The Double-Edged Sword Role of ROS in Cancer

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Abstract

In this chapter, how increased levels of reactive oxygen species (ROS) that have been detected in almost all types of cancers play a role in its development as well as therapeutic management have been discussed. ROS is a by-product of many metabolic cycles and processes within a cell. But the powerful antioxidant

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system of the cell keeps a check on ROS levels to maintain the normal function of cells. However in abnormal cases, like that of tumorigenesis, ROS plays an important role. ROS-mediated oxidative stress hampers cell cycle and causes DNA lesions like other known carcinogens, e.g., polycyclic aromatic hydrocarbons. The type of ROS generated, the location, and pathway followed for its generation, as well as the intracellular concentration is important for the cellular functions of ROS in cancer. Studies suggest that ROS interferes in pathways which involve transcription factors including NF-kB, AP-1, p53, HIF, Wnt, and Nif2 by upregulation or downregulation. The role of ROS in activation of oncogenic pathways leads up to transformation of a normal cell to oncogenic cells like survival, proliferation, angiogenesis, metastasis, therapeutic resistance, and stem cell survival. However, a growing body of evidence also suggests that ROS plays an important role in tumor inhibition too. That is why many anticancer agents are known to exploit the cytotoxic effects through ROS to activate the destructive cellular cycle like apoptosis, autophagy, necrosis, etc. which lead up to its death. From chemotherapies to targeted therapies, involvement of ROS coupled with some other therapeutic elements is being studied under clinical trials all over the world.

Keywords

Tumorigenesis · Angiogenesis · Metastasis · ROS · Cellular signaling · Cancer stem cells

Introduction

Cells with abnormal growth anywhere in the body with risk of spreading to other body parts cause cancer disease. There are over 100 types of cancer. It can be caused by various factors, most of which is tobacco and excessive alcohol consumption, lack of physical activity, obesity, unhealthy diet and lifestyle pattern infections, etc. There are various treatments for cancer which includes surgery, radiation therapy, chemotherapy, targeted therapy, etc. Cancer is a genetic problem which can be caused by both intrinsic (inherited mutations, hormones, and immune conditions) and extrinsic (tobacco, diet, radiation, and infectious organisms) factors. These elements interfere with some other important factors like proto-cx, tumor suppressor genes, and DNA repair genes through various biomolecules. Biomolecules that are less stable and highly reactive influence the signaling pathways through various transcription factors like nuclear factor-κB (NF-κB), hypoxia-inducible factor (HIF)-1α, kinases, signal transducer and activator of transcription (STAT)-3, various growth factors, cytokines, and other proteins. One such biomolecule is reactive oxygen species (ROS). It is a biological intermediate which is produced in all organisms during the course of aerobic cellular metabolism. ROS shows important beneficial effects at low levels, but

accumulation of ROS can cause several health disorders including carcinogenesis. Many researchers have observed and documented how ROS plays an important role in carcinogenesis. Examples of ROS include various molecular oxygen-based reactive molecules and free radicals, e.g., peroxides ($RO_2^{\bullet}$), superoxide ($O_2^{\bullet-}$), hydroxyl radical ($^{\bullet}OH$), etc. (Liou and Storz 2010). It has been noticed that low levels of ROS can be beneficial as they help in triggering the killing response of immune cells to microbial invasion, and it also helps in maintaining homeostasis and cell signaling (Devasagayam et al. 2004). However, excessive accumulation can cause damage to cellular membranes and even promotes cancer. There is a system of enzymatic and nonenzymatic antioxidants (antioxidant system) which eliminates ROS in the human body by maintaining the equilibrium of production and neutralization of ROS by help of various enzymes like catalase (CAT), glutathione S-transferase (GST), superoxide dismutase (SOD), glutathione reductase (GR), glutathione peroxidase (GPx), and glutathione (GSH) (Das and Roychoudhury 2014). If this equilibrium is disturbed then oxidative stress is caused which may initiate carcinogenesis (Snezhkina et al. 2019).

Oxidative stress can cause activation of transcription factors like NF-κB, AP-1, p53, HIF-1α, PPAR-γ, β-catenin/Wnt, and Nrf. These transcription factors are involved in expression of over 500 different genes. These genes are further involved in functioning of growth factors, inflammatory cytokines, chemokines, cell cycle regulatory molecules, tumor suppression including *p53*, and anti-inflammatory molecules (Fig. 1) (Uehara and Tanaka 2018). ROS also interferes with the signaling of apoptosis in cancer cells. There is evidence which indicates that oxygen radicals are involved in intercellular and intracellular signaling. In cultured cells, addition of ROS leads to an increased rate of DNA replication and cell multiplication. Hence, it can be said that ROS might function as mitogen. Also because of ROS's involvement in various signaling pathways which are mainly responsible for cellular transformation, inflammation, tumor proliferation, and invasion of cancer, its accumulation is harmful (Schieber and Chandel 2014). Any normal cell can get converted into a cancerous cell. The development of cancer mediated by ROS involves various signaling molecules.

Cancer cells are different from normal cells in the sense they exhibit greater oxidative stress because of the carcinogenic stimulation, and increased metabolic and malfunctioning mitochondrial activity, endoplasmic reticulum, and cell membranes. These changes become a main cause of respiratory dysfunction, decrease in coupling efficiency of the mitochondrial ETC, and increase in electron leakage and ROS generation (Rohrmann et al. 2013). Most common hallmarks of cancer cells are modulation in redox balance and deregulation of redox signaling (Wallace 2012). Because of this, various types of DNA damage take place which includes base damage, base modification, DNA double-strand breaks, the activation of oncogenes, gene amplification, rearrangement of DNA sequence, DNA single-strand breaks, and DNA miscoding lesions (Levine et al. 2017). Most of the treatment methods of cancer like chemotherapy stimulate ROS stress. Hence, ROS has a contradictory nature.

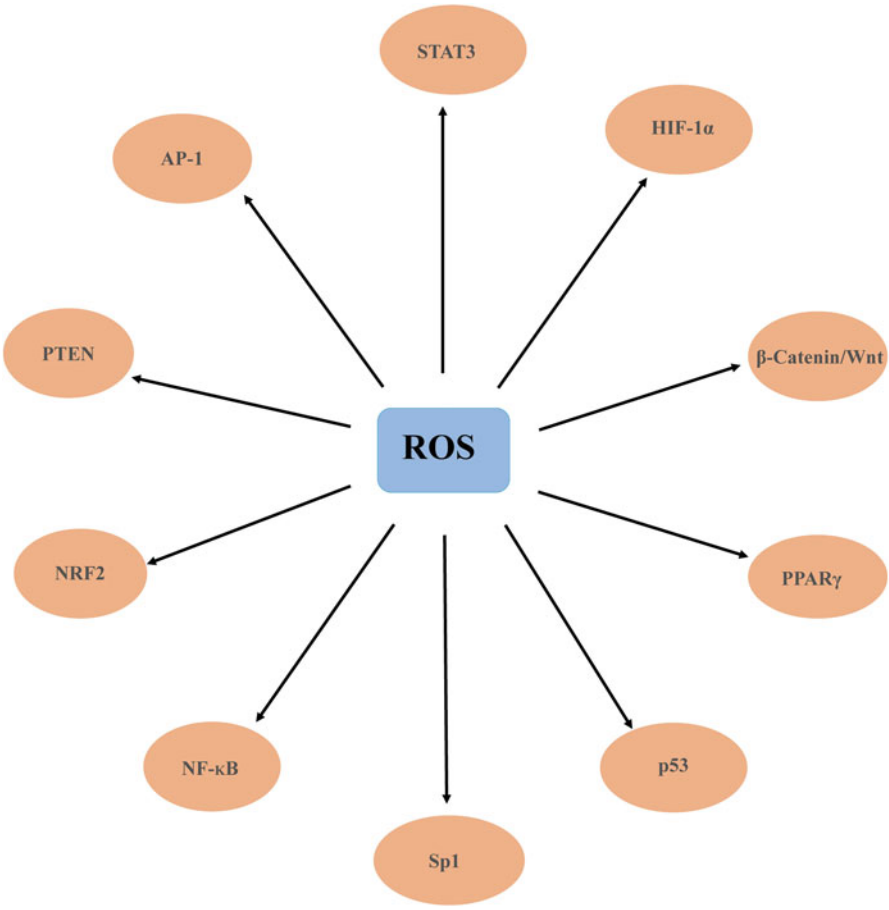

Fig. 1 Transcription factors regulated by ROS having role in different types of cancer

Role of ROS in Tumor Promotion

In oncology, transformation is a process whereby normal cells acquire properties of malignant cells. This causes activation of oncogenes and inactivation of tumor suppressing genes.

Cancer cells are characterized by presence of increased ROS level with respect to normal cells. This can be caused by abnormal oxidative mitochondrial pathways (Tafani et al. 2016). ROS plays a role in every step of development of cancer, from initiation, promotion, proliferation, progression, and finally further infection. Cancers like melanoma, glioma, hepatoma, leukemia, breast cancer, pancreatic cancer, cancer of bladder and colon, lung and prostate cancer, etc. are known to be caused by an increase in cellular ROS (Fig. 2) (Prasad et al. 2017).

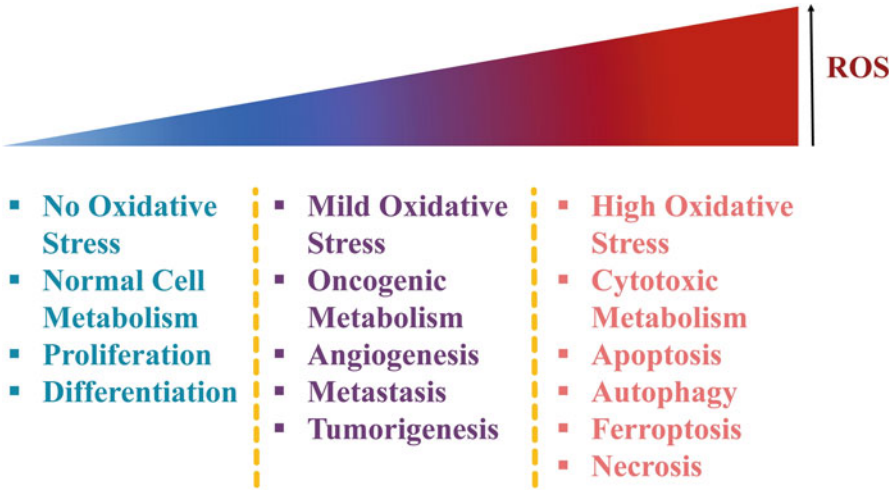

Fig. 2 Effect of increasing levels of ROS on cellular metabolism

Tumorigenesis Via Cell Signaling

Most characteristic conditions, or behavior that increases the likelihood of cancer, signal the cells by producing ROS. ROS, as a result, stimulates transcription factors such as tumor protein p53, activator protein 1 (AP-1), nuclear factor erythroid 2–related factor 2 (Nrf2), nuclear factor-κB (NF-κB), and hypoxia-inducible factor-1α (HIF-1α). These factors control the cellular machinery involved in transformation and tumor cell death or survival, growth, invasion, migration, and angiogenesis.

The NFE2-related factor 2 (Nrf2) belongs to the leucine zipper (bZip) transcription factors' basic cap "n" collar (CNC) subfamily. It increases proteolysis in proteasomes by its interaction with Kelch-like ECH-associated protein 1 (KEAP1), a cytoplasmic protein, and the Cul3-based E3 ubiquitin. KEAP1 has redox-sensitive cysteine residues that get oxidized by H_2O_2 in high ROS condition. This stops NRF2 association and subsequent degradation. NRF2 gets stabilized under oxidative stress; this helps the nucleus in triggering the expression of numerous genes, GPXs, CAT, and PRXs like antioxidants, detoxification enzymes, and enzymes involved in GSH synthesis. PPP and serine biosynthesis pathways are also affected by the activation of Nrf2, which again ultimately increases NADPH production. Stimulation of Nrf2 is seen in cancers like breasts, lungs, etc. NRF2 activation is triggered by mutations in KEAP1 and NRF2 as well as activation of oncogenes such as Kras and Myc (Ma 2013).

Another pathway called PI3K/AKT signaling pathway plays an important role in the control of cell proliferation, programmed cellular death, and oncogenesis. Programmed to promote the survival and growth in response to extracellular signals, it promotes ROS production by complimenting the energy metabolism in the mitochondrial region by its downregulation. This pathway also affects the activity of

AMPK by inhibiting it and increasing levels of ROS. Inactivation of ACC1/2 via AMPK is fundamental for maintaining balance of NADPH and ROS level. According to various studies, it was observed that cellular pathways like PI3K/Akt/mTOR and MAPK/ERK pathways are activated by H_2O_2-mediated activation of Ras and growth factor signaling. H_2O_2 also plays a role in inactivation of PTEN signaling cascades. A group of researchers also demonstrated that mitochondrial DNA haplogroup related to breast cancer promotes abnormal growth via ROS-mediated AKT activation. Cysteine thiol phosphatases such as protein-tyrosine phosphatase 1B (PTP1B), phosphatase and tensin homolog (PTEN), and protein phosphatase 2 (PP2) are oxidized in a reversible manner by H_2O_2. This results in loss of their activity, and the activation of the PI3K/Akt/mTOR signaling pathway. (Aggarwal et al. 2019)

Tumorigenesis in Response to ROS-Mediated Activation of Factors and Other Genetic Changes

Hypoxia-inducible factors (HIFs) are activated in cancer cells to adapt to their oxygen-deficient microenvironment (because of mROS). This is essential for cell survival, growth, and proliferation (Semenza 2012). Metastasis is aided by the stabilization of HIF1 during lack of adequate oxygen supply. This is achieved by oxidation of prolyl hydroxylase domain protein 2 (PHDP2), a key oxygen sensing protein, by H_2O_2 (Brewer et al. 2015). H_2O_2-mediated oxidation of targeted protein is proposed to have the floodgate model as the possible mechanism. The floodgate mechanism involves a pathway in which oxidation leads to disabling of various enzymes that help in hunting cellular ROS by hyperoxidation or phosphorylation, thereby causing an increase in H_2O_2 which ultimately causes protein oxidation locally (D'Autréaux and Toledano 2007).

When overexpressed, a transcription factor called FOXM1 is seen in tumors and it regulates growth and metastasis of cancer cells (Luo et al. 2016). Overexpression of FOXM1 causes decreased cellular concentration of ROS in cancer cells by increasing the function of enzymes, such as catalase, SOD2, and PRDX3 (Park et al. 2009).

Carcinogenic K-Ras mutations increase the chances of development of pancreatic, colorectal, and lung cancer by increasing generation of mitochondrial ROS of acinar cells of pancreas with high metabolism (Liou et al. 2016).

Different epigenetic changes like methylation and inactivation of tumor suppressor genes also play a role in inducing ROS production and thereby tumorigenesis. Researchers have shown that H_2O_2 negatively regulates E-cadherin's (a cell adhesion molecules and tumor suppressor) expression in hepatocellular carcinoma cell. When promoter region of E-cadherin is hypermethylated by histone deacetylase 1 (HDAC1) and DNA methyl transferase 1 (DNMT1), it loses its tumor suppression quality (Lim et al. 2008). Decreased expression of E-cadherin have shown a complementary relationship with increased metastasis in hepatic carcinoma (Endo et al. 2000). In SNU-407 human colorectal carcinoma, downregulation of runt-related

transcription factor 3 (RUNX3) tumor suppressor mRNA and protein are also caused by H_2O_2-mediated oxidative stress (Kang et al. 2012). ROS also facilitates tumorigenesis later than activation of oncogenes or inactivation of tumor suppressor genes. Like, mutating Ras oncogene leads to stimulation of ROS generation, which causes further transformation (Maciag et al. 2004). Also, it was seen that loss of p53 in the mouse prostate cancer model caused increased levels of cytokine release and ROS generation (Komarova et al. 2005). Various cancer cells have shown presence of inactivated breast cancer 1 (BRCA1), Von Hippel-Lindau (VHL), cyclin-dependent kinase inhibitor 2A (CDKN2A), and retinoblastoma (Rb) as a result of ROS-mediated epigenetic interference (Toyokuni 2008).

Angiogenesis

Formation of new blood capillaries is called angiogenesis. This process involves the differentiation of cells which line the insides of blood vessels (called endothelial cells) and their migration. Angiogenesis involves chemical signaling. It is an essential event for neoplastic growth, proliferation, and metastasis. Evidence has shown that ROS plays a key role by generation of H_2O_2 via NOX4. This helps in angiogenesis. NOX2 helps in avoiding apoptosis (Datla et al. 2007; Peshavariya et al. 2009). ROS monitors vascular endothelial growth factor (VEGF) signaling by involvement of transcription factor HIF-1α. This leads to the upregulation of VEGF and VEGF receptor expression (Xia et al. 2007). Another method involves activation of VEGF receptors to various angiogenic signaling pathways such as PI3K/Akt and MAPK pathways (Jing et al. 2012). Inhibition of prolyl hydroxylase stabilizes HIF1α, which in turn leads to VEGF activation (Klimova and Chandel 2008).

Hypoxia-induced mitochondrial ROS stabilizes the HIF-α subunit, which is oxygen sensitive, by showing PHD2 activity. When oxygen supply is enough then HIF-α is degraded by hydroxylation via PHD2 followed by recognition from the E3 ubiquitin ligase von Hippel–Lindau protein. But during oxygen scarcity, the HIF-α protein dimerizes with the HIF-β subunit. This dimer gets translocated to the nucleus to subsequently induce expression of genes that will trigger factors responsible for endothelial generation (for angiogenesis), such as VEGF and genes for metastasis, glycolysis, and cell survival (Semenza 2012). Stabilization of HIF-α by ROS-monitored mechanism is observed to promote the tumorigenesis of few oncogenic cells. In solid tumors, production of less NADPH via pentose phosphate pathway in case of low glucose condition causes increase in ROS level. As a result, the antioxidant capacity of such cells is increased. To avoid tumor cell death via ROS, AMPK is activated to promote NADPH production and consumption of NADPH is reduced by preventing degrading processes that require NADPH. The reduced levels of AMPK have been observed in oncogenic transformation prevention. In case of hypoxia, the one-carbon metabolism enzyme SHMT2 is upregulated by HIF which promotes serine catabolism and NADPH production in mitochondrial matrix (Reczek and Chandel 2017).

Apart from all this, p53, redox factor-1 (Ref-1), matrix metalloproteinases (MMPs), BNF-κB, cyclooxygenase-2 (COX2), etc. are regulated by ROS (Ushio-Fukai and Nakamura 2008).

Metastasis

Metastasis is a key event for cancer. It is performed by following compound cellular signaling pathways responsible for controlling dynamics of cytoskeleton and its interaction with the extracellular matrix. It was seen that H_2O_2 plays a key role in directing metastasis (Tochhawng et al. 2013). Another method by which ROS is involved in promotion of tumor is by degradation of proteins glycosaminoglycan (GAG) and other extracellular components (Nikitovic et al. 2013). As stated earlier, increase in ROS results in activated oncogenes. This causes high metabolism in cancer cells. This in turn may result in activation of Wnt/β-catenin pathway where regulation of c-Myc is facilitated by Wnt/β-catenin and causes increased metastatic potential (Steelman et al. 2008). Metastasis is also governed by endogenous growth factors and cytokines (Wells 2000). NOX or mitochondrial generation of ROS is also induced by various growth factors such as hepatocyte growth factor (HGF), vascular endothelial growth factor (VEGF), and cytokines like tumor necrosis factor-α (TNF-α). This leads to invasiveness of cancerous cells (Tochhawng et al. 2013). ROS generation mediated by growth factors have shown to regulate ERK1/2 activity, like HGF-mediated ROS generation regulates ERK1/2 activation which causes invasion of renal carcinoma cells (Lee et al. 2014). Urokinase plasminogen activator (uPA) is a serine protease which is involved in cellular invasion and its expression is also induced by ROS and mediated by HGF via ERK1/2 activation pathway. Since it is involved in cell invasion, it stimulates the invasiveness of human gastric plasminogen activator (Lee et al. 2009). ROS-induced ERK1/2 activation is also seen and observed in deferoxamine (iron chelator)-induced breast cancer cell invasion (Liu et al. 2014). VEGF-driven SOD3 expression has also shown tumor synthesis and metastasis in breast tumor (Wang et al. 2014). However, ROS also plays a role in metastasis inhibition. Anchorage independence and escape from programmed cell death leads to directly proportional increase in metastatic potential. Higher metastatic potential is attained by undergoing some metabolic changes to increase the antioxidant power of cancer cells. Likewise, cytosolic IDH1-dependent carboxylating reduction triggers anchorage-independent growth. This is done by increasing the production of mitochondrial NADPH and lowering mitochondrial ROS levels. Metastasis (for longer distance) is promoted by the NADPH-generating enzymes of the one-carbon metabolism pathway mediated by folate in human melanoma cells. Hence, these ROS reducing pathways can be investigated and exploited for therapeutic purposes (Reczek and Chandel 2017).

There are certain cases of chemotherapy resistance in some patients. This is a major step back in cancer treatment. Multidrug resistance protein 1 called P-glycoprotein, e.g., ATP-binding cassette (ABC) transporter protein, helps in

removal of certain anticancer agents from cells (Ambudkar et al. 2003). Upregulation of P-gp expression in hepatoma cells have been observed to be mediated by ROS (Ledoux et al. 2003).

Role of ROS in Tumor Suppression

As mentioned already, ROS plays a key role in cancer treatment and therapy by initiating different cycles of cell death like autophagy, apoptosis, etc.

Apoptosis

Apoptosis is an orderly cascade process in which cellular contents are repacked into small vesicles for disposition by immune cells. It is the most common cell death type. There are two types of apoptosis:

1. The death receptor–dependent apoptosis called extrinsic apoptosis
2. The mitochondrial-dependent apoptosis called intrinsic apoptosis

Extrinsic apoptosis follows Fas pathway and TNFα (tumor necrosis factor α). Recent study showed that ROS generated by atmospheric gas in the plasma extracellularly activates TNFα signaling and triggers extrinsic apoptosis in melanoma cancer cells (Ishaq et al. 2014). Mitochondrial apoptotic pathway helps in antitumor character of ROS (Dewangan et al. 2017). FAS ligand (FasL) and TNF-related apoptosis-inducing ligand (TRAIL) have receptors to which they are glued together to form a death inducing signaling complex with FADD and TRADD as adaptor protein and a procaspase (procaspase 8, mostly). For example, the FAS receptor (FasR), the TNF receptor (TNFR), and the death receptors (DR4 and DR5) are few receptors that form death-inducing ligand-receptor ligation. This results in activation of the caspase sequential order and apoptosis (Galadari et al. 2015). A study showed the susceptibility of colorectal cancer cells to SN-38 is increased by chloroquine. This activates ROS-mediated mitochondrial apoptotic pathway (Chen et al. 2017).

Autophagy

Autophagy (type II programmed cell death) is an orderly, regulated degradation and recycling of cells and its components by itself in the lysosome. Autophagy has been considered as a self-regulatory adaptive method for survival by cell, but autophagy can also function as a tumor suppressor mechanism as well. It has also been observed that autophagy compliments anticancer drugs (Zou et al. 2012). Excess ROS can trigger autophagic cell death (Zhao et al. 2016). H_2O_2 generated by mitochondria incites autophagy in malignant glioma cells when treated with sanguinarine (Pallichankandy et al. 2015).

Necrosis

Necrotic cell death is a programmed type of cell death in response to injury, chemicals, radiation, organelle swelling, and membrane rupture. ROS controls necrosis by NOX-mediated mechanism. Riboflavin kinase forms a complex with NOX1 and TNFR in TNF-ALPHA treatment which causes NOX-mediated ROS generation (Dixon et al. 2012). Some evidence has shown that ROS has a controlling influence on signaling proteins RIP1 and RIP3 which participate in necroptosis. Mitochondrial ROS regulates RIP1 by autophosphorylating it which results in its oxidation and formation of intermolecular disulfide bonds. This activates RIP3 into necrosome to trigger necroptosis (Zhang et al. 2017b). ROS generated in mitochondrial ETC also induces calcium-mediated formation of RIP1/RIP3 complex and causes necroptosis in human colon cancer cells (Sun et al. 2017b). The types of ROS involved in necroptosis and their methodology is yet to be studied and determined.

Ferroptosis

Ferroptosis is iron-dependent programmed cell death. In ferroptosis, the accumulation of lipid peroxides is seen. It is both genetically and biochemically different from other forms of programmed cell death cycles such as apoptosis. Ferroptosis involves complex iron-dependent cellular pathways for ROS formation and lipid oxidation. If glutathione (GSH)-dependent antioxidant defense system is suppressed, ferroptosis is triggered by some other biomolecules. Studies have shown that a lot many anticancer drugs mediated anticancer effects via ferroptosis. For instance, the antimalarial drug artesunate works as an inducer of ferroptosis to exert anticancer effects. Salinomycin prevents iron translocation, thereby inducing iron depletion and finally inducing ferroptosis against cancer stem cells (Hamaï et al. 2017). Recently, a study has also shown that cisplatin causes ferroptosis in lung cancer cells. Heat shock protein B1 (HSPB1) is also involved in ferroptosis. It was demonstrated that phosphorylation of HSPB1 via protein kinase C (PKC) downregulates the ferroptosis in cancer cells with RAS by reducing iron consumption and lipid ROS production (Stockwell et al. 2017). A kinase inhibitor called Sorafenib is being used for the treatment of hepatocellular carcinoma and renal cell carcinoma by inducing ferroptosis in cancer cells. An artemisinin-based drug called artesunate also initiates ferroptosis in adenocarcinoma of pancreatic ducts (Louandre et al. 2013, 2015).

Chemosensitization

As stated earlier, P-gp is a multidrug resistance protein that helps the cells in escaping drug-induced cell via death-inducing signaling complexes. At increased doses of drugs, ROS can also negatively regulate the P-gp expression. P-gp expression is increased in Caco-2 human colon cancer cells, induced by low concentrations of H_2O_2. On the contrary, when concentration is increased significantly, P-gp

expression is decreased (Terada et al. 2014). On the same lines, it was studied that catalase upregulates P-gp expression in human liver cancer cells (HepG2 cell) (Li et al. 2006). The cytotoxicity of carboplatin and 5-fluorouracil in MCF-7 cells is promoted by hyperglycemia via downregulated expression of P-gp (Pandey et al. 2011). In summary, ROS are implicated in both chemoresistance and chemosensitization.

ROS in Cancer Stem Cells

Cancer stem cells (CSCs) are a small, distinguished type of cells that can undergo differentiation, self-renewal, and tumorigenicity and are capable of driving and maintaining various types of malignancies. These small subpopulation of cells are found in tumors. CSCs are involved in various processes that lead to neoplastic development of cells, such as invasion, metastasis, angiogenesis, and resistance to chemotherapy (Li et al. 2007). However, CSCs usually have low levels of intracellular ROS content (Shi et al. 2012). The reason behind this low level of intracellular ROS could be the reduced imbalance in redox balance. Another reason could be the enhanced ROS scavenging antioxidant systems in the CSCs. In gastrointestinal CSCs, GSH synthesis was enhanced and system xc^- was activated, which caused reduced ROS levels (Ishimoto et al. 2011). In breast tumors, reduced ROS level due to CSCs was observed (Diehn et al. 2009). This contradicting feature of CSCs from cancer cells is responsible for recurrence and reemergence of tumorigenicity of cells even after chemo and radiotherapy (Shi et al. 2012). It was hinted that the hike in survival potential of CSCs of prostate tumors even after ionizing radiation therapy may be due to their low vulnerability to ROS-induced cellular damage (Kim et al. 2013). Unfortunately, the lack of insights in the methodology involved in the redox regulation in CSCs have led to lack in development of insights in its role in therapy.

Role of ROS in Cancer Therapies

The cancer cells are clever cells that are quick to adapt to the high ROS in an oncogenic microenvironment by involving mechanisms of ROS detoxification in their survival. This leads to increased dependency on antioxidant systems, thereby creating a functional cross talk between oncogenic cells and antioxidant defense systems. This puts forth a challenge to study different strategies that can disturb this cross talk by increasing the intensity of oxidative stress in the presence of some selected metabolic inhibitors (Harris and Brugge 2015).

Roles of ROS in Molecular Targeted Therapies

Incorporating ROS in personalized medical therapy for cancer treatment like molecular targeted therapies has garnered a lot of research interest. The idea is to increase

ROS levels by molecular targeted drugs in drug-resistant oncogenic cells to provide therapeutic relief to the patient (Zou et al. 2017).

Roles of ROS in Targeted Tyrosine Kinase Therapies

This treatment mainly includes monoclonal antibodies (mab) like trastuzumab, cetuximab, pertuzumab and bevacizuma, and small molecule inhibitors like axitinib, sorafenib, pazopanib, erlotinib, afatinib, and gefitinib. A number of studies show that these mab and other molecules produce anticancer effects via ROS. Trastuzumab can induce ROS generation against HER2 to contribute to treatment of breast cancer. Another recombinant mab specific to epidermal growth factor receptor (EGFR) called cetuximab is used against colorectal carcinoma. Cetuximab promotes ROS production by decreasing the amount of glutathione. EGFR inhibitors gefitinib and erlotinib when coupled help in treatment of non-small-cell lung carcinoma (NSCLC). Gefitinib and erlotinib complimentarily stimulate KEAP1 and suppress NRF2 to increase ROS levels in NSCLC cells via enhanced mitochondria-dependent apoptosis. Salinomycin and gefitinib induce ROS-mediated disturbance in membrane potential of mitochondria and lysosome, and also apoptosis. Lung cancer cells developed an enhanced immunity against resistance to gefitinib after treatment with gefitinib for prolonged time periods (Teppo et al. 2017). Gautam et al. showed that another anticancer drug called sunitinib when paired with chloroquine increases ROS levels to induce cell apoptosis (Gautam et al. 2017).

It is important to point out that we cannot give a conclusion on which ROS plays what role in targeted therapies as the probes used for examining the ROS levels are the fluorogenic probes DCFH. DCFH is not actually a useful specific probe for the H_2O_2 and $O_2^{\bullet-}$ (Bonini et al. 2007; Wardman 2007).

Roles of ROS in Cancer Chemotherapy

Chemotherapeutic drugs involves damaging of the DNA directly to induce cell death in carcinogenic cells. Some chemotherapeutic drugs exploit ROS for antitumor effects. Chemotherapeutic drugs cause ROS accumulation which causes disturbance in redox balance to show anticancer effects (Magda and Miller 2006). ROS-mediated DNA promotes p53 accumulation. It also activates p53/bax signaling pathway which induces oncogenic cellular apoptosis in mitochondrial-dependent manner (Ray et al. 2016).

Roles of ROS in Radiotherapy

Radiotherapy is a cancer treatment that uses high doses of radiation to kill cancer cells and shrink tumors, mostly solid tumors, by damaging the cellular DNA. Ionizing radiation also generates oxidative stress by producing ROS through water

radiolysis. This destabilizes cancer cells structure and DNA by forming stable DNA peroxides and finally cellular death. During radiotherapy, water radiolysis induces ROS production, mainly hydroxyl radicals and few other radicals. Cells that divide more rapidly are more sensitive to radiotherapy than slowly dividing cells (Dayal et al. 2014). Radiotherapy induces oxidative stress that makes cells in G2 phase and M phase of mitosis more sensitive to it than compared with cells in S phase (Yu et al. 2018). Certain chemicals are used to increase this sensitivity for therapeutic purpose. For example, coroglaucigenin inhibits the expression of antioxidant molecules to promote the production of hydroxyl radicals. This increases the sensitivity of lung cancer cells to radiation therapy (Sun et al. 2017a). Another drug, auranofin, enhances the sensitivity of cells of breast cancer by causing the suppression of enzyme thioredoxin reductase, causing an increase in the level of hydroxyl radicals. In nasopharyngeal oncogenic cells, salinomycin is used to sensitize the cancer cells to radiotherapy by inactivating Nrf2 and increasing hydroxyl radical generation (Zhang et al. 2017a).

Conclusion

Growing bodies of evidence show that in the development of different types of cancer, altered pathways of cellular metabolism play an important role. The impact of oxidative stress on cancer initiation, progression, promotion, infection, and response to therapy is both positive and negative. ROS generation can promote tumorigenesis, and can also increase the vulnerability of cancer cells to various death-inducing pathways by signaling. Both sides of the role of ROS have been exploited for treatment and cure of cancer. The area regarding the use of ROS for therapeutic purposes is still grey with debates about whether involvement of ROS modulation is clinically beneficial or not for cancer treatment. A coming-of-age model is increasing the level of ROS to a toxic level may provide a platform for cancer treatment by making the cells more prone to various programmed death cycles and chemosensitization. This model provides a logical approach to the usage of both properties of reactive oxygen species. Antioxidants coupled with anticancer drugs can also be revolutionary in this field. The methods for exploiting these dual effects of ROS in a therapeutic sense are still being studied and observed in various clinical trials all over the world.

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