads to increased activity of Akt/PI3K pathway and promotes tumor cell survival (Hoxhaj and Manning 2019). Mitochondrial fusion and fission are important functional dynamics that regulate mitochondrial functions, and imbalance in these dynamics participates in tumorigenesis. Smooth fission ensures the equivalent distribution of daughter mitochondrial cells for mitosis, and this is achieved by the recruitment of Dynamin-1-like protein (Drp1) to the outer mitochondrial membrane (Moro 2019). Fusion is also an important dynamic, important for the oxidative metabolic process and balanced

entry into S-phase and mediated by mitofusin-1 (Mfn-1), mitofusin-2 (Mfn-2), and optic atrophy-1 (Opa-1) (Moro 2019). However, under the influence of increased ROS, studies have shown the involvement of fusion in delaying cytochrome c and apoptosis, leading to tumor survival (Moro 2019). Apart from the aforementioned attributes, increased cytosolic Ca^{2+} is another consequence of mitochondrial dysfunction. The endoplasmic reticulum is the storehouse of Ca^{2+} and its release into the cytoplasm is regulated by the inositol trisphosphate receptor (IP3R), whereas reuptake is primarily regulated by voltage-dependent anion channel (VDAC) and mitochondrial calcium uniporter (MCU) (Wang et al. 2018). However, mitochondrial dysfunction impairs VDAC and MCU, resulting into increased cytosolic Ca^{2+} levels. Increased cytosolic Ca^{2+} exerts significant oxidative stress and causes activation of calpain proteases and Ca^{2+}/calmodulin-dependent protein kinases that activate MAPK and promote nuclear translocation of NF-kB, leading to persistent inflammation, evading of apoptosis, angiogenesis, and the overall event of tumorigenesis (Moro 2019). Thus, there occurs substantial evidence for the role of mitochondrial dysfunction and ROS in tumorigenesis via altering biogenesis, mitophagy, fission-fusion dynamics, and increased cytosolic Ca^{2+}(Moro 2019; Wang et al. 2018).

Antioxidant and Prooxidant: Dichotomous Approach Toward ROS in Tumorigenesis

From the above-discussed sections, it is much evident that ROS is a major player in tumorigenesis (Prasad and Srivastava 2020). Additionally, use of anticancer drugs also induces significant oxidative stress and exerts a deleterious effect on healthier cells/organs (Iqubal et al. 2019c). Therefore, use of antioxidant will showcase a potent anticancer effect and also protects the body from the side effect of anticancer drugs. Use of antioxidants has been reported to prevent the progression of cancer (Iqubal et al. 2019c). Use of antioxidants such as flavonoids or polyphenols maintains the equilibrium between excessively produced ROS and endogenously produced antioxidant detoxifying enzymes. In our lab, we explored the cardioprotective potency of naturally occurring molecules against anticancer drug-induced cardiotoxicity (Iqubal et al. 2019b; Khan et al. 2014). We observed that the use of doxorubicin and cyclophosphamide induces significant oxidative stress and causes cardiotoxic manifestations, whereas the use of naturally occurring antioxidant drugs exerts potent cardioprotective properties (Iqubal et al. 2019a; Iqubal et al. 2019b; Khan et al. 2014). Additionally, Nrf2 gets downregulated during the progression of cancer, and the use of Nrf2 agonist or Nrf2 activator exerts significant antitumor effect (Wang et al. 2013). Similarly, quercetin has been explored for its antitumor potential because of its antioxidant properties (Zhang et al. 2020). However, there also exists a controversial role of antioxidant therapy in cancer. Use of exogenous antioxidant maintains the balance of antioxidant level, reduces ROS level, and exerts antioxidant effect. However, there are growing shreds of evidence that show increased sensitization of tumor cells toward chemotherapy by ROS and the use of these antioxidants also increases the level of ROS and potentiates the chemotherapeutic effect (prooxidant therapeutics) (Ahmad et al. 2005; Zhang et al. 2020). It

always remains a challenge to select antioxidant or prooxidant therapy which is one of the major limitations in the success of such therapy in cancer. Several anticancer drugs such as arsenic trioxide, doxorubicin, vincristine, and 5-FU increase the level of ROS and exert a potent antitumor effect. Radiation therapy, when combined with arsenic trioxide, exhibits a superior antitumor effect as compared to individual therapy, and the reason was found to be increased ROS production (Chiu et al. 2012). Piperlongumine is a naturally active molecule reported to inhibit rapidly dividing breast cancer cells via increasing ROS levels and via stimulation of apoptotic and other signaling pathways (Raj et al. 2011). Interestingly, no significant toxic effect was observed on healthy cells (Raj et al. 2011). This gives a positive indication that rationally designed drugs can be a therapeutic approach to exert prooxidant effect without damaging healthy cells. However, more detailed studies are needed to understand the pro-oncogenic and anti-oncogenic effect of ROS along with the understanding of selective target that can be used for antioxidant or prooxidant effects of drugs. There also exists a need for clear-cut classification of types of tumors that should be exposed to antioxidant or prooxidant therapy along with a clear understanding of the amount of ROS needed for the antitumor effect.

Conclusion

There are a number of factors responsible but ROS plays the most vital role because of its multifactorial pathogenic role. Increased level of ROS, reduced antioxidant enzymes, and reduced Nrf2 level not only trigger this event but actively participate in tumor initiation, progression, and invasion. This involves various pathways such as NF-kB, MAPK/ERK ½, Akt/PI3K/mTOR, and p53 leading to angiogenesis as well as other tumorigenic events. Apart from these signaling pathways, ROS also alters various mRNA and causes mitochondrial dysfunction that further alters bioenergetic mechanism-induced ER stress and causes mitophagy, fission-fusion dynamic, and calcium dysregulation that eventually causes tumorigenesis. Thus, looking into the pathological role of ROS, various studies have shown the antitumor effect of antioxidants such as curcumin, resveratrol, quercetin, etc. Other studies have conversely shown the anticancer effect of ROS via its chemosensitizing and direct antitumor effect. Thus, there occurs dichotomous role of ROS, and it's a matter of debate and research that at what circumstances antioxidant or ROS should be used for therapeutic purpose. Additionally, research are being conducted to find clinically active antioxidant drugs and threshold level of ROS to act as antitumor agent with better safety profile.

References

Aggarwal V et al (2019) Role of reactive oxygen species in cancer progression: molecular mechanisms and recent advancements. Biomol Ther 9:735

Ahmad A, Syed FA, Singh S, Hadi S (2005) Prooxidant activity of resveratrol in the presence of copper ions: mutagenicity in plasmid DNA. Toxicol Lett 159:1–12

Bost F, Kaminski L (2019) The metabolic modulator PGC-1α in cancer. Am J Cancer Res 9:198
Boutwell RK, Sivak A (1973) The function and mechanism of promoters of carcinogenesis. CRC Crit Rev Toxicol 2:419–444
Braglia L, Zavatti M, Vinceti M, Martelli AM, Marmiroli S (2020) Deregulated PTEN/PI3K/AKT/mTOR signaling in prostate cancer: still a potential druggable target? Biochim Biophys Acta (BBA) Mol Cell Res:118731
Carmeliet P, Jain RK (2000) Angiogenesis in cancer and other diseases. Nature 407:249–257
Chen J, Wang A, Chen Q (2017) SirT3 and p53 deacetylation in aging and cancer. J Cell Physiol 232:2308–2311
Chierto E, Cristinziano G, Sapone F, Meffre D, Jafarian-Tehrani M (2020) Effect of Etazolate on ROS production after tBHP-induced oxidative stress in oligodendroglial 158 N cell line. React Oxyg Species 9:22-35–22-35
Chiu H-W, Chen Y-A, Ho S-Y, Wang Y-J (2012) Arsenic trioxide enhances the radiation sensitivity of androgen-dependent and-independent human prostate cancer cells. PLoS One:7
Daniel P et al (2017) PI3K activation in neural stem cells drives tumorigenesis which can be suppressed by targeting. CREB bioRxiv:143388
Dolcet X, Llobet D, Pallares J, Matias-Guiu X (2005) NF-kB in development and progression of human cancer. Virchows Arch 446:475–482
Dunn KL, Espino PS, Drobic B, He S, Davie JR (2005) The Ras-MAPK signal transduction pathway, cancer and chromatin remodeling. Biochem Cell Biol 83:1–14
Fang J, Zhou Q, Liu L-Z, Xia C, Hu X, Shi X, Jiang B-H (2007) Apigenin inhibits tumor angiogenesis through decreasing HIF-1α and VEGF expression. Carcinogenesis 28:858–864
Feitelson MA et al (2015) Sustained proliferation in cancer: mechanisms and novel therapeutic targets. In: Seminars in cancer biology. Elsevier, pp S25–S54
He J et al (2012) Reactive oxygen species regulate ERBB2 and ERBB3 expression via miR-199a/125b and DNA methylation. EMBO Rep 13:1116–1122
Hong B, van den Heuvel PJ, Prabhu V, Zhang S, El-Deiry W (2014) Targeting tumor suppressor p53 for cancer therapy: strategies, challenges and opportunities. Curr Drug Targets 15:80–89
Hoxhaj G, Manning BD (2019) The PI3K–AKT network at the interface of oncogenic signalling and cancer metabolism. Nat Rev Cancer:1–15
Huang M, Xin W (2018) Matrine inhibiting pancreatic cells epithelial-mesenchymal transition and invasion through ROS/NF-κB/MMPs pathway. Life Sci 192:55–61
Iqbal MA, Arora S, Prakasam G, Calin GA, Syed MA (2019) MicroRNA in lung cancer: role, mechanisms, pathways and therapeutic relevance. Mol Asp Med 70:3–20
Iqubal A et al (2019a) Molecular mechanism involved in cyclophosphamide-induced cardiotoxicity: old drug with a new vision. Life Sci 218:112–131
Iqubal A et al (2019b) Nerolidol attenuates cyclophosphamide-induced cardiac inflammation, apoptosis and fibrosis in Swiss Albino mice. Eur J Pharmacol 863:172666
Iqubal A, Sharma S, Najmi AK, Syed MA, Ali J, Alam MM, Haque SE (2019c) Nerolidol ameliorates cyclophosphamide-induced oxidative stress, neuroinflammation and cognitive dysfunction: plausible role of Nrf2 and NF-κB. Life Sci 236:116867
Je JH, Lee JY, Jung KJ, Sung B, Go EK, Yu BP, Chung HY (2004) NF-κB activation mechanism of 4-hydroxyhexenal via NIK/IKK and p38 MAPK pathway. FEBS Lett 566:183–189
Kashyap D, Tuli HS, Sak K, Garg VK, Goel N, Punia S, Chaudhary A (2019) Role of reactive oxygen species in cancer progression. Curr Pharmacol Rep 5:79–86
Khan G, Haque SE, Anwer T, Ahsan MN, Safhi MM, Alam M (2014) Cardioprotective effect of green tea extract on doxorubicin-induced cardiotoxicity in rats. Acta Pol Pharm 71:861–868
Khromova N, Kopnin P, Stepanova E, Agapova L, Kopnin B (2009) p53 hot-spot mutants increase tumor vascularization via ROS-mediated activation of the HIF1/VEGF-A pathway. Cancer Lett 276:143–151
Li A, Wang J, Wu M, Zhang X, Zhang H (2015) The inhibition of activated hepatic stellate cells proliferation by arctigenin through G0/G1 phase cell cycle arrest: persistent p27Kip1 induction by interfering with PI3K/Akt/FOXO3a signaling pathway. Eur J Pharmacol 747:71–87

Li R, Quan Y, Xia W (2018) SIRT3 inhibits prostate cancer metastasis through regulation of FOXO3A by suppressing Wnt/β-catenin pathway. Exp Cell Res 364:143–151
Li T et al (2019) Ribociclib (LEE011) suppresses cell proliferation and induces apoptosis of MDA-MB-231 by inhibiting CDK4/6-cyclin D-Rb-E2F pathway. Artif Cells Nanomed Biotechnol 47:4001–4011
Liou G-Y, Storz P (2010) Reactive oxygen species in cancer. Free Radic Res 44:479–496
Liu Y et al (2015) Role of miR-182 in response to oxidative stress in the cell fate of human fallopian tube epithelial cells. Oncotarget 6:38983
Luo K-W, Chen W, Lung W-Y, Wei X-Y, Cheng B-H, Cai Z-M, Huang W-R (2017) EGCG inhibited bladder cancer SW780 cell proliferation and migration both in vitro and in vivo via down-regulation of NF-κB and MMP-9. J Nutr Biochem 41:56–64
Mori K, Uchida T, Yoshie T, Mizote Y, Ishikawa F, Katsuyama M, Shibanuma M (2019) A mitochondrial ROS pathway controls matrix metalloproteinase 9 levels and invasive properties in RAS-activated cancer cells. FEBS J 286:459–478
Moro L (2019) Mitochondrial dysfunction in aging and cancer. J Clin Med 8:1983
Nguyen TT, Ung TT, Li S, Lian S, Xia Y, Park SY, Do Jung Y (2019) Metformin inhibits lithocholic acid-induced interleukin 8 upregulation in colorectal cancer cells by suppressing ROS production and NF-kB activity. Sci Rep 9:1–13
Paunkov A, Chartoumpekis DV, Ziros PG, Sykiotis GP (2019) A bibliometric review of the Keap1/Nrf2 pathway and its related antioxidant compounds. Antioxidants 8:353
Pei X et al (2019) Oenothein B inhibits human non-small cell lung cancer A549 cell proliferation by ROS-mediated PI3K/Akt/NF-κB signaling pathway. Chem Biol Interact 298:112–120
Prasad S, Srivastava SK (2020) Oxidative stress and cancer: chemopreventive and therapeutic role of Triphala. Antioxidants 9:72
Raj L et al (2011) Selective killing of cancer cells by a small molecule targeting the stress response to ROS. Nature 475:231–234
Reid MA, Wang W-I, Rosales KR, Welliver MX, Pan M, Kong M (2013) The B55α subunit of PP2A drives a p53-dependent metabolic adaptation to glutamine deprivation. Mol Cell 50:200–211
Shaw AT et al (2011) Selective killing of K-ras mutant cancer cells by small molecule inducers of oxidative stress. Proc Natl Acad Sci 108:8773–8778
Storz P (2013) Oxidative stress in cancer. In: Oxidative stress and redox regulation. Springer, pp 427–447
Tang Z et al (2016) CREB-binding protein regulates lung cancer growth by targeting MAPK and CPSF4 signaling pathway. Mol Oncol 10:317–329
Teng J-F et al (2020) Polyphyllin VI induces Caspase-1-mediated pyroptosis via the induction of ROS/NF-κB/NLRP3/GSDMD signal axis in non-small cell lung cancer. Cancers 12:193
Wang Q et al (2013) Nrf2 is associated with the regulation of basal transcription activity of the BRCA1 gene. Acta Biochim Biophys Sin 45:179–187
Wang C et al (2017) Cholesterol enhances colorectal cancer progression via ROS elevation and MAPK signaling pathway activation. Cell Physiol Biochem 42:729–742
Wang L et al (2018) Calcium and CaSR/IP3R in prostate cancer development. Cell Biosci 8:16
Yang W, Lu Z (2013) Regulation and function of pyruvate kinase M2 in cancer. Cancer Lett 339:153–158
Zhang K et al (2018) miR-9 regulates ferroptosis by targeting glutamic-oxaloacetic transaminase GOT1 in melanoma. Mol Carcinog 57:1566–1576
Zhang X, Huang J, Yu C, Xiang L, Li L, Shi D, Lin F (2020) Quercetin enhanced paclitaxel therapeutic effects towards PC-3 prostate cancer through ER stress induction and ROS production. Onco Targets Ther 13:513