Review

Paradoxical action of reactive oxygen species in creation and therapy of cancer

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A great number of comprehensive literature believe that reactive oxygen species (ROS) and their products play a significant role in cell homeostasis maintenance, tissue protection against further insults by controlling cells proliferation through inducing apoptosis, and defending against cancer. ROS is believed to be like a potential double-edged sword in both cancer progression and prevention. Although at low and moderate levels ROS affect some of the most essential mechanisms of cell survival such as proliferation, angiogenesis and tumor invasion, at higher levels these agents can expose cells to detrimental consequences of oxidative stress including DNA damage and apoptosis that result in therapeutic effects on cancer. Understanding the new aspects on molecular mechanisms and signaling pathways modulating creation and therapy of cancers by ROS is critical in development of therapeutic strategies for patients suffering from cancer. This paper presents a general overview and rationale of paradoxical action of ROS in creation and therapy of cancer, tests to be used, and examples of how it may be applied.

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1. Introduction

Cancer, medically termed as malignant neoplasm, includes a wide spectrum of over 100 types of different diseases that can afflict humans with various etiologies and epidemiological distributions. The eruption of this condition is initiated by uncontrollable reproduction of cells in a specific part of the body and also failure in the mechanisms of cell death. Malignant tumors, formed by rapid division of cancerous cells, can invade and destroy surrounding tissues and organs. Spreading of cancerous tumors to distant parts of the body through the lymphatic system or bloodstream is known as metastasis (Michael et al., 2009). Cancer is a major public health problem in the United States and many other parts of the world, with regard to the fact that it is responsible for a mortality of 1 in 4 deaths in US; the disease is ranked as the second cause of death after cardiac health problems (Rebecca Siegel and Ahmedin, 2012).

Most risk factors associated with cancer interact with cells through the generation of oxidative stress. This is a condition in which reactive oxygen species (ROS) and/or free radicals, namely superoxide (O$_2^-$), hydroperoxyl radical (H$_2$O$_2$), and hydroxyl radical (OH$^-$), are produced intra- or extra-cellularly, and induce toxic impacts on cells. Oxidative stress can play an important role in the pathogenesis or treatment of cancer diseases. A great number of comprehensive literature believe that ROS and their products are responsible for important signaling functions, and play a significant role in cell homeostasis maintenance, tissue protection against further insults by developing preconditioning, gene transcription regulation, controlling cell proliferation by inducing apoptosis, and defending against cancer (Becker, 2004; Martin and Barrett, 2002; Nakashima et al., 2003). Under physiologic conditions, cells control ROS levels by the use of scavenging systems that balance ROS generation and elimination. But under oxidative stress conditions, excessive ROS can damage cellular proteins, lipids, and DNA, leading to fatal damages to cell that may contribute to carcinogenesis (Fausto, 2006).

2. Reactive oxygen species

ROS, as byproducts of oxygen metabolism, are constantly produced in the human body and removed by antioxidant defense. High chemical reactivity is a prominent characteristic of these chemical agents; therefore, these factors are believed to be toxic in cellular life (Auten and Davis, 2009). Given the higher stability of paired electrons positioned in an orbital compared to single electrons, it is expected that radicals express a higher degree of reactivity than non-radicals (Halliwell, 1991a). The interest to radical agents rose in 1970s after the discovery of superoxide dismutase (SOD) in 1968. Removal of superoxide radicals, a species with an extra electron than the oxygen molecule, is enhanced by SOD enzymes (Ebrahim et al., 2012; Jouroukhin and Gozes, 2011). Although ROS play a positive role in normal physiological pathways and necessary functions, their excess concentrations and overproduction are immensely toxic to cellular life by damaging macromolecules such as DNA and proteins. This potential toxicity is magnified in the presence of transition metal-ions such as iron or copper (Arumou and Halliwell, 1991). Briefly, along with the traditionally known damaging impact on cells, ROS are also considered as modulators of pathologic processes.

3. Controversy of free radical hypothesis

Although ROS was first implicated as deleterious and destructive in events such as ischemia reperfusion (IR) injury, later findings revealed that these agents could also be implicated in the processes involved in maintenance of homeostasis (Becker, 2004; Feinendegen, 2002). They can play an important role in preventing diseases through supporting the immune system, intervening in cell signaling and mediating apoptosis. On the other hand, they can also be responsible for carcinogenesis and cardiovascular diseases by damaging valuable macromolecules in cells (Alizadeh et al., 2012; Alizadeh and Mirzabeglo, 2013; Faghihi et al., 2012; Finkel and Holbrook, 2000; Holbrook and Ikeyama, 2002; Martin and Barrett, 2002; Nakashima et al., 2003). Under physiologic conditions, cells control ROS levels by the use of scavenging systems that balance ROS generation and elimination. But under oxidative stress conditions, excessive ROS can damage cellular proteins, lipids, and DNA, leading to fatal damages to cell that may contribute to carcinogenesis (Fausto, 2006).
Recent evidence emphasized that ROS production is so vital in normal physiological process, especially for appropriate immuno-competence and activation of several signal transduction pathways (Holbrook and Ikeyama, 2002). There are several studies reporting unsuccessful results in applying antioxidants in order to restore the function of tissues suffering from pathologies in which ROS play the most important role (Domijan et al., 2014; Sheu et al., 2006). Some other studies described diverse side effects arising from antioxidant administration as well, which is opposed to the anticipated effects of these substances (Horakova et al., 1992). Some epidemiological studies supporting this idea, demonstrated that ischemic heart disease and specific types of cancer were inversely related to the endogenous antioxidant condition (Gey et al., 1991). Other investigations also indicated that antioxidant therapy might even be deleterious in conditions such as lung cancer or cardiovascular diseases (Omenn et al., 1996). Armario and his colleagues showed that despite inhibition of lipid peroxidation in the gastric mucosa by vitamin E or allopurinol, ischemia-reperfusion-induced lesions were not decreased at all (Armario et al., 1990). Many studies believed that excessive ROS production is a consequence of tissue injury rather than the cause of damage itself. It has been indicated that the peak of hydroxyl radical concentration did not match with cell injuries (Khalid and Ashraf, 1993). There are also disappointing clinical evidence indicating the failure of antioxidant supplementation for oxidative stress-associated pathologies such as cancer, cardiovascular diseases, coronary artery diseases, Alzheimer’s disease, and chronic obstructive pulmonary disease (COPD) (Gilgun-Sherki et al., 2003; Lonn et al., 2005; Tousoulis et al., 2005; Vivekananthan et al., 2003). The idea of antioxidants therapy has some controversies and the ideal antioxidant strategy is still unidentified. We believe that with regard to different pathophysiology of diseases in which ROS are involved, selection of the exact antioxidants and methods of introduction to body directly affects the success or failure in the final results. On the other hand, the local life style of patients may be the underlying interfering factor responsible for controversial results of cohort studies with same method. Furthermore, despite the difficulties in measuring the bioavailability of tissue antioxidants in targeted organs, this may clear the conventional paradoxical notions about antioxidant therapy if done with standard criteria in large studies.

4. ROS sources

Mitochondrion is perhaps the most important in vivo source of ROS (Fig. 1). The mitochondrial electron transport chain (ETC) has several redox centers, which may leak electrons to molecular...
oxygen, serving as the primary producer of ROS in most of the tissues (Ott et al., 2007). Mitochondrial ETC generates primary ROS at two complexes, I and III. H2O2 and O2− radicals are produced from ROS in a number of cellular reactions and by different enzymes, e.g., lipoxygenase (LOX), nicotinamide adenine dinucleotide phosphate (NADPH) oxidase, xanthine oxidase (XO), SOD, and peroxidase (Blokhina et al., 2003; Finkel and Holbrook, 2000a). Lipids, by peroxidation of unsaturated fatty acids in the membranes are the main cellular components which are susceptible to damage by free radicals (Blokhina et al., 2003; Saki et al., 2013).

XO is a major source of O2− which can generate H2O2 as well. This enzyme is present in endothelial cells and at higher levels in blood circulation. The interactions of neutrophils and endothelial cells play an important role directly in ROS production through converting the enzyme, xanthine dehydrogenase (XD), into XO. It has been discovered that XO is placed in coronary endothelial cells but cannot be found in the human cardiac myocytes (Gunther et al., 1999).

Cytochrome P450 enzymes are other potent sources of ROS production, particularly in the liver. These are a group of membrane-bound enzymes named the cytochrome mixed function oxidases, mostly present in the endoplasmic reticulum as constituents of a multi-enzyme system. This system comprises the flavin adenine dinucleotide/flavin mononucleotide (FAD/FMN)- including NADPH-P450 reductase and cytochrome b5. Although most of them are localized in the endoplasmic reticulum, they are mainly found in the mitochondria (Anandatheerthavarada et al., 1999; Ortiz de Montellano, 1995).

Recent evidences suggested that peroxisomes have a significant part in both the production and scavenging of ROS in the cell, especially hydrogen peroxide (H2O2) (Stolz et al., 2002; Zwacka et al., 1994). An appealing characteristic of peroxisomes is their capacity to reproduce and multiply, or be degraded as a reaction to oxidative stress though very specific pathways (Svingen et al., 1979; Young and McEneny, 2001). Like other free-radical chain reactions, the cycle only terminates when a free radical reacts with another free radical to produce two neutral molecules. In the absence of antioxidants, such reaction happens when the membranes have undergone a great deal of damage, resulting in high concentrations of free radicals and increasing the chance for interaction between two free radicals (Yin et al., 2011). If the lipid peroxidation process is not terminated in a short period, considerable measures of oxidative damage are dealt to the cell membrane (Yajima et al., 2009a). Moreover, products of lipid peroxidation process, such as malondialdehyde, are proved to be carcinogenic and mutagenic since they can deteriorate the DNA (Niedernhofer et al., 2003). Also, targeting lipid peroxidation is a characteristic of some virulent invaders. Oxidatively damaged DNA and lipid have been detected in HCV core-protein-transgenic mice while presenting robust accumulation of ROS (Machida et al., 2006). In an evolutionary cycle to control lipid peroxidation, organisms have developed defense systems such as antioxidants to terminate the chain reaction before it can damage the membranes. Certain vitamins (e.g., Vitamin E) and enzymes such as glycosyn phosphorylase, SOD and Catalase play a major role in early termination of lipid peroxidation chain reaction (Huang et al., 2002; Zimmermann et al., 1973).

5. Lipid peroxidation

Lipid peroxidation is the oxidative process of lipid degradation by ROS that are either by products of cellular metabolic reactions or oxidative stress (Marnett, 2002; Young and McEneny, 2001). Uncontrolled reaction of ROS with the present membrane lipids, especially polyunsaturated fatty acid chains, will lead to a myriad generation of free radicals produced in chain reaction that renders deterioration of the membranes (Feeney and Berman, 1976). The process consists of three chief steps in resemblance to all free-radical chain reactions: Initiation, Propagation, and Termination (Fig. 2) (Marnett, 1999). In the initiation phase of the peroxidation chain reaction, a ROS molecule reacts with an unsaturated fatty acid chain. The products of the initiation step are a fatty-acid radical and a humble water molecule (Dix and Aikens, 1993). Since the fatty-acid radical is not a stable molecule, in the propagation phase, it tends to react with molecular oxygen (O2) to produce a lipid-proxyl radical. This generated molecule then reacts with yet another unsaturated fatty acid and produces another fatty-acid radical in addition to a lipid peroxide. The fatty-acid radical takes part in another cycle of the same series of reactions and produces more free radicals and lipid peroxides in a continuous chain reaction. This step is responsible for the vast deterioration of cellular membranes induced by ROS during oxidative stress (Svingen et al., 1979; Young and McEneny, 2001). Like other free-radical chain reactions, the cycle only terminates when a free radical reacts with another free radical to produce two neutral molecules. In the absence of antioxidants, such reaction happens when the membranes have undergone a great deal of damage, resulting in high concentrations of free radicals and increasing the chance for interaction between two free radicals (Yin et al., 2011). If the lipid peroxidation process is not terminated in a short period, considerable measures of oxidative damage are dealt to the cell membrane (Yajima et al., 2009a). Moreover, products of lipid peroxidation process, such as malondialdehyde, are proved to be carcinogenic and mutagenic since they can deteriorate the DNA (Niedernhofer et al., 2003). Also, targeting lipid peroxidation is a characteristic of some virulent invaders. Oxidatively damaged DNA and lipid have been detected in HCV core-protein-transgenic mice while presenting robust accumulation of ROS (Machida et al., 2006). In an evolutionary cycle to control lipid peroxidation, organisms have developed defense systems such as antioxidants to terminate the chain reaction before it can damage the membranes. Certain vitamins (e.g., Vitamin E) and enzymes such as glycosyn phosphorylase, SOD and Catalase play a major role in early termination of lipid peroxidation chain reaction (Huang et al., 2002; Zimmermann et al., 1973).
6. ROS and carcinogenesis

6.1. Oxidant stress and inflammation

Inflammation is classified into two major stages: acute and chronic. As the initial stage, acute inflammation is mostly due to the “respiratory burst” caused by mast cells and leukocytes as a response against an exogenous invasion (Reuter et al., 2010). However, in the chronic stage the increased release of mediators such as metabolites of arachidonic acid, cytokines, and chemokines by inflammatory cells recruit inflammatory cells to the site of damage which will ultimately result in a higher level of ROS production and predispose the host to various chronic illnesses, including cancer (Grievinkov et al., 2010; Kamp et al., 2011). The more this vicious cycle of sustained inflammation and oxidative stress persists, the higher the risk of carcinogenesis will be (Shacter and Weitzman, 2002). As an example of the synchronous development of cancer and inflammation progression, interleukin (IL)-1 and IL-6 proinflammatory cytokine tyrosines can be induced by various malignant cells with rat sarcoma (Ras) oncogene (Bhauik et al., 2009). On the other side of the cycle, certain antioxidants and steroids that have the ability to inhibit the phagocyte respiratory burst can regress tumor promotion (Fabiani et al., 2001). Besides contributing to genomic instability, ROS can specifically trigger certain pathways involved in tumor proliferation (Weinberg et al., 2010). For example, as an episode of inflammation induced carcinogenesis, nitrosative stress can activate AP-1, a redox-sensitive transcription factor, which promotes cell division and transformation (Matthews et al., 2007; Schonthaler et al., 2011). Although NO− is a short lived free radical, the expression of the inducible nitric oxide synthase isoform (iNOS) by inflammatory cytokines such as tumor necrosis factor (TNF)-α and interferon-γ can produce deleterious damages (Tyagi et al., 2012). The up-regulation and the activity of iNOS has been associated to tumor angiogenesis in human colorectal cancer (Gianchi et al., 2003; Gochman et al., 2012). Matrix metalloproteinase (MMP)-2 and -9, a subgroup of MMPs which play critical role in collagen degradation and tumor invasion, are activated under prolonged oxidative stress and inflammation (Gencer et al., 2013). Redox signaling of a number of metastatic cascade steps are also regulated by the inflammatory molecule electrophile cyclopentenone prostaglandin (Diers et al., 2010). Studies have indicated that ROS are involved in the release of vascular endothelial growth factor (VEGF) and fibroblast growth factor (FGF) into tumor environment by inflammatory cells (Blanchetot and Boonstra, 2008). Hence, it can be concluded that carcinogenesis can be affected by both the oxidative stress and ROS induced inflammation (Fig. 3). There is also a probability that patients who are presenting a chronic higher level of inflammation and oxidative stress in minor conditions e.g., skin conditions such as acne and psoriasis (Poupard et al., 2013; Sutcliffe et al., 2007) are more prone to develop cancers in elderly in comparison to normal individuals. Therefore, controlling inflammation in such patients may prevent future risks.

Several of the inflammatory cells including mast cells, macrophages, eosinophils, and neutrophils which are believed to participate in the inflammatory response have been shown to release ROS after activation by a variety of stimuli (Gillisien et al., 1997; Reid et al., 2003). Superoxide dismutase plays an important role in conversion of O2, produced by inflammatory cells, to H2O2 (Shuvaev et al., 2011). HOCI, a potent oxidant, is formed in neutrophils myeloperoxidase from H2O2 and chloride ions (Eisrich et al., 1998). O2 can also be released from eosinophils in response to different allergic or parasitic related factors such as complement fragments, IgG, IgE, platelet-activating factor (PAF) and the lipid mediator (Dri et al., 1991). Interaction of eosinophil products including cationic proteins, eosinophil peroxidase (EPO) and major basic protein are correlated to epithelial damage; EPO and hydrogen peroxide are two arms of a cytotoxic system in the presence of halide ions. This phenomenon is vastly studied in asthma attack in which blood vessels containing purified amounts of PAF activated eosinophil granulocytes directly rushes to airway epithelial cells. The initiation of the attack may be linked to arachidonic derived chematic factors stimulated by increased levels of ROS (Wenzel, 1997). As a noticeable reduction of in vitro damage is observed after applying of catlase, it is suggested that the reaction is mostly mediated by ROS (Barnes, 1990). Nasal epithelium is also exposed to this injurious effect of H2O2 and EPO (Holm et al., 1999). In late phases of allergen exposure ROS may contribute to attraction of macrophages and also mucus hypersecretion (Barnes, 1990). While blood histamine and vessel diameter rises in a high ROS cellular context, it is therefore much more reasonable that oxygen inhalation will ultimately lead to infiltration of neutrophils (Scherhorn et al., 1999). Lacroix et al. study on the injured spinal cord demonstrated that delivery of interleukin 6 (IL6) can increase neutrophil and macrophage infiltration which in turn inhibits axonal growth (Lacroix et al., 2002). Studies proved that IL6 is a modulator of both ROS generation and neutrophil trafficking (Fielding et al., 2008; Sung et al., 2000). Assays of cell detachment and digestion of cell surface proteins in excessive neutrophil mediated inflammatory reaction are an index of anokis, a special form of cell death promoted by disconnection of anchorge-dependent cells from extracellular matrix (ECM) (Li et al., 1999). A direct relationship between intracellular ROS and cell detachment coincided by a burst in number of inflammatory cells describes the significant role of neutrophils in induction of tissue damage (Harlan et al., 1981; Rafiq et al., 2006). Ischemia-reperfusion injury of tissues characterized by microvascular impairment and enhanced leukocyte infiltration is frequently associated with formation of ROS (Grisham and Granger, 1988), in vivo studies represent that the reperfusion of ischemic tissues which is concomitant with release of leukocyte generated ROS, mainly inflammatory cells NADPH oxidase, may result in substantial damage of different tissues including epithelial cells of interstitial mucosa and hepatic mesenchymal cell structure (Jaeschke et al., 1990; Simpson et al., 1993).

6.2. Influence of ROS on the cell cycle

A broad range of external cellular factors including growth factors and extracellular matrix factors are known which take pivotal parts in regulating the cell cycle. Cyclins and cyclin dependent kinases (CDKs) are two classes of molecules with major impact on cell cycle. On the upper level cell division cycle (Cdc)-25 phosphatases and CDK-inhibitors (CKIs) control the activity rate of cyclins and CDKs by reversible phosphorylation. Recent researches have shed light on the synchronous production of ROS with the normal cell cycle and their key role as a vital modulator (Takahashi et al., 2004). It has become clear that the ROS produced downstream of growth factor receptors induce ubiquitination by intermediate phosphorylation of CDKs and other regulatory factors such as epidermal growth factor receptor (EGFR) (Colavitti et al., 2002). In addition, activation of EGFR results in increased transcription and translation of molecules in intracellular signaling pathways involving cyclins and CDKs and also escalated ROS content which proves a synergistic correlation of the mentioned agents. Despite the studies that have clarified the role of ROS as a positive progression factor in cell cycle process, conspicuous results of recent studies assert that generation of ROS from different sources may have contrasting consequences in adjusting the cell cycle and the related enzymatic systems, considerably the Cdc-25 (Boonstra and Post, 2004). Apparently, the exact effects of ROS on both phosphorylation and ubiquitination depends on cellular and molecular context including the amount and the
specific types of ROS produced and also cell cycle associated enzymes (Verbon et al., 2012). Feher et al. have provided evident data of positive effects of oxidative stress and ROS on the acceleration of Auxin-mediated G0-to-G1 phase in plant cell cycle mechanism (Fehér et al., 2008). A study of the outcomes of oxidative stress on neurodegenerative disorders by Klein et al. affirmed both cell cycle and ROS as team players in progression of the disease, and concluded that cell cycle in neurons can be repressed by ROS (Klein and Ackerman, 2003). In summary, it is apparent that ROS may play an important role in activation of growth factor receptors, and thus modulate cell cycle progression in cancer (Fig. 4). Considering the reactions of different tumor cell lines for survival, studies on behavior of cancers in response to changes in ROS level and arrested proliferation are required to reveal weather halted cell cycle may affect other factors such as tumoral invasion and metastasis in long terms.

6.3. Angiogenesis induced by ROS

Inadequacy in oxygen supply to cells by prolonged hypoxia leads to cell death. As new blood vessels are atypical or the blood flow is poor, tumors often turn to be hypoxic. Under this condition, cells upregulate signaling pathways which activate proliferation and angiogenesis. These pathways have been adapted in cancer cells effectively which allow tumors to stay viable and grow under such conditions (Harris, 2002). The reperfusion of hypoxic tissue can rise the free oxygen radical levels (Prabhakar, 2001). ROS produced by hypoxia/reoxygenation not only have detrimental effects in cells such as tissue injury, but also have an important role in vascular angiogenesis (Fig. 5) (Maulik and Das, 2002).

Angiogenesis is essential for both physiological processes like embryonic development and wound healing and pathological changes such as cancer, diabetic retinopathy, and atherosclerosis (Maulik and Das, 2002; Tonnesen et al., 2000). Necessary events in the process of angiogenesis include endothelial cell migration, proliferation, and tube formation. ROS may directly play a part in all these events, as H2O2 has been indicated to be involved in proliferation and migration of endothelial cells and to mediate lymphocyte-activated tubulogenesis (Fig. 5) (Maulik and Das, 2002; Soares et al., 2014). Furthermore, low concentrations of superoxide was shown to accelerate endothelial cell migration and tube formation in an in vitro model of angiogenesis (Stone and...
ROS also act as mediators of angiogenic growth factors, such as VEGF (Kuroki et al., 1996). It has been demonstrated that NADPH oxidase not only induces the expression of VEGF (Arbiser et al., 2002) but also regulates VEGF-induced angiogenesis (Ushio-Fukai et al., 2002a, 2002b). Previous studies also revealed that ROS can enhance the production of angiogenic factors such as IL-8 and VEGF. Besides, ROS elevate the secretion of the MMP-1 by tumor cells leading to neovascularization within the tumor microenvironment (Brown et al., 2000; Milligan et al., 1996).

Another mechanism by which oxidative stress affect the blood supply within the tumor is by triggering vasodilatation. ROS activated heme oxygenase-1, results in production of carbon monoxide, or induces iNOS leading to nitric oxide (NO) production. Both carbon monoxide and nitric oxide are vasodilators (Milligan et al., 1996). It is also worth knowing that ROS may increase the risk of tumor metastasis by promoting vascular permeability, either by direct endothelial cell injury or by the up-regulation of iNOS or heme oxygenase-1 (Brown and Bicknell, 2001). It has been reported that the activity of the ROS-generating enzyme Nox1 is essential for up-regulation of VEGF, a powerful stimulator of tumor angiogenesis (Komatsu et al., 2008) and also VEGF receptors such as VEGF-R1 and VEGF-R2 are considerably induced in vascular cells in Nox1-expressing tumors. Nox1 is a potent activator of the angiogenic switch, elevating the tumor vascularity and regulating molecular markers of angiogenesis. Matrix metalloproteinase activity, another indicator of the angiogenic switch, also is stimulated by Nox1. Recent evidence reported that Nox1 induction of VEGF is reduced by co-expression of catalase, showing that hydrogen peroxide signals take part in the angiogenic switch pathway (Arbiser et al., 2002).

It has been investigated that free radicals can increase levels of HIF-1, a transcription factor responsible for cell adaptation to hypoxia and in charge of the oxygen-dependent expression of VEGF gene (Chandel et al., 1998), implying that oxidative stress in carcinoma cells might cause increased HIF-1 induction during hypoxia condition leading to further production of VEGF (Chandel et al., 2000; Richard et al., 2000). As a final point, ROS production is a consequence of cell exposure to alternate hypoxia-reoxygenation condition which per se represents a potent pro-angiogenic stimulus.

6.4. ROS and tumor cell invasion

Cell migration and invasion of tumor cells are the inceptive determinant elements in metastasis and prognosis of cancer. Through a complex multistep process and a series of cellular events involving cytoskeletal changes which result in attachment of tumor cells to the basement membrane, degradation of the extracellular matrix and migration through the degraded stroma, cells detach from the initial tumor location and metastasize to remote areas (Liotta and Stetler-Stevenson, 1990). Stimulation of cell surface receptors or amplification of intracellular signals triggers signaling pathways that maintain and provoke these changes. ROS are classified among the factors that participate in

Fig. 4. Mediators involved in ROS regulation of cell cycle.

Fig. 5. Hypoxia/Reperfusion induction of ROS and involvement of mediators in neovascularization and angiogenesis.
tumor cytoskeletal dynamics and invasion (Fig. 6) (Friedl and Alexander, 2011). It has been suggested that various pathways in the family of MAPK are activated in consequence to stimulation of receptor tyrosine kinases (RTKs) by ROS during cell migration (Hurd et al., 2012). In response to elevation of growth factor content and thus stimulation of the related receptors such as platelet derived growth factor (PDGF) and VEGF, the rise in the levels of the specific types of generated ROS leads to enhanced cell migration (Ushio-Fukai et al., 2002a, 2002b). Cellular protrusions are the initial steps in alteration of actin cytoskeleton and promotion of cell migration. As a downstream production of different signaling molecules that contribute to dynamicity of cytoskeleton, ROS are considered as both direct and indirect agents affecting cell migration. For example, an increase in Rac 1 induction of actin polymerization is significantly correlated to heightened levels of available superoxide (Moldovan et al., 1999). ROS are known to have superior control via stimulation slingshot (SSH) proteins on the activity of cofillin, an agent that can regulate actin remodeling and de-polymerization by modulating the formation of lamellipodia which are a leading class of actin rich protrusions in cell migration (Kim et al., 2009). Studies report that prior to tumor cell migration, localization of high amounts of cofillin to lamellipodia can help tumor cell migration (Friedl and Gilmour, 2009; Lai et al., 2008). Apart from lamellipodia formation, neurites and F-actin are also other cellular protrusion that ROS are considered as their essential regulating factors (Munnalai and Suter, 2009). Migration of tumor cells is followed by cellular adhesion process where integrins are proven to be central modulators by formation of focal adhesion, a connection between extracellular matrix (ECM) and tumor cell cytoskeleton (Svineng et al., 2008). Integrin signaling is accompanied by phosphorylation of focal adhesion kinase (FAK), Paxillin and p130Cas, which is mediated thorough oxidative burst and ROS generation from multifarious sources notably Nox, mitochondria and lipoxigenases (Gozin et al., 1998; Taddei et al., 2007). In addition, MMPs comprise more than 26 types of zinc dependent endopeptidases with the ability to degrade ECM components that have been associated to several steps of cancer cell invasion (Egeblad and Wahl, 2002; Overall and López-Otín, 2002). There are now considerable amount of documents on ROS regulated expression of MMPs in diverse types of cancers including pancreatic cancer, glioblastoma and breast cancer (Ellenrieder et al., 2000; Inoue et al., 2010; Nakopoulou et al., 2003). Ma et al. (2013) explored mitochondrial respiratory defect involvement in cancer pathogenicity using Rotenone inhibitor of electron transport complex-I to generate breast cancer clones. In comparison to parental cells, increased ROS and higher migration were expressed in the cancerous clones. Further observations depicted antioxidants such as polyethylene glycol (PEG)-catalase and Mito-TEMPO as potent inhibitors of tumor invasive behaviors. As an example in animal models, expression of human catalase gene (mCAT) could significantly reduce invasion of breast cancer cells in transgenic (MMTV-PyMT) mice that develops metastatic breast cancer (Coh et al., 2011). Conspicuously, HIF-1-a and VEGF are the up-regulators of ROS (Ma et al., 2013). In a study by Allili et al. in vivo melanoma tumor invasion in immunodeicient mice was subsided due to treatment with redox active cerium oxide nanoparticles (CNPs) (Allili et al., 2013). Luanpitpong et al. demonstrated the role of caveolin-1 (Cav-1) as a collaborator to cancer aggressive invasion of human lung carcinoma H460 cells. Cav-1 is regulated by intracellular ROS; however, its expression varies depended on the interaction of Cav-1 and different types of ROS. Hydrogen peroxide and superoxide anion inhibits cell migration by down regulation of Cav-1 while invasion is provoked by hydroxyl radical stimulation of Cav-1 (Luanpitpong et al., 2010). These results emphasizes that ROS can be controlled to prevent invasion and end stage cancer.

6.5. ROS adaptation by cancer cells

Depending on the present molecular context ROS can exert their heterogeneous chemical properties by regulating a wide range of downstream pathways (Fig. 7). Although at low and moderate levels ROS affect some of the most essential mechanisms of cell survival such as proliferation and homeostatic signaling, at higher levels these agents can expose cells to detrimental consequences of oxidative stress including DNA damage, gradual senescence and also apoptosis caused by mitochondrial...
permeabilization and the release of cytochrome c (Gao et al., 2007; Giannoni et al., 2005; Nakashima et al., 2003; Ramsey and Sharpless, 2006). Therefore, in an attempt to counteract these deleterious aftermaths of ROS overload which may unstable the cell viability, stressed cells trigger a variety of adaptation mechanisms by utilizing redox buffering systems and antioxidants (Fruehauf and Meyskens, 2007). Reduced glutathione (GSH) and thioredoxin (TRX) are two NADPH dependent antioxidants that restrain ROS cytotoxic effects and prevent the possible irreversible impairments by lowering the excessive levels of ROS. Regarding the oncogenic mutations that increase anomalous metabolism, escalated rates of ROS are a primary component of highly proliferative cancer cells environment. Thus, to impede this accumulation of ROS and maintain a higher level of tolerance, tumor cells initiate vast up-regulation of multiple antioxidant systems such as SOD, catalase, and peroxidases (Sundaresan et al., 1995; Vaughn and Deshmukh, 2008). Hence, with regard to this synergetic increase in both ROS content and antioxidants, it can be concluded that cancer cells control oxidation state by balancing the involved factors at higher levels than normal cells. However, loss of tumor suppressors in development process of cancer cells may provoke aberrant metabolism and disturb the cellular control over the stabilization of redox state. For example the deletion of TSC2 tumor suppressor hyperactivates mammalian target of rapamycin (mTOR) which in turn leads to inordinate translation and production of ROS (Li et al., 2010; Ozcan et al., 2008). Similar imbalances in regulation of the oxidation state have been observed in the absence of other tumor suppressors such as PTEN (Nogueira et al., 2008). In a comparable theory, PS3 plays an important role in cellular defense against oxidative stress by mediating the transcription of nuclear factor-erythroid 2-related factor 2 (Nrf2) an up-regulator of several detoxifying antioxidants (Budanov et al., 2004; Liu et al., 2008, 2009). Studies have demonstrated that DJ1 as an anti-degrading factor in neural tissues may stimulate tumorigenesis by balancing oxidative stress and preventing ROS induced apoptosis (Kahle et al., 2009). Given that in the absence of these tumor suppressors additional oxidative stress may induce selective killing of malignant cells, therefore, it is hypothesized that these mutations can be clinically exploited by targeting tumor cells with vast amounts of ROS.

7. Oxidative therapy against cancer

Although oxidative stress, considered as the imbalance of ROS production and its clearance by antioxidant defenders, is the primary cause of injury to cellular components and thus cell death, recent researches propose that selective induction of oxidative stress might be utilized as an advantageous factor in certain conditions (Dawson and Kouzarides, 2012). As tumor cells are distinguished by abnormal oxidation state and extensive amounts of ROS generation, investigating the possibility of selective targeting of cancer by drugs that can differentiate uncontrollable tumor cells from normal cells may be an efficient method in eliminating malignancies (Tandon et al., 2005). Hileman et al. study results of intracellular O$_2$$^\cdot$ content and apoptosis analyses, depicted that the more the oxidative stress increases, the more the cancerous cells relies on antioxidant enzymes (Hileman et al., 2004). An outlook on the killing mechanism of mainstream treatment methods of cancer, including irradiation and chemotherapeutic drugs, notified researchers that overproduction of ROS is a common pathway in all these leading therapies. Exhausted capacity of adaptive defenses such as SOD and other antioxidants stroke the forerunners of cancer therapy with the idea of applying ROS generating chemicals and also antioxidant inhibitors to enhance the prognosis of patients by triggering apoptosis in cancer cells (Kong et al., 2000; Kong and Lillehei, 1998). Further studies conducted on ovarian cancer and human leukemia cells demonstrated that the stability of cancer cells can be much more easily disturbed thorough 2-methoxyestradiol (ME) mediated SOD inhibition than normal cells (Aguilo et al., 2012; Brown et al., 2009). With regard to the most recent and available data, it is evident that targeting the oxidative stress inside the cancer by exposing the cells to further ROS can be the proverb of a new generation of experimental cancer therapies that can be used alone or in combination with previous methods.

7.1. Cell death by ROS increment

ROS are believed to be like a potential double-edged sword in preventing and slowing the progression of disease. Temporary changes in ROS concentration in the body can affect activity of signal transduction pathways leading to either cell proliferation, or to apoptosis and necrosis, regarding to the dosage and duration of ROS and also the type of cell. Normally, low doses can be mitogenic, while medium doses of ROS cause transient or permanent growth arrest, and high doses usually lead to cell death caused by apoptosis or necrosis (Holbrook and Ikeyama, 2002). This issue becomes more notable when precise concentration of ROS are required for normal cell function such as promoting apoptosis of precancerous or transformed cells (Martindale and Holbrook, 2002). For instance, some chemotherapy drugs and radiation therapies targeting cancer cells by producing high levels of ROS could possibly be interfered by antioxidant supplements (Seifried et al., 2004).

Disproportionate ROS levels may harm major cellular components such as DNA, proteins, lipids and membranes. Subsequent to mitochondrial lipid and protein oxidation, permeability of the mitochondrial membrane increases, and then the coupling efficiency of the electron transport chain is altered, leading to further production of free radicals, and the release of cytochrome c, and finally activating the apoptosis which depends on caspases (Conklin, 2004). It has been reported that the key mechanism by which oxidant agents may kill cells is the activation of apoptosis. More evidence showed that the oxidative stress can stimulate c-Jun N-terminal kinase (JNK) and caspases to initiate apoptotic cell death (Conde de la Rosa et al., 2006; Czaja et al., 2003) and this pathway can be attenuated through activation of protein kinase C and extracellular-signal-regulated kinase (ERK) 1/2 leading to down-regulation of JNK (Singh and Czaja, 2007). In some cases, the high concentrations of ROS may prevent apoptosis at a caspase level and switch the process toward necrosis (Chandra et al., 2000). This diversion is essential in solid tumors and requires extensive amounts of ROS, a reduction of ATP and some changes in the mitochondrial ETC (Lee et al., 1999). Therefore, different ROS
can cause necrosis via mechanisms involving mitochondrial permeability transition pore (mPTP) opening, breakdown of the mitochondrial membrane potential and ATP reduction (Nieminen et al., 1997). Nonetheless, the extracellular ROS do not trigger the mPTP but they make effects via oxidation of NADPH, Ca^{2+} uptake and mitochondrial ROS production (Nieminen et al., 1997). Taken together, these results support the important pathophysiological role of ROS in discrete apoptotic signaling pathways.

7.2. ROS-induced autophagy and cancer therapy

Autophagy, also called a process of ‘self-eating’, is an evolutionary conserved pathway and one of the nonapoptotic cell death mechanisms (Huang and Klionsky, 2002; Klionsky, 2007), which serves a housekeeping role and include engulfment of damaged or incorporated organelles into double membrane vesicles named autophagosomes. It has been illustrated that autophagy participates in several diseases such as cancer, infectious diseases, myopathies, and neurodegenerative disorders (Komatsu et al., 2006; Zhou and Spector, 2008). Recent evidence demonstrated that chemotherapeutic drugs induce autophagy in different kinds of cancer cells (Table 1) (Levine and Kroemer, 2008). On one hand, autophagy plays a crucial role in cell protection that allows cells to survive against cytotoxic agents; on the other hand, it can lead to cell death called autophagic cell death (Kondo and Kondo, 2006; White and DiPaola, 2009). Numerous works have described the beginning of autophagic mechanism in response to oxidative stress. Autophagy under these situations may serve to remove oxidized and damaged proteins and organelles, and thus maintain cellular survival. Nevertheless, if the oxidative damage is wide, autophagy can provide a way to cell death (Scherz-Shouval and Elazar, 2009).

In spite of description of ROS-induced autophagy, only a few direct factors that adjust autophagy under oxidative stress are discovered so far. In a study by Eisenberg-Lerner and Kimchi, death-associated protein kinase (DAPK) and protein kinase D (PKD) were introduced as the regulators by revealing their role in the process of autophagy in general, and particularly during oxidative stress (Eisenberg-Lerner and Kimchi, 2012a). Later, it was emphasized that both PKD and DAPK were needed for the induction of autophagy during oxidative stress, and that PKD proceeds as a downstream effector of DAPK in ROS-induced autophagy (Eisenberg-Lerner and Kimchi, 2012b). Recently, autophagy was identified to be regulated by mitochondrial superoxide (Chen et al., 2009). Based on Liu’s study, oxidized low-density lipoproteins upregulate proline oxidase (POX) to start ROS-dependent autophagy, commonly via the generation of superoxide which is a specific member of ROS produced by POX (Liu et al., 2005).

Several studies showed that ROS formation may mediate apoptosis and/or autophagy induction in several types of cancer cells (Wang et al., 2011; Wong et al., 2010). In this case, it has been found that curcumin can induced autophagy in HCT116 human colon cancer cell mediated by ROS generation (Lee et al., 2011). A study indicated that Resveratrol effectively suppressed the growth of HT-29 and COLO 201 human colon cancer cells through involvement of Caspase-8/Caspase-3-dependent apoptosis which is also mediated by ROS-triggered autophagy (Miki et al., 2012). It was also shown that under starvation situations, cells produce ROS, specifically H_2O_2, which is essential for autophagosome formation and autophagic degradation as a survival pathway (Scherz-Shouval et al., 2007).

Autophagy is often involved in several anticancer treatments that are armed against uncontrollable division of tumor cells by vast generation of ROS (Kimmelman, 2011). Photokilling of cancer cells is conducted thorough irradiation of visible light to photosensitizing agents accumulated in the specific cell organelles. This process, also called photodynamic therapy (PDT) renders a photophysical reaction in the targeted areas that leads to production of multiple cytotoxic ROS (Dolmans et al., 2003; Kessel et al., 2012). Depending on the molecular context of PDT target tissues, different signaling pathways of autophagy induction can result in contrasting responses (Reiners et al., 2010). Decreased accumulation of oxidatively damaged macromolecules and increased clearance of nonfunctional cellular agents have been observed after PDT-induced autophagy. Silencing of autophagy-related protein-5 (Atg5) or Atg7, two critical autophagy genes, is related with increased apoptotic photo killing; thus, it can be concluded that autophagy may contribute to cancer defending mechanisms against ROS-based anticancer therapies (Dewaele et al., 2011).

Table 1
The common pro-autophagy drugs on cancer cells.

<table>
<thead>
<tr>
<th>Drugs</th>
<th>Targeted cells</th>
<th>Outcomes</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arsenic trioxide</td>
<td>Malignant glioma cells, leukemia cells, ovarian</td>
<td>Dose-dependent cell arrest in all cell types</td>
<td>Gousetis et al. (2010), Kanzawa et al. (2003), and Smith et al. (2010)</td>
</tr>
<tr>
<td>(As2O3)</td>
<td>Carcinoma cells</td>
<td>Induced cytotoxicity and concentration-dependent death in multiple cell lines</td>
<td>Egger et al. (2013) and Geng et al. (2010)</td>
</tr>
<tr>
<td>Chloroquine</td>
<td>Melanoma cell lines, glioma cells</td>
<td>Induction of autophagy while preventing oxidative stress as an antioxidant</td>
<td>Han et al. (2012) and Kim et al. (2012)</td>
</tr>
<tr>
<td>Curcumin</td>
<td>Endothelial cells, oral cancer cells</td>
<td>Induction of autophagy</td>
<td>Wei et al. (2010) and Zhang et al. (2009)</td>
</tr>
<tr>
<td>Fulvene C60</td>
<td>Normal and drug-resistant cancer cells</td>
<td>Killing both normal and drug resistant cell lines</td>
<td>Giannopoulou et al. (2009) and Kalofonos et al. (2009)</td>
</tr>
<tr>
<td>Panitumumab (EGF-Rantibody)</td>
<td>Colon cancer cells</td>
<td>Reduced cell proliferation</td>
<td>Marino et al. (2010)</td>
</tr>
<tr>
<td>Esomeprazole</td>
<td>Melanoma cells</td>
<td>Induced accumulation of ROS as well as autophagosomes</td>
<td>Liu et al. (2011) and Tang et al. (2010)</td>
</tr>
<tr>
<td>Recombinant Human MCM1</td>
<td>Leukemia cells, pancreatic cancer cells</td>
<td>Increased expression of the autophagic markers and autophagosome formation</td>
<td>Puisant et al. (2010), Scarlatti et al. (2008), Signorelli et al. (2009)</td>
</tr>
<tr>
<td>Resveratrol</td>
<td>Breast cancer cells, myelogenous leukemia cells,</td>
<td>Triggers death and arrests cell proliferation while promoting autophagy</td>
<td>Cao et al. (2008) and Yamamoto et al. (2008)</td>
</tr>
<tr>
<td>Suberylanilide</td>
<td>Chondrosarcoma cells, Hela S3 cells</td>
<td>Induction of nonapoptotic cell death with autophagy</td>
<td>Hermann-Antosiewicz et al. (2006)</td>
</tr>
<tr>
<td>Sulfasphane</td>
<td>Prostate cancer cells</td>
<td>Increased autophagy</td>
<td>Bursch et al. (1996) and De Medina et al. (2009)</td>
</tr>
<tr>
<td>Tamoxifen</td>
<td>Mammary carcinoma cells</td>
<td>ROS-dependent inhibition of cell replication and vast accumulation of autophagic vacuoles</td>
<td>Kanzawa et al. (2004) and Milano et al. (2009)</td>
</tr>
<tr>
<td>Temozolomide</td>
<td>Malignant glioma cells</td>
<td>Inhibition of cell viability, remarkable increase in disruption of cell cyle and autophagic cell death</td>
<td>Fu et al. (2010)</td>
</tr>
</tbody>
</table>
Mroz et al., 2011). Although the mentioned pro-survival function of autophagy can be provoked in particular conditions, further investigations have demonstrated that PDT-mediated autophagy in cancer cells with defective apoptotic pathways navigates the cell into pro-death settings. This data suggest that autophagy is the primary requirement of efficacious photo killing in tumor cells with inoperative cell death mechanisms; therefore, the ultimate aftermath of autophagy in PDT is directly dependent on the functionality of cell apoptotic systems (Kessel et al., 2006).

As a promising treatment for patients with acute promyelocytic leukemia, arsenic trioxide (ATO) mechanism is mostly relied on a multiplex of ROS generating pathways that induce apoptosis (Beauchamp et al., 2011). However, studies are suggestive of ATO-mediated autophagic cell death besides other oxidative rendered apoptotic cascades (Goussetis et al., 2013). Recent studies have shown a disruption in Bcl-2/Bcl-XL expression along with loss of mitochondrial membrane potential in several tumor cell lines (Momeny et al.; Selvaraj et al.; Zheng et al., 2010). Enlightening the role of ATO in malignant glioma autophagic cell death, Kanzawa et al. have reported BNIP3 as a central factor (Kanzawa et al., 2005). Hence, it can be concluded that Bcl-2/Bcl-XL down-regulation and BNIP3 up-regulation are potent targets of autophagy. A study by Chiu et al. revealed that ATO induced inhibition of Survivin, a protein overexpressed in glioma cells, could trigger both apoptosis and autophagy in human glioma cell line U118-MG (Chiu et al., 2011). An in vitro investigation of human T-lymphocytic leukemia and myelodysplastic syndrome cell lines has indicated the up-regulation of Beclin-1 as an involved mechanism in ATO autophagic cell death (Qian et al., 2007). However, analysis of ATO induced death on transforming growth factor-β signaling mediators in ovarian cells by Smith et al. demonstrated the role of SmoN in promotion of ATO mediated autophagic cell survival as a beclin-1-independent pathway (Smith et al., 2010). With regard to the diversity of ATO triggered pathways in apoptosis and autophagy mediated cell death, it seems that ATO response is cell type dependent.

In addition, 2-methoxyestradiol (2-ME) has potent anti-angiogenic, anti-proliferative and ROS generating capacities, as a result of its potential in inhibiting both mitochondrial respiratory chain and SOD (Fukui and Zhu, 2009). Assessments of the in vitro antimitotic activities of 2-methoxyestradiol-bis-sulphamate on the non-tumorigenic MCF-12A breast epithelial cell line endorse the differential death mechanisms of this agent (Visagie and Joubert, 2012). 2-Ethyl-3-O-sulphamoyl-estra-1,3,5 [16-tetraene (ESE-16)], a novel 2-ME analogue is designed to improve anti-mitotic activities of 2-methoxyestradiol-bis-sulphamate on the non-tumorigenic MCF-12A breast epithelial cell line (Duechler et al., 2008; Han et al., 2005). Enhanced responses against tumor cells in synergetic use of BSO and melphalan supports the strategy of combining GSH-depleting agents with chemotherapeutic drugs as a successful method (Anderson et al., 2000), Kimani et al. reported that combination of antioxidant inhibitors and photodynamic therapy (PDT) may result in higher levels of ROS generation and apoptosis in MCF-7 cancer cells compared to PDT alone (Kimani et al., 2012), Considering that the overall oxidative state of cell is controlled by both ROS production and elimination; hence, appropriate results in killing cancer cells depends on using a proper combination of antioxidant inhibitors and ROS generating factors.

8.1. ROS-generating drugs as chemotherapeutics in cancer

Higher level of oxidative stress in tumor cells compared to normal tissue cells makes it reasonable that production of a cancerous tissue is more dependent to defense systems such as antioxidants than normal cells (Hileman et al., 2004). Based on this notion researchers have set strategies to increase ROS content, weather by attenuating the prominent role players of the cancer defense line or by raising the ROS level as a direct target (Pelicano et al., 2004). Currently, several anticancer therapeutic agents are known which can stimulate oxidative stress, and thus kill tumor cells in a preferential manner. Cisplatin, arsenic trioxide, anthra-cyclines and bleomycin are among the most common applied ROS generators in therapy strategies (Zhang et al., 2009). Cisplatin is a highly effective ROS generator and the first member of a class of anti-cancer drugs, called platinum containing agents. The platinum atom of this chemical substance can mediate DNA cross linking by binding to guanine bases and promoting apoptosis as a result (Huang et al., 1995). Studies suggest that TNF-α induced ROS production and also activation of NADPH oxidase, which has a notable effect on signaling pathways of oxidative stress, are two

8. Approaches to generate ROS as cancer therapy

The possession of higher capacity in coping with ROS induced stress might make the use of direct or indirect ROS generators possible in preferential killing of cancer cells and ameliorating the therapeutic course of the disease toward a selective method (Diehn et al., 2009). Hence, in order to inflict cancer cells with lethal damages of oxidative stress, treatment strategies trigger apoptosis by either utilizing ROS producing agents or striking of tumor cell strongholds (Benhat et al., 2002). The use of ROS generating drugs is now widespread as the main or adjuvant method in treating cancer; therefore, these agents are reviewed in a separate topic. Penetration of cell defend lines can be achieved in two principal ways: inhibition of antioxidants or demotion of intrinsic capacity of buffering oxidants (Fruehauf and Meyeskens, 2007). Clinical trials have observed a remarkable attenuation of ROS insults in confront with antioxidant systems. Shi et al. in a recent study of formaldehyde-induced toxicity in A549 human lung cancer cell line reported reduced levels of ROS and DNA-protein cross-links with selenium pretreatment (Shi et al., 2012). A study of rat alveolar macrophages by Pathania et al. demonstrated suppressed ROS content in response to vitamin E supplementation (Pathania et al., 1999). Thus several researches have tested the ability of antioxidant inhibitors such as ATO and 2-ME in compromising cellular ROS coping potentials (Han et al., 2010; She et al., 2007). Involvement of these agents in multi biological pathways may also enable them to deploy their toxic effects by interfering in cellular reducing buffering systems as well as further ROS generation. For instance, besides inhibiting glutathione peroxidase antioxidant, ATO can also render vast generation of ROS via impairing the mitochondrial respiratory chain (Dai et al., 1999; Lu et al., 2007). GSH as the leading component of thiol buffer plays an important role in survival of tumor cells targeted by cisplatin or ATO (Balendirian et al., 2004; Galluzzi et al., 2012). Increased apoptotic effect of ATO in cells such as HL-60 and arsenic resistant NB4 cell lines is also observed in its concomitant use with Buthionine sulfoximine (BSO) that can lower GSH content by inhibiting glutamylcysteine synthetase (Duechler et al., 2008; Han et al., 2005). Enhanced responses against tumor cells in synergetic use of BSO and melphalan supports the strategy of combining GSH-depleting agents with chemotherapeutic drugs as a successful method (Anderson et al., 2000). Kimani et al. reported that combination of antioxidant inhibitors and photodynamic therapy (PDT) may result in higher levels of ROS generation and apoptosis in MCF-7 cancer cells compared to PDT alone (Kimani et al., 2012). Considering that the overall oxidative state of cell is controlled by both ROS production and elimination; hence, appropriate results in killing cancer cells depends on using a proper combination of antioxidant inhibitors and ROS generating factors.
probable mechanisms of cisplatin mediated cell death (Kim et al., 2010; Woo et al., 2000). The remarkable efficacy of ATO in inhibiting acute promyelocytic leukemia growth led to further investigations on the acting mechanisms of this agent. Although its exact site of action is still not completely clear, the drug is supposed to disrupt mitochondrial membrane permeability by increased production of intracellular ROS, notably superoxides (Choi et al., 2002). Daunorubicin and Doxorubicin are two members of anthracycline family which trigger apoptosis by increasing intracellular ROS. Daunorubicin ROS products interact with JNK/ Stress-activated protein kinase (SAPK) pathway while doxorubicin causes mitochondrial damage by immense generation of superoxide and hydrogen peroxide (Laurent and Jaffrézou, 2001; Zhang et al., 2012). Studies demonstrate that bleomycin initiates oxidative stress induced cell death by regulating caspase-8 and caspase-9. In late phases Fas can also amplify this mechanism in epithelial cell apoptosis (Wallach-Dayan et al., 2006).

8.2. Radiation induced-ROS as cancer therapy

A vast number of researches suggest that the potential of cancerous tumors in neutralizing and defending against ROS agents can directly affect their chemotherapy resistance level. Therefore, diverse anti-cancer drugs have been developed to increase the ROS production as a direct target or by interaction with ROS scavenging enzymes. Efforts in investigation of solutions intensifying radiation therapy have helped researchers develop two strategies, dose escalation of radiation therapy and introduction of a new class of cytotoxic chemotherapy drugs called radiosensitizers and enhancers. Among all these anti-cancer agents Procarbazine, Buthionine sulfoximine (BSO), and Motexafin gadolinium (MGd) are well known examples that some have been even approved by the U.S. Food and Drug Administration (FDA) (Renschler, 2004). Procarbazine was one of the first developed drugs with ROS-generating properties (Berneis et al., 1963). It is hypothesized that the production of hydrogen peroxide by oxidation of procarbazine in aqueous solution plays a critical role in the cytotoxic mechanism of this agent as a new class of tumor inhibiting compounds (Gupta et al., 2012). Early screening conducted in mice and rats showed the procarbazine ability in prevention of transplantable tumors growing including Walker carcinoma 256 and Ehrlich carcinoma and also reduction of Ehrlich ascites cells mitoses (Schwartz et al., 1967). The MOPP regimen, a combination of Mustargen with Oncovin, Procarbazine and prednisone, has achieved long term remissions in more than 80% of patients. The synergistic effects of Procarbazine with radiation therapy has ranked it as a top selection drug primarily for the treatment of Hodgkin’s and non-Hodgkin’s lymphomas, and also brain tumors since its early approval. Considering that procarbazine can easily pass the blood brain barrier with a balanced concentration between plasma and cerebrospinal fluid, its effects has been widely researched in brain tumors, especially in patients with glioma (Armand et al., 2007). Despite limited usage, malignant melanoma, multiple myeloma, and lung cancer has also been targeted by Procarbazine (Falkson et al., 1965).

BSO is an efficacious and specific inhibitor of gamma-glutamyl-cysteine synthetase which is commonly up-regulated in tumors resistant to chemotherapy (Griffith, 1982; Renschler, 2004).

In phase I of clinical trials, it was claimed that the administration of BSO is safe and notable reduction of GSH level (< 10% of pretreatment value) in cancer patients was seen (Bailey et al., 1997). In a similar conclusion, depletion of GSH content of tumor cells increases the sensitivity to agents mediating cytolysis such as sesquiterpene lactones, platinum compounds, alkylators and also ROS generated by acute phase leukocytes (Arrick et al., 1983; Meijer et al., 1992). A recent research carried out by Maeda et al. suggested that the combination of BSO and arsenic trioxide (As₂O₃), a potent ROS generator that has been shown to enhance clinical remission in cases of acute promyelocytic leukemia, can result in successful treatment of advanced solid tumors (Yang et al., 1999). In vitro studies of this combination has also shown improvements in inhibition of growth in cell lines of prostate, lung, bladder, colon, cervix, and kidney cancers (Yang et al., 1999).

MGd is an aromatic macrocycle with radio-enhancing properties which has a unique effect on increasing the whole brain radiotherapy index in brain metastases (Khuntia and Mehta, 2004). In the molecular pathway, MGd targets and inhibits oxidative stress-related antioxidant enzymes such as thioredoxin reductase. It is a hydride donor in re-reduction of GSSG to GSH, therefore, also is suggested to act as an indirect antioxidant by restoring the antioxidative power of glutathione. Hence, it is thought that the interactions of MGd will ultimately lead to a reduced repair ability of cancerous cells in a clash against oxidative damage induced by radiation and an increase in responsiveness to a variety of tumor treatments (Bradley et al., 2013a; Hashemy et al., 2006). In vitro studies on cancer cell lines of multiple sources have shown that in redox cycling and presence of oxygen, MGd forms superoxide and several other ROS by accepting electrons from various cellular reducing metabolites. The improving effects of MGd combination treatment with ionizing radiation have been observed in some controlled clinical trial investigations (Magda and Miller, 2006). Mehta et al. in a phase III randomized trial assessed neurocognitive function and survival rate in two groups of patients receiving whole brain radiation with or without MGd (Mehta et al., 2003). There were no remarkable differences in the overall results; however, neurologic findings demonstrated MGd treatment benefits and an improved time to neurologic progression in all patients (Mehta et al., 2003). To improve the final outcomes, Bradley et al. administered MGd prior to daily radiation, in phase II of a clinical trial; in contrast to the study conducted by Mehta et al., the results did not support the role of this radiosensitizing agent in enhancing the survival rate of pediatric patients suffering from newly diagnosed intrinsic pontine gliomas (Bradley et al., 2013b).

9. Targeting mitochondrial ROS as cancer therapy

Mutated cancer cells with associated disruption of mitochondrial transport chain are more likely to exhibit chronic states of metabolic oxidative stress and thus have a higher susceptibility to ROS induced apoptosis (Li et al., 2013). This escalated level of tumor cells sensitivity can be exploited for selective demolition of cancer cells (Dilda and Hogg, 2005). Mitocans are a group of anti-cancer drugs able to specifically target and destabilize cancer cells mitochondria as their Achilles heel for tumor destruction by unleashing the apoptogenic potential that can suppress tumor growth (Neuzil et al., 2007). Superoxide formation is induced as a frequent aftermath of mitocans specific for the mitochondrial electron transport chain inhibition, resulting in the preferential killing of cancer cells. Furthermore, the rare mutations of macro-molecular complexes of the electron transport chain is an indication of useful targets for anti-cancer drug development (Rohlena et al., 2013). Siedlakowski et al. have reported Pancratistatin ability in selective induction of mitochondrial apoptosis in human breast cancer cells lines MCF-7 and Hs-578-T compared to non-cancerous counterparts thorough mitochondrial membrane permeabilization, increased levels of ROS and decreased ATP (Siedlakowski et al., 2008). Investigation of BTG2 pathways, a tumor suppressor gene associated to cancer cell metastasis formerly, in A549 and PC3 cell lines showed that BTG2...
overexpression can synchronously increase Src reduction state and inhibit ROS production by being localized in mitochondria. Downregulation of Src-FAK signaling also resulted in suppressed cell migration (Lim et al., 2012). Compound K modulation of Bax and Bcl-2 in HT-29 human colon cancer cells can disrupt mitochondrial membrane potential and activate caspase 3 and 9. As a result, the intracellular generation of ROS led to a mitochondria-dependent apoptosis (Lee et al., 2010). Emodin-mediated apoptosis in human tongue cancer cells is another example of caspase-9 and caspase-3 cell death concomitant with the release of cytochrome c from mitochondria and ROS generation (Lin et al., 2009). Together the emerging of recent researchers on mitochondria anti-cancer drugs which mostly are dependent on imbalances of mitochondrial membrane potential and ROS production emphasizes the crucial role of mitochondria as an organelle that can be targeted in selective killing of cancer cells. Thus, the continuous research in this area will lead to introduction of novel drugs with the least adverse effects on normal cells.

10. Antioxidants in oxidative therapy against cancer

Although ROS formation is the primary mechanism of most chemotherapy drugs against cancer, unfortunately, normal tissues are also exposed to the serious side effects of these agents (Wiseman, 2005). For example, both cisplatin and anthracycline as two common drugs of current chemotherapeutic strategies can develop severe complications including nephrotoxicity, peripheral neuropathy and cardiotoxicity (Monsuez et al., 2010; Rajeswaran et al., 2008). Thus, to counteract ROS side effects and alleviate the deterioration of normal tissues, a toxicity neutralizing method which can enable much more patients to undergo chemotherapy regimens, may be considered beneficial in combination with chemotherapy (Lindley et al., 1999). While investigating the correlation between ROS, cancer and serum levels of many other related factors, contrasting levels of antioxidants in different tumors intrigued researchers to a new influencing factor on cancer development that could be beneficial in chemotherapy (Table 2) (Conklin, 2000). A few reports show that some types of cancers have elevated levels of antioxidants; however, studies propose that this increased activity of antioxidant systems is a response of cancerous cells to the chemotherapeutic agents (Jarvinen et al., 2000; Portakal et al., 2000). Biochemical evaluations demonstrated that in most human cancers antioxidant levels are below the normal range. Increased oxidative stress and low antioxidant status are even seen before the initiation of oncology treatment in patients with tongue and lung cancers (Klarod et al., 2011; Sharma et al., 2009). Therefore, it may be possible that the diminished amounts of antioxidants are able to make cells susceptible to carcinogenic agents. The debate arises from the role of antioxidants as preventive agents of cancer progression and their efficacy on altering patients’ prognosis has become the focal point of several researches (Borek, 2004). The inverse relation of cancer risk with consumption of dietary supplements and foods with high levels of antioxidants is shown in some epidemiological and interventional studies. Selenium, carotenoids, vitamin E and C are among antioxidants that are supposed to have anti-cancer effects (Borek, 2004; Watson and Leonard, 1986). Antioxidants such as vitamin E, D and C can cause selective induction of apoptosis in tumor cells but not in normal cells and prevent metastatic spread and angiogenesis (Hong et al., 2007; Mathiasen et al., 1999; Patacsi et al., 2012). Han et al. reported an attenuated risk of pancreatic cancer in association with dietary intake of selenium (Han et al., 2013). In another study Gopalakrishna et al. have focused on the crucial role of selenium as an inhibitor of tumor promoting signals generated by enzymes such as protein kinase C. The study concluded that advanced malignancies may be developed by a resistance to selenium (Gopalakrishna and Gundimeda, 2002). Vitamin E succinate inhibition of colon cancer growth and metastases both in vitro and in vivo via tumor apoptosis and suppression of cell proliferation was manifested in a clinical trial by Barnett KT et al. (Barnett et al., 2002). An analysis of eight cohort studies conducted by Eliassen et al. proved that high levels of circulating carotenoids may reduce the risk of breast cancer (Eliassen et al., 2012). Adverse side effects of cancer therapy to normal cells such as fibrosis and mucositis were shown to be improved by patients using vitamin E and C (Citrin et al., 2010). On the other hand, there are also opposing data to the positive properties of antioxidants as a cancer adjuvant treatment. To compare the enhancing effects of antioxidants with placebo Bjelakovic et al. reviewed all available related randomized trials in prevention of gastrointestinal cancers. No evidence was found in accordance with the antioxidants cancer prevention hypothesis. On the contrary, results showed an overall increase of mortality in patients consuming antioxidant supplements (Bjelakovic et al., 2004). Cortes Jofre et al. also did not recommend the use of any combinations of vitamin supplements to decrease lung cancer mortality in healthy people. There are some documents revealing a small increase of mortality and risk in lung cancer patients who are either smokers or exposed to asbestos and use beta carotene supplements (Cortes Jofre et al., 2012). Although studies have achieved valuable results from trials, determining the ability of antioxidants to reduce cancer risk has not been totally conclusive and further research on this topic is still needed to resolve the current controversies.

11. Conclusion

ROS is a potential double-edged sword in progression and prevention of cancers (Fig. 8). Regarding to the dosage and duration of

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Table 2:
The common antioxidants involved in clinical cancer trials.

<table>
<thead>
<tr>
<th>Antioxidant supplement</th>
<th>Targeted patients</th>
<th>Outcome</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vitamin A</td>
<td>Lung cancer</td>
<td>No difference in survival of smokers, protective in nonsmokers</td>
<td>Feskanich et al. (2000) and van Zandwijk et al. (2000)</td>
</tr>
<tr>
<td>Vitamin C</td>
<td>Terminal cancer patients</td>
<td>Lower scores for appetite loss, fatigue, nausea/vomiting and pain</td>
<td>Yeom et al. (2007)</td>
</tr>
<tr>
<td>Vitamin E</td>
<td>Primary cancer prevention</td>
<td>No overall benefit in mortality, may increase all-cause mortality in high dose</td>
<td>Lee et al. (2005) and Miller et al. (2005)</td>
</tr>
<tr>
<td>Selenium</td>
<td>Prostate cancer</td>
<td>Reduced risk, no prevention</td>
<td>Etminan et al. (2005) and Lippman et al. (2009)</td>
</tr>
<tr>
<td>Coenzyme Q10</td>
<td>End-stage cancer patients</td>
<td>Improved survival</td>
<td>Hertz and Lister (2009)</td>
</tr>
<tr>
<td>Melatonin</td>
<td>Solid tumor cancer patients</td>
<td>Substantial reduction in risk of death with no severe adverse events</td>
<td>Mills et al. (2005)</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>Colorectal cancer</td>
<td>Reduced recurrence rate of colon neoplasia</td>
<td>Hoenschi et al. (2008)</td>
</tr>
</tbody>
</table>

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ROS and also the type of cell, all process of cell life including proliferation, or apoptosis and necrosis are specifically influenced by temporary changes in ROS concentrations and the affected signal transduction pathways. It can be concluded that cancer cells control oxidation state by balancing the involved factors at higher levels than normal cells. Thus, to impede this accumulation of ROS and maintain a higher level of tolerance, tumor cells initiate vast up-regulation of multiple antioxidant systems such as SOD, catalase, and peroxides. Combination therapies in cancer patients could be achieved in order to either increase the intensity of ROS, or to perform different cytotoxicity mechanisms. Therefore, there is an opportunity to extend handling strategies in which ROS can either induce or inhibit cancers in different conditions.

Declaration of interest

The author(s) report no conflicts of interest. The authors alone are responsible for the content of the paper.

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