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Natural Compound-Generated Oxidative Stress: From Bench to Bedside

Aloran Mazumder and Marc Diederich

Additional information is available at the end of the chapter

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Abstract

Oxidants are constantly generated in a biological system as a result of physiological processes. However, an imbalance between oxidants and antioxidants can lead to a pathophysiological condition known as oxidative stress. Natural compounds as inducers of oxidative stress are able to modulate physiological functions of cancer cells leading to cell death or survival. This chapter aims at providing an overview of pro- and antioxidant activities of natural compounds related to cancer and related therapies.

Keywords: natural compounds, cancer, oxidative stress, clinical use

## 1. Natural compound anticancer agents

In the search of improved cytotoxic agents against cancer, natural compounds possess advantages with regard to availability, low toxicity, and suitability for oral application and metabolite likeliness [1]. Moreover, new technologies of combinatorial chemistry and high-throughput screening are used to design different synthetic drugs with natural compounds that serve as templates for development of novel molecules with enhanced biological properties.

In 1960, the National Cancer Institute (NCI) began a large-scale screening program for anti-tumor agents, and 35,000 plant species samples were tested primarily on mouse leukemia cells [2, 3]. The most promising drug to emerge from this program was paclitaxel, a microtubule disruptive agent obtained from the bark of the Pacific yew *Taxus brevifolia*. This finding served as the springboard for further investigations with natural compounds, and in the late 1960s, vinblastine and vincristine were reported from *Catharanthus roseus*. Both drugs major-

ly contributed to long-term remission and cures for childhood leukemia, Hodgkin's lymphoma, testicular teratoma, etc. Other anticancer agents to enter clinics, which are derived from natural sources, include etoposide, which has been proven as an effective treatment against testicular teratoma and small cell lung cancer, whereas teniposide was shown to be effective against acute lymphocytic leukemia (ALL) and neuroblastoma in children and non-Hodgkin's lymphoma [1]. A comprehensive study published on new medicines approved by US Food and Drug Administration between 1981 and 2010 revealed that 34% of those medicines based on small molecules were either natural products or a direct derivative which mainly included statins, immunosuppressant, and tubulin-binding anticancer drugs [4, 5].

Natural compound constituents demonstrated anticancer activity according to a combination of epidemiological and experimental studies [6]. Mechanistic insights underlined that the chemotherapeutic potential of these agents may be a combination of antioxidant, anti-inflammatory, immune-promoting, cytostatic, differentiating, and cytotoxic effects. Altogether, natural compounds efficiently prevent initiation, promotion, and progression of cancer development thus interfering with all 10 hallmarks and enabling characteristics of cancer [7–10].

Increasing technological advancements led to the development of better purification techniques with defined molecular assays, which can efficiently exclude "distracting molecules" such as tannins and saponins, thereby increasing the chances of identifying the critical agent with specific anticancer activity. The diverse bioactivity potential of natural compounds can be related to the huge structural diversity existing in nature. This compound repertoire is available for further modifications to improve the therapeutic potential of lead compounds. In addition, combinatorial biosynthesis further modulates the functional groups of lead compounds and can be complemented with high-throughput screening, computational chemistry,
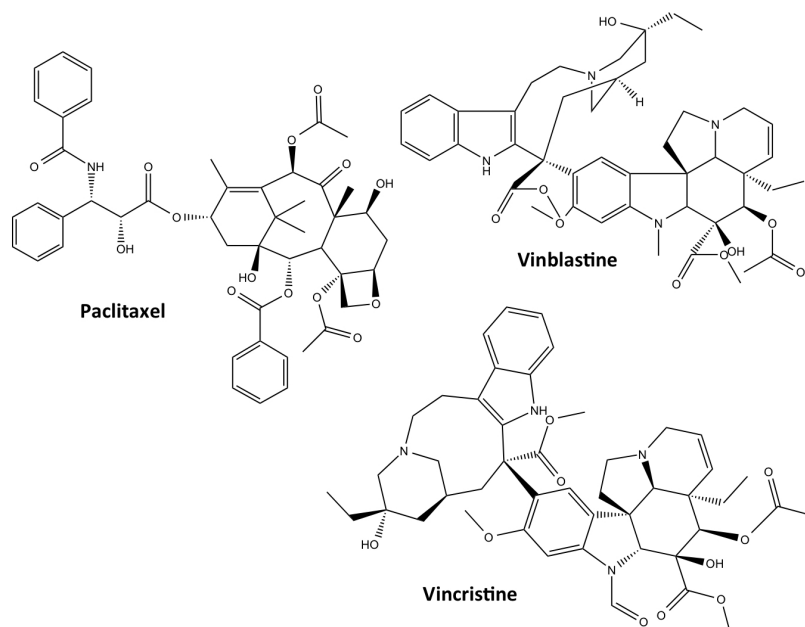
Figure 1. Molecular scaffolds of plant anticancer agents. Molecular structures were drawn with ChemDraw13 (Perkin Elmer).

and bioinformatics to generate structural analogues with improved pharmacological activity and reduced toxicity [1] (**Figure 1**).

2. Natural compounds as scavengers of free radicals

Oxidants are constantly generated in a biological system as a result of physiological processes. However, an imbalance between oxidants and antioxidants can lead to a pathophysiological condition known as oxidative stress [11]. In light of this knowledge, oxidative stress has been defined as perturbations in redox homeostasis. Broadly, the cellular redox level is regulated by three different systems, two of which are dependent on glutathione that includes glutathione (GSH), glutathione reductase (GR), glutathione peroxidases (GPX), and glutathione S-transferases (GST) [12–14]. Glutathione undergoes oxidation to form glutathione disulfide (GSSG), thereby reducing the disulfide bonds of cytoplasmic proteins to cysteine and protects the cell against oxidative stress [15]. Under normal conditions, GSH exists in reduced form due to constitutive activity of GR. GSTs act as detoxifying enzymes that conjugate GSH to various electrophilic compounds [16].

Reactive oxygen species (ROSs) have been reported in both solid and hematopoietic cancers where they are associated with tumor development and progression [17, 18]. However, cancer cells also express antioxidant proteins to detoxify ROS, suggesting that the fine-tuning of intracellular ROS signaling is critical for cancer. Therefore, understanding the susceptibility of cancer cells to oxidative signals could open new therapeutic window for rational design of new anticancer agents [19]. In addition to their well-characterized effects on cell division and viability, cytotoxic agents can induce oxidative stress by modulating levels of ROS such as the superoxide anion radical, hydrogen peroxide, and hydroxyl radicals. Eukaryotic cells have highly organized pathways to orchestrate the many extracellular stimuli received and convert them into specific physiological processes. This classical cascade also termed as signal transduction pathways includes a series of events occurring constitutively and initiated by interaction of a ligand with its receptor on the cell membrane. ROS in this cascade have been proposed as second messengers in the activation of signaling events that lead to survival or death [20]. Moreover, redox-sensitive cysteine residues are known to sense and transduce changes in cellular redox status caused by ROS production and the presence of oxidized thiols. Various dietary phytochemicals have been shown to exhibit beneficial effects including the prevention of cancer by modulating the cellular redox status by acting as either an antioxidant or pro-oxidant. They function as detoxifying enzyme inducers, which mainly include phenolic and sulfur-containing compounds. Phenolic compounds are classified as polyphenols or flavonoids, whereas sulfur-containing compounds may be classified into isothiocyanates and organosulfur compounds. Epigallocatechin-3-gallate (EGCG) from green tea, curcumin [21–24] from turmeric, and resveratrol [25, 26] from grapes are the classical examples of polyphenols, whereas flavonoids include quercetin from citrus fruits [26–28] and genistein from soya. Isothiocyanates represent a group of compounds such as sulforaphane from broccoli and phenethyl isothiocyanate from turnips. Organosulfur compounds mainly include diallyltetrasulfide derived from garlic [29–34]. Cells respond to these phytochemicals by a non-classical

receptor-sensing mechanism of electrophilic chemical stress characterized as "thiol-modulated cellular signaling" events leading to gene expression commending the pharmacological activity (**Figure 2**).
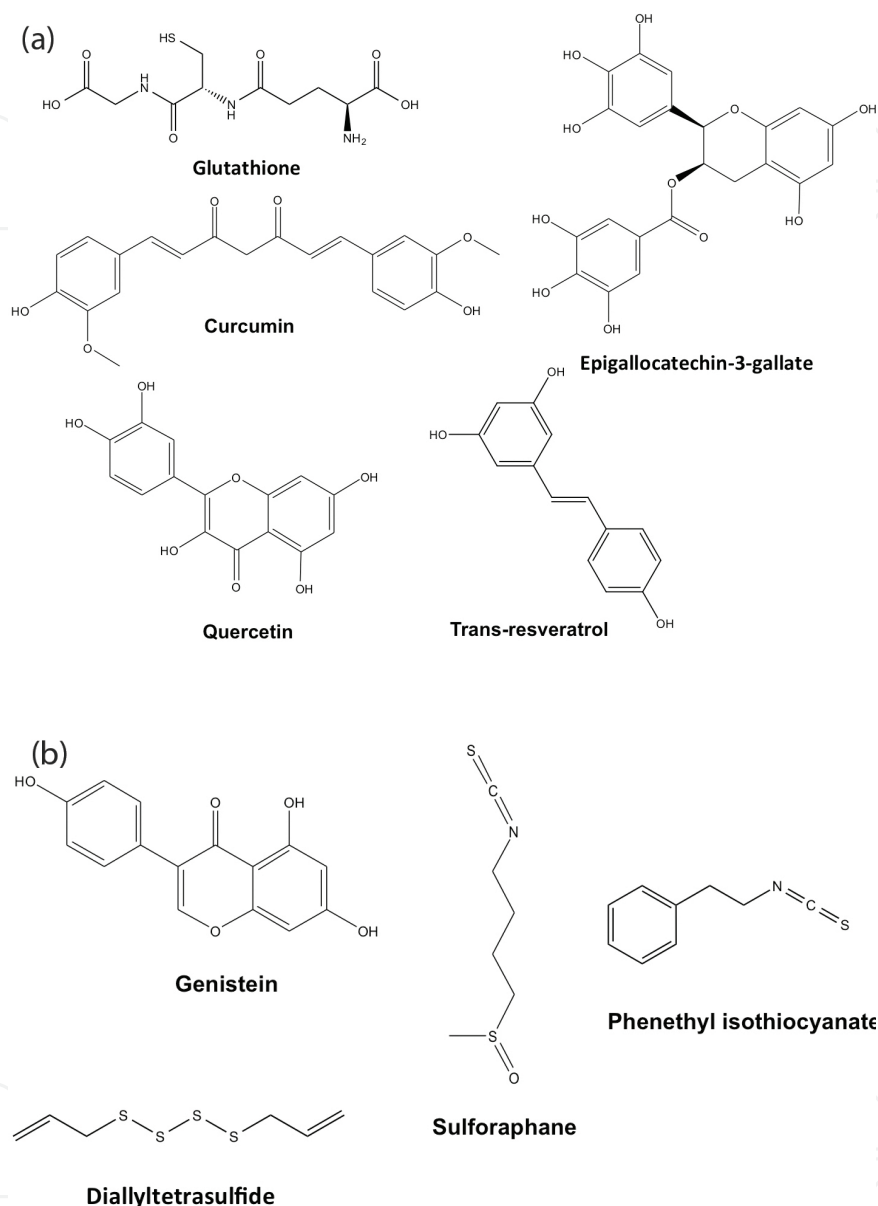
Figure 2. Natural compounds as scavengers of free radicals. Molecular structures were drawn with ChemDraw13 (Perkin Elmer).

## 3. Survival pathways activated by free radicals

ROSs are tumorigenic as elevated levels of ROS-sensitive signaling pathways have been implicated in various cancers where they are involved in sustenance of cell growth, prolifer-

ation, survival, migration, and by inducing DNA damage leading to formation of genetic lesions initiating tumorigenesis [35, 36]. Low levels of hydrogen peroxide (H_2O_2) stimulation have been shown to propagate cell proliferation in an array of cancer cell types. Role of hormones in endocrine cancers is well documented. In hormone-dependent breast cancer cells, one of the functions of estrogen is to translocate to mitochondria, thereby initiating mitochondrial ROS production that can be impaired by inhibition of mitochondrial uniporter, which prevents estrogen-induced cell proliferation [37, 38]. Sodium arsenic in MCF-7 was shown to mimic the effect of estrogen and potentiated S phase progression and proliferation by inducing ROS production and ROS-related depolarization of the mitochondrial membrane [39]. Moreover, estrogen-induced cell proliferation of MCF-7 was strongly inhibited by antioxidants such as *N*-acetyl-L-cysteine (NAC) or mitochondrial blockers of protein synthesis such as chloramphenicol [40]. ROS generation was shown to augment G1/S transition by increasing the expression levels of cyclins D1, D3, E1, E2, and B2 [41]. In contingent to these finding, cytochrome P450B1-mediated conversion of estrogen to a putative carcinogenic metabolite 4-hydroxyestradiol in human mammary epithelial cells MCF-10 leads to intracellular ROS production and neoplastic transformation. ROS overproduction was shown to activate IκB kinase (IKK) signaling with increased nuclear translocation and NF-κB activity [42].

Since deregulation of NF-κB is related to increased cell survival, proliferation, and development of drug resistance in different cancers, series of work conducted in this direction showed that NF-κB is a redox-regulated sensor for oxidative stress and is activated by low doses of H_2O_2 [43, 44]. In MCF-7 cells, interleukin (IL)-1β stimulation of NF-κB is partially regulated by H_2O_2-mediated activation of NF-κB inducing kinase (NIK)-mediated phosphorylation of IKKα [45]. Moreover, overexpression of manganese superoxide dismutase (MnSOD) in MCF-7 cells completely abolished tumor necrosis factor (TNF) α-mediated NF-κB activation, IκBα degradation, p65 nuclear translocation, and NF-κB-dependent reporter gene expression [40]. In other forms of cancer such as oral squamous carcinoma, a mild difference in endogenous ROS functions as a physiological signaling modulator of the NF-κB signaling cascades through its ability to activate NIK [46]. Besides solid tumors, redox regulation of NF-κB has also been implicated in hematopoietic cancers. Our group for the first time reported that in U937 cells, melatonin a pineal hormone might induce ROS generation, which ultimately is involved in transactivation of NF-κB-promoting survival of these cells [47–50]. Moreover, myeloid leukemia, which often maintains a high intracellular ROS level and uses redox signal for survival, is sensitive to NF-κB inhibition since NF-κB is involved in moderating the ROS level, which prevent activation of c-Jun N-terminal kinase (JNK) and cell death [51–54] (**Figure 3**).

Apart from NF-κB, ROS-mediated regulation of tyrosine phosphatases, protein tyrosine kinases, and receptor tyrosine kinases, which is critical for cell survival and cancer such as mitogen-activated protein (MAP) kinase/extracellular-regulated kinase (Erk) cascade and phosphoinoside-3-kinase (PI3K)/Akt-regulated signaling cascade, is well documented in the literature [55, 56]. Activation of MAPK/Erk1/2, which is mediated through growth factors, and K-ras is functionally linked to increased cell proliferation. Several studies have shown how ROS activate Erk1/2 pathway by modulating and activating its upstream target such as Ras. For instance, oxidative modification at its cysteine 118 residue leads to the inhibition of GDP/

GTP exchange [57]. Moreover, ROS activates p90[RSK] that acts as an upstream kinase of Erk1/2 [58, 59]. In ovarian cancer, sustained Erk1/2 activity was linked to increased concentration of endogenous ROS resulting from ubiquitination and loss of endogenous mitogen-activated protein kinase phosphatase 3 (MKP3), which negatively regulates Erk1/2 [58, 59].


N-acetyl-L-cysteine   **Melatonin**
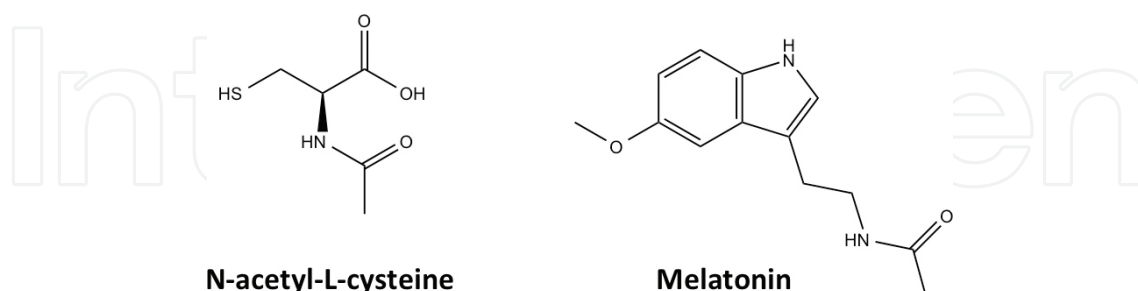
Figure 3. Molecular scaffolds of physiological antioxidants Molecular structures were drawn with ChemDraw13 (Perkin Elmer).

Oxidative stress regulation of PI3K/Akt pathway has been implicated in different cancers. In ovarian cancers, H_2O_2 produced in response to epithelial growth factor signaling (EGF) activates Akt and p70 S6k1, a substrate of Akt involved in regulating protein synthesis [60]. In pancreatic cancer PANC-1 cells, NADPH oxidase (NOX)-4-mediated generation of intracellular ROS was related to survival of these cells, which undergo apoptosis in response to diphenylene iodonium (DPI), an inhibitor of NOX that inhibited superoxide production and impaired levels of phosphorylated Akt [61]. Moreover, benzo(a)pyrene (BaP), a known mammary carcinogen in rodents, increased cell proliferation in human mammary epithelial cells MCF-10A through H_2O_2 generation and activation of epidermal growth factor receptor (EGFR), Akt, and ERK phosphorylation, which was strongly inhibited by NAC treatment [62].

4. Reactive oxygen species contribute in tumor progression

Intracellular redox status aids tumor progression by modulating the processes of metastasis, angiogenesis, survival of cells under hypoxic conditions, and maintenance of cancer stem cell (CSC) subpopulation [63]. Decreased cell adhesion to extracellular matrix, anchorage-independent survival, and invasion of tumor cells are well documented to be influenced by ROS [64]. Perturbation of mitochondrial respiratory chain in breast cancer cells leads to generation of a cellular subpopulation with increased levels of ROS, which are highly metastatic and maintain increased invasive property in vivo [65]. ROS induction was shown to influence overexpression of chemokine CXCL14 through the activator protein (AP)-1-signaling pathway and promote cell motility through elevation of cytosolic Ca^{2+} by binding to the inositol 1,4,5 triphosphate receptor on the endoplasmic reticulum [65]. DNA methylation and histone modification leading to epigenetic silencing of superoxide dismutase (SOD)-2 alter the expression of antioxidant enzyme MnSOD, which promotes invasion of breast cancers [66].

Moreover, a decreased MnSOD level was also associated with increased pancreatic tumor invasion [67]. Degradation of the extracellular matrix (ECM) and activated matrix metallo-proteinases (MMPs) are a prerequisite of cancer cell migration and invasion. Binding of several integrins to the ECM results in increased expression of several MMP proteins. Since integrins signal by a vast array of kinases, phosphatases, GTPases, and transcription factors, it is likely that an elevated level of ROS has an effect on integrin-mediated signaling. Several studies reported the inactivation of critical phosphatases such as protein tyrosine phosphatase (PTP)-PEST (PTPN12), SHP-2 (Src homology 2 [SH2] domain-containing non-transmembrane PTP), and low molecular weight protein tyrosine phosphatases (LMW-PTPs) by oxidation [68]. Catalase, a H_2O_2 scavenger, binds SHP-2 and growth factor receptor-bound protein-2 (Grb2) adapter protein upon integrin ligand binding and therefore protects them against H_2O_2-mediated oxidation [69]. In non-transformed intestinal epithelial cells, elevated ROS increased the expression of $\alpha2\beta1$-integrin, which subsequently increased the levels of cyclooxygenase-2 (COX-2) and promoted cell migration [64]. These results also suggest a mechanism where ROS-induced modulation of ECM promotes cancer formation in intestinal epithelial cells. ROSs have also been implicated in promoting tumor progression by modulating the processes involved in epithelial mesenchymal transition (EMT). Several transcription factors, which promote metastasis such as AP-1, Ets, Smad, and Snail, are regulated by ROS, inducing an effect on upstream target molecules involved in activation of these transcription factors such as protein kinase (PK) C and PTPs [70].
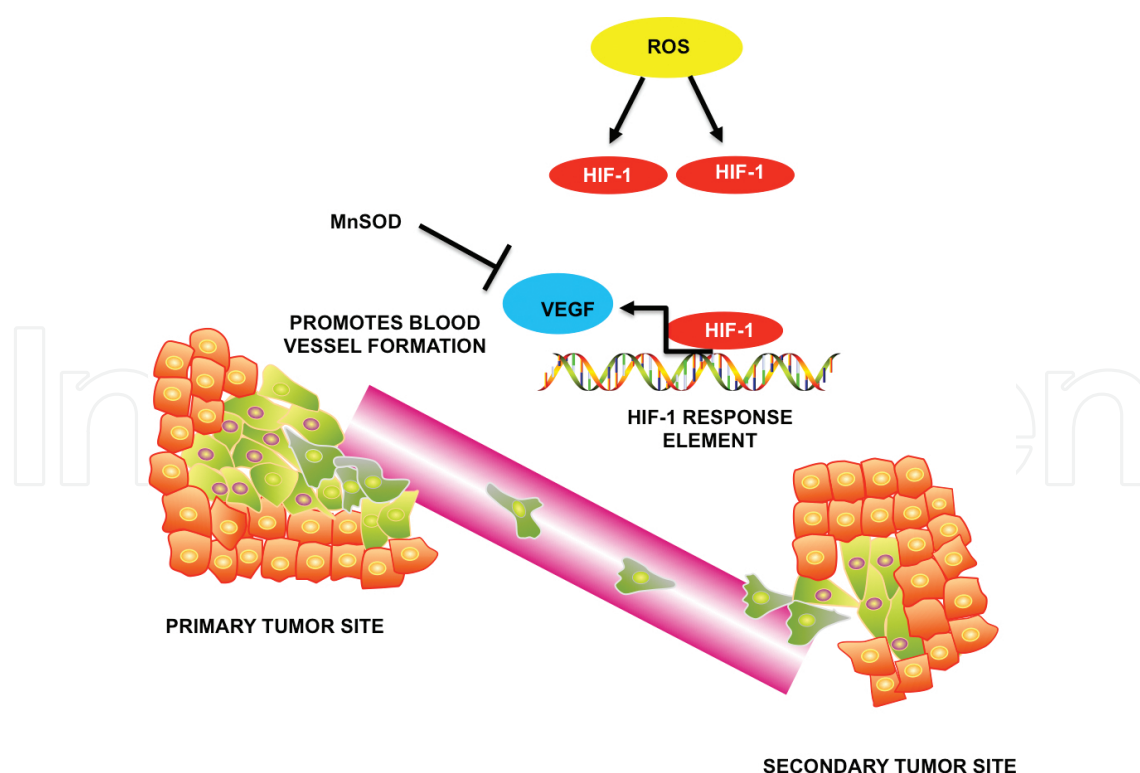
Figure 4. Molecular mechanisms of hypoxia affected by natural compounds. Scheme was drawn with ScienceSlides Suite 2105 (Visiscience).

In a given tumor mass, cancer cells often are exposed to an environment with reduced levels of tissue oxygen, a condition known as hypoxia. Prolonged limitation in oxygen supply can result in cell death. Therefore, cancer cells often undergo genetic and adaptive changes that contribute to a malignant phenotype and adopt characteristics of an aggressive tumor. Cancer cells mimic a phenomenon known as the "Warburg effect" that is to switch to anaerobic glycolysis when adequate oxygen supply is absent [71]. ROSs have been implicated to facilitate the tumor survival under hypoxic conditions by modulating different transcription factors involved. Hypoxia inducible transcription factor (HIF)-1 is most widely studied for its role in tumor promotion under hypoxic conditions. HIF-1 is a heterodimer that consists of hypoxic response factor HIF-1α and constitutively expressed aryl hydrocarbon receptor nuclear translocator (ARNT) also known as HIF-1β [72]. Under reduced oxygen levels, HIF-1 binds to hypoxia response elements, thereby activating hypoxia response genes such as the pro-angiogenic vascular endothelial growth factor (VEGF) [73]. Moreover, HIF-1 has been shown to regulate expression of all enzymes of the glycolysis pathway as well as glucose transporters GLUT1 and GLUT3 [74]. In human breast carcinoma, increased MnSOD activity is reported to inhibit HIF-1α along with suppression VEGF protein that impaired tumor metastasis [75]. Suppression of endogenous ROS by NADPH oxidase inhibitor DPI and mitochondrial electron chain inhibitor rotenone decreased HIF-1 induction and VEGF expression in ovarian and prostate cancer cells [75]. Moreover, growth factor such as epidermal growth factor (EGF)-induced ROS production may lead to activation of AKT/p70S6K1 pathway resulting in increased expression of VEGF stimulating tumor angiogenesis [60] (**Figure 4**).

In any given tumor, subpopulations of cells have the ability to self-renew and drive tumorigenesis. This population of cells is termed as cancer stem cells (CSCs), which are isolated from most cancers such as hematopoietic, breast, lung, colon, etc. CSCs are characterized by the expression of specific stem cell markers and are of clinical relevance as they are highly drug resistant and mostly initiate recurrence after chemo- or radiotherapy [76]. Studies have shown that normal hematopoietic and epithelial stem cells maintained a lower level of ROS than mature progeny to prevent cellular differentiation and maintain long-term cellular self-renewable. Similarly, CSCs unlike cancer cells have reduced level of ROS. Moreover, compared to tumor cell counterparts, CSCs showed increased expression of enzymes, which are associated with ROS scavenging [76]. Particularly, glutathione synthetase that is involved in glutathione synthesis is upregulated along with Forkhead transcription factor (FOXO)-1 to confer resistance to oxidative stress in hematopoietic stem cells [77]. Also, activation of antioxidant response that is frequently reported in CSCs prevents DNA damage in these cells exposed to ionizing radiations, thereby protecting CSCs against irradiation-induced cell death [78]. Based on these findings, it is widely accepted that cancer recurrence in response to withdrawal of conventional therapies is majorly dependent on existence of a resistant CSC subpopulation within the patients. Therefore, further identification of key molecular drivers that regulate the redox balance in CSCs might provide a possibility to eliminate these cells, which may contribute in overcoming the limitations of cancer relapse in future.

## 5. Cell death pathways activated by reactive oxygen species

As mentioned above, cancer cells in particular generate increased ROS levels; now severe accumulation of cellular ROS in response to chemotherapy may induce cell cycle arrest, senescence, or lethal toxicity inducing apoptosis [79]. Electrons leaking from the respiratory complexes in mitochondria are a major source for ROS production [80]. For instance, As_2O_3 which impair the function of respiratory chain increases the production of superoxide ions [65]. Alternatively, drugs, which act as redox cyclers such as anthracyclines daunorubicin and doxorubicin, react with cytochrome p450 reductase and NAD(P)H dehydrogenase [quinone] 1(NQO1) in the presence of reduced NADPH to generate superoxide in the presence of oxygen [81].

Apoptosis is linked to an increase in mitochondrial oxidative stress that causes a series of hallmark events such as release of cytochrome c followed by caspases activation ultimately leading to cell death. Sodium salicylate and non-steroidal anti-inflammatory drugs were reported to induce apoptosis in cancers such as colon, breast prostate, and leukemia through ROS production and activation of intrinsic cell death pathway measured by cleavage of caspase-9 and caspase-3 [82]. However, apoptosis was subsequently that a Rac1-NADPH oxidase-dependent pathway is activated in response to treatments that produce ROS and triggers apoptosis [82]. Mitochondrial release of H_2O_2 has been associated with activation of different stress kinases such as c-Jun N-terminal kinase (JNK) and p38. In response to ROS production, JNK mediates phosphorylation and downregulation of anti-apoptotic proteins B-cell lymphoma (Bcl)-2 and Bcl-extra large (xL) [79]. Moreover, several studies reported that both Bcl-2 and Bcl-xL antagonize ROS generation and protect cells against apoptosis [44, 83]. p38 MAPKs are also implicated in apoptosis induction in response to increased ROS production [84]. p38 is activated through apoptosis signal regulating kinase (Ask)-1. Activity of Ask-1 is dependent on a redox-regulated protein thioredoxin that in its reduced form binds to and conserves Ask-1 in an inactivated form. Increased ROS production uncouples thioredoxin from Ask-1 leading to its activation and phosphorylation of p38 required for TNFα-mediated apoptosis [84]. Studies conducted on L929 fibrosarcoma cells revealed that mitochondrial ROS play a key role in inducing TNFα cytotoxicity presumably by ROS-mediated caspase activation and cell death [85]. Moreover, TNF receptor associated factor 4 (TNFR4), a component of the TNF signaling chain, binds to NADPH and activates JNK suggesting different mechanisms by which death receptors induce ROS activation in cells [86]. Additionally, different studies have reported the significance of ROS-mediated signaling pathway regulated by protein kinase D1. PDK1 is activated by direct binding to Src and by phosphorylation, which promotes proliferation [35]. Inhibition of this pathway sensitizes cancer cells to ROS. Furthermore, beyond the conventional therapy to induce cytotoxicity to cancer cells and overcome the limitations associated with therapy resistance and risk of developing metastatic phenotype, recent advancement is made to explore the phenomenon of senescence, which inhibits the proliferation of cancer cells and restricts them in a dormant phase [87]. Senescence in cancer cells is mainly characterized by increased activity of β-galactosidase along with modulation of several cell cycle regulators such as cyclin-dependent kinases (CDKs), p16, and p27 [87]. Different

polyphenolic compounds extracted from artichokes (*Cynara cardunculus*) or ginseng (*Panax ginseng*) were described to trigger ROS-dependent senescence.

6. Pathological alterations triggered by free radicals

Intracellular ROS generation may lead to damage of cellular macromolecules such as DNA, proteins, and lipid bilayer. Studies have indicated that H_2O_2 is not very reactive towards DNA; however, the damage to DNA is mainly caused by hydroxyl ions that are generated by the Fenton reaction where transition metals such as iron or copper donate or accept free electrons during intracellular reactions [88]. H_2O_2 acts as a catalyst in the reaction in the formation of free radicals. The generated hydroxyl ions are highly diffusible and lead to DNA damage like oxidation, single-, and double-strand breakage. Under normal physiological conditions, such DNA defects are repaired by base excision repair (BER) or nucleotide excision repair (NER). Cells unable to repair the DNA lesions undergo apoptosis to ensure that the mutations are not passed on during cell division. However, failure in either process of DNA repair or apoptosis may harbor the possibility of formation of cancerous growth.
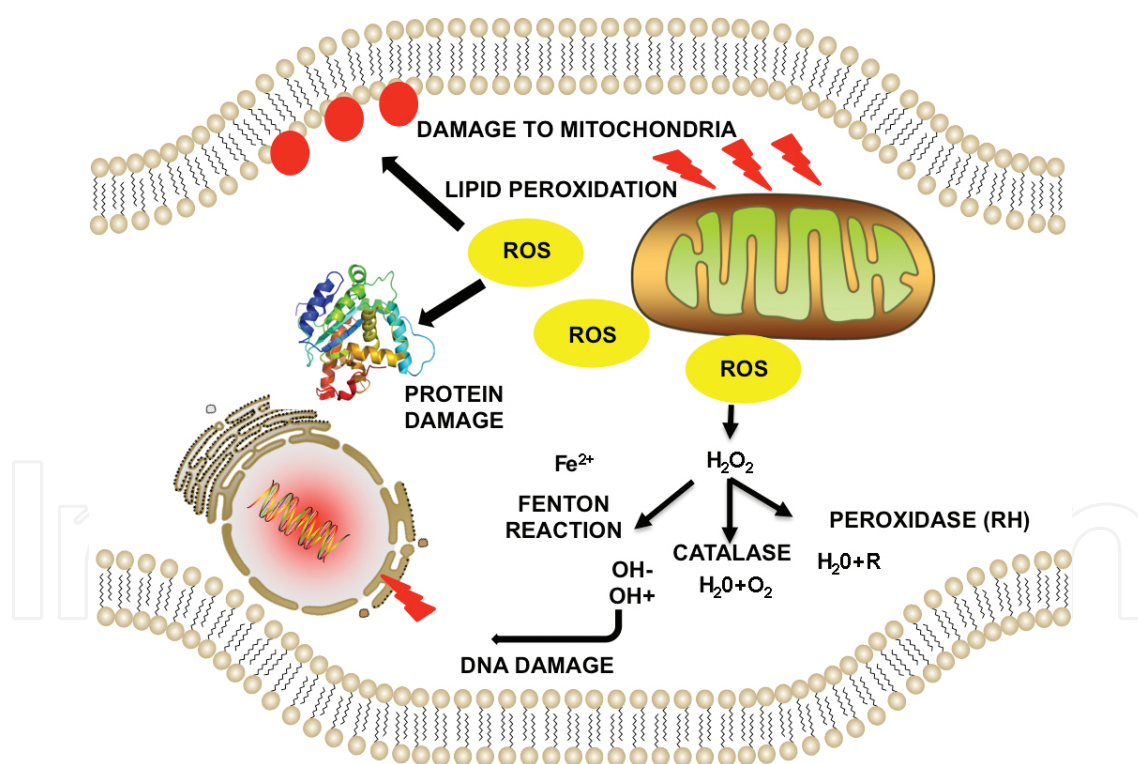
Figure 5. Molecular mechanisms of ROS-induced macromolecule damage. Scheme was drawn with ScienceSlides Suite 2105 (Visiscience).

ROS-mediated damage of proteins is mainly associated with modifications in specific amino acid residues leading to altered function [89]. Beside, some ROS-mediated modifications of protein also includes increased protein carbonylation, nitration of tyrosine and phenylalanine

residues or formation of cross-linked and glycated proteins [89]. The oxidized amino acid residues in proteins may influence their activity in a signal transduction pathway. For instance, oxidation of phosphatases within the catalytic sites impairs their enzymatic activity [90].

Moreover, ROSs react with polyunsaturated or polyunsaturated fatty acids to trigger lipid peroxidation that has also been used as a tumor biomarker in clinical studies [91]. For instance, in colorectal cancer patients, the presence of thiobarbituric acid reactivates has been linked to high levels of lipid peroxidation [63] (**Figure 5**).

7. Natural compounds as pharmacological antioxidants

It has been reported in several studies that dietary phytochemicals can interfere with every stage of cancer development. Therefore, antioxidant functions of phytonutrients have been investigated thoroughly for their role in pathophysiology associated with cancer. Dietary antioxidant compounds with significant anticancer activity mainly include anthocyanidins (and their glycosides termed anthocyanins) from berries [92], catechins from green tea, curcumin from turmeric, genistein from soy, resveratrol from grapes and red wine, all-trans lycopene from tomatoes [93], indole-3-carbinol from broccoli, sulforaphane from asparagus, quercetin from red onions and apples. Beside this, carotenoids, flavonoids, and isothiocyanates have also exhibited strong antioxidant properties.
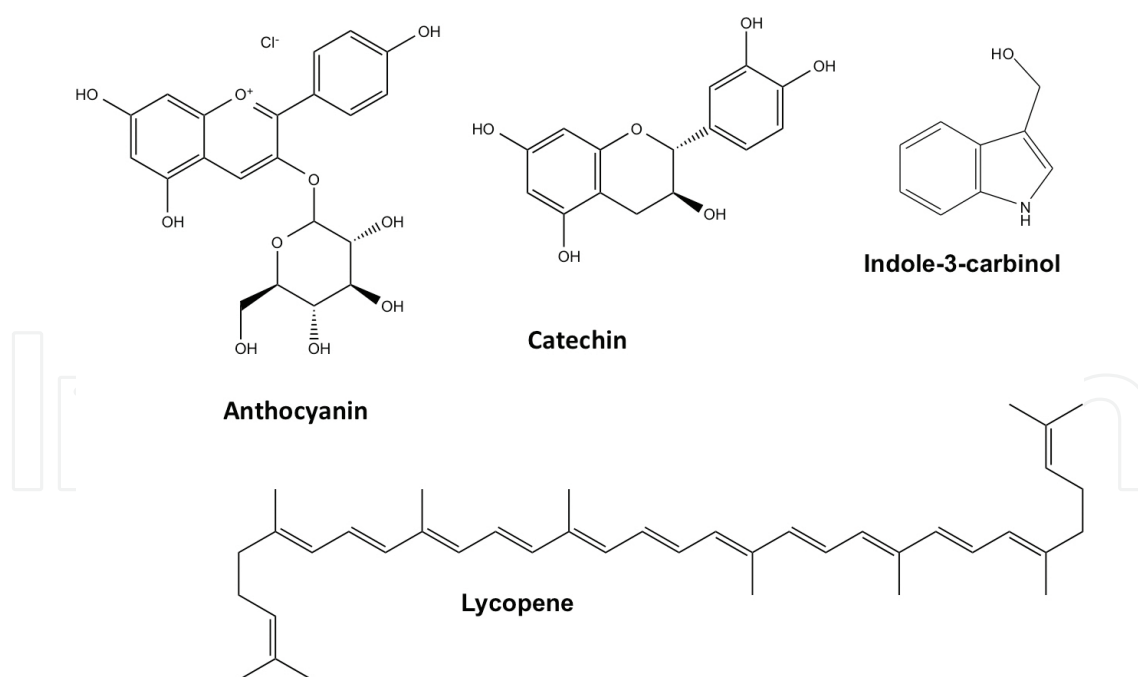
Figure 6. Pharmacological antioxidants of plant origins. Molecular structures were drawn with ChemDraw13 (Perkin Elmer).

Epigallocatechin gallate (EGCG) is the most abundant catechin found in green tea and curcumin-induced anticancer activity promoting cell cycle arrest, polyamine synthesis, and

affecting transglutaminase (TG) activity along with regulation of signaling pathways mediated by NF-κB, AP-1, and MAPKs [94]. In a recent study, EGCG was shown to inhibit cell proliferation of cervical carcinoma Hela cells by promoting depolymerization of cellular microtubule and disrupting tubulin-microtubule equilibrium. Spectroscopic analysis revealed that EGCG bound to the α-subunit of tubulin at the interphase of α- and β-heterodimers preventing colchicine binding to the colchicine-binding site [95]. Also, in osteosarcoma cells, EGCG treatment induced cell cycle arrest, promoted apoptosis, and inhibited growth of transplanted tumors *in vivo* by regulating miR1/c-MET interaction [96] (**Figures 6** and **7**).
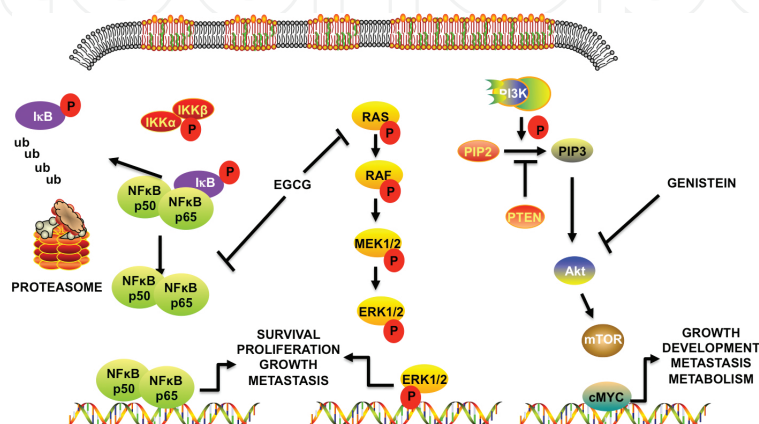
Figure 7. Molecular mechanisms involved in ROS-triggered survival. Scheme was drawn with ScienceSlides Suite 2105 (Visiscience).

Eugenol (4-allyl-2 methoxyphenol) is a naturally occurring phenolic compound that exhibits antioxidant properties. The antioxidant activity of eugenol was evaluated by the extent of protection offered against free radical-mediated lipid peroxidation using both in vitro and in vivo studies [97]. The chemopreventive and anticancer role of eugenol was evaluated on *N*-methyl-*N′*-nitro-*N*-nitrosoguanidine (MNNG)-induced gastric cancer in Wistar rats by analyzing the markers of apoptosis, invasion, and angiogenesis. Rats exposed to MNNG developed gastric cancer with upregulation of pro-invasive and angiogenic factors. Eugenol inhibited cell proliferation by suppression of NF-κB signaling. Apoptosis in these cells following eugenol treatment was mitochondrial pathway mediated that decreased the expression of Bcl-2, following release of cytochrome c and caspases activation. Anti-angiogenic and inhibition of invasion was evidenced by decreased expression of VEGF, its receptor VEGFR1 changes in the activities of MMPs and the expression levels of MMP-2 and MMP-9, VEGF, VEGFR1, tissue inhibitor of metalloproteinases (TIMP)-2 and reversion-inducing cysteine-rich protein with kazal motifs (RECK), a metastasis inhibitor [97].

Several studies aim toward proving the anticancer properties of flavonoids on an array of cancer cell types. Hirano and co-workers tested the anticancer activity of 28 flavonoids on human acute myeloid leukemia (AML) cell line HL-60. Eight of these flavonoids showed strong inhibition of cell proliferation with IC_{50} values in a nanomolar range [98]. In contingent to this finding, Kuntz et al. showed strong inhibition of proliferation induced by flavonoids on two colon cancer cell models with Caco-2 displaying features of small intestinal epithelial cells and

HT-29, resembling colonic cryptic cells [99]. Moreover, in vivo studies on mice strongly inhibited the growth and metastatic potential of melanoma cells B16-BL6 in response to flavonoid treatment [100].

Epigenetic modifications resulting in heritable changes into gene expression without changing the DNA sequence have been marked as key player in promoting cancer [101]. The most common types of epigenetic modifications that may contribute to tumor promotion are DNA methylation and histone acetylation or methylation. Antioxidant compounds mainly isoflavones, flavonols, and catechins have shown to modulate epigenetic features, thereby showing antitumor activity [102–104]. EGCG was shown to affect DNA methyltransferase by inhibiting DNMT and reactivating tumor suppressor genes RARα, p16, and O^6-methylguanine methyltransferase in esophageal cancer KYSE 510 cells [105]. Treatment with caffeic acid (3,4-dihydroxycinnamic acid) or chlorogenic acid [106] of hormone-dependent MCF-7 and hormone-independent MDA-MB-231 breast cancer cell lines partially inhibited the methylation of promoter region of the RARβ gene, thereby restoring its function [107]. Furthermore, studies also indicated that dietary antioxidants such as genistein, quercetin, parthenolide, and lycopene may affect DNA methylation status of different genes associated with cancer [108–111].

In addition to this, synergistic or additive effects of phytochemicals could be achieved when administered along with conventional chemotherapy or radiation therapy. This could be explained due to the fact that phytochemicals, which target different biochemical pathways, may enhance the efficacy of conventional therapies. Moreover, different studies have reported the synergistic cytotoxicity on different cancers when phytochemicals are administered together. Apple extracts and quercetin 3-β-D-glucoside combination showed synergistic antiproliferative effect on MCF-7 breast cancer cells [112]. Genistein a major phytoestrogen which has higher affinity for ERβ compared to ERα showed synergistic cytotoxicity in combination with indole-3-carbinol in HT-29 cells by simultaneously inhibiting Akt phosphorylation and progression of autophagic process [113]. Combination of δ-tocopherol and resveratrol showed strong inhibition of HMC-1 mastocytoma cell proliferation. The two compounds together strongly inhibited Ser473-phosphorylation of Akt, thereby reducing its activity compared to individual treatment [114]. Gagliano et al. suggested that the use of quercetin in combination with other antioxidants such as resveratrol or sulforaphane might be a novel approach for the treatment of human glioma, which has poor clinical prognosis in both adults and children [115].

Additionally, pharmacological implications of polyphenols have also been explored with respect to inhibition of cancer stem cells and self-renewal. It has been demonstrated that polyphenols can efficiently target pathways such as Wnt/β-catenin, Hedgehog, and Notch, which are critical for cancer stem, cells self-renewal [116]. Sulforaphane has been demonstrated to target cancer stem cells by modulating the pathways such as NF-κB, Hedgehog, and Wnt/β-catenin in different cancers such as breast, pancreas, and prostrate and has been proposed as an adjuvant of chemotherapy in different pre-clinical studies [117, 118]. As discussed earlier, cancer stem cells are characterized by a glycolytic metabolism with lower mitochondrial respiration compared to the tumor cells. Therefore, a proposed strategy to counteract CSCs

population is to impair their metabolism by inhibiting glycolysis or by forcing CSCs into mitochondrial metabolism and oxidative phosphorylation. To this purpose, polyphenols have been implicated to regulate the cancer metabolism. For instance, EGCG in human breast cancer have been shown to target the 5′ adenosine monophosphate-activated protein kinase (AMPK) pathway, which is involved in maintaining cellular energy status, cell cycle, and protein synthesis [119] (**Figures 8** and **9**).
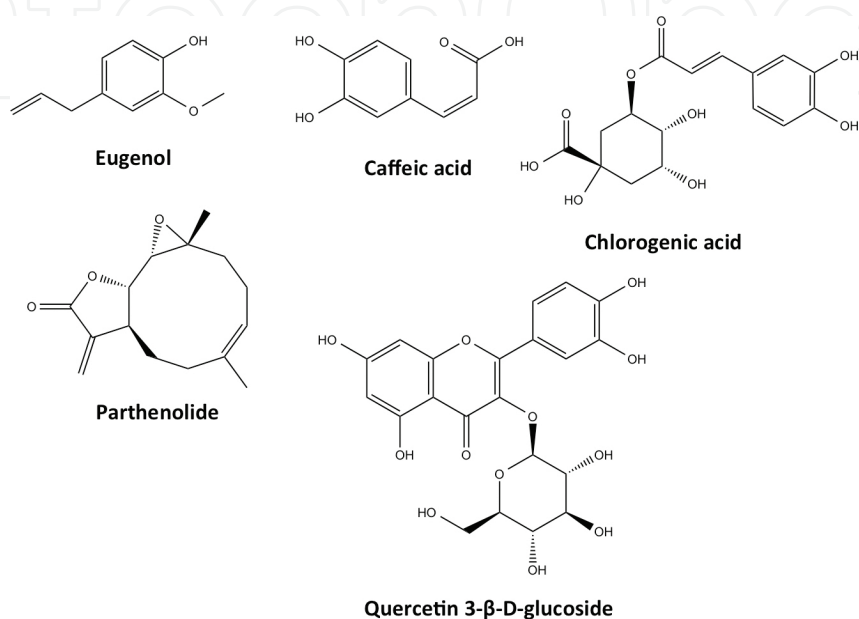
Figure 8. Pharmacological antioxidants of plant origins. Molecular structures were drawn with ChemDraw13 (Perkin Elmer).
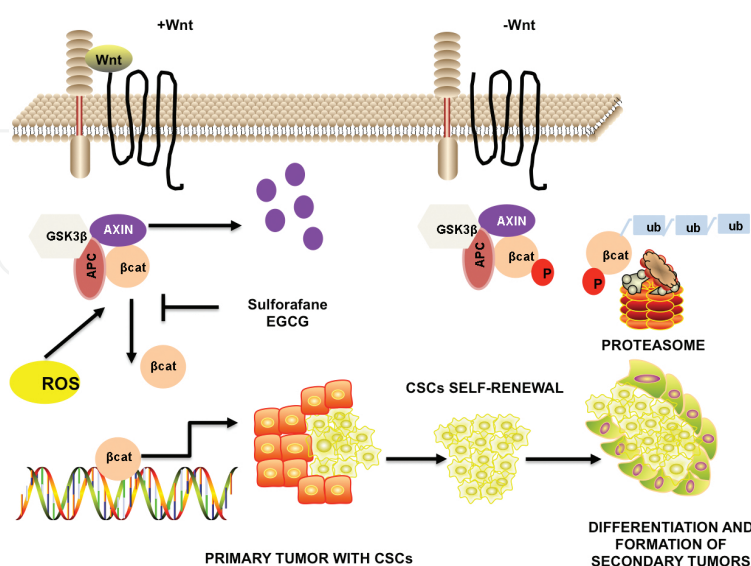

Figure 9. Molecular mechanisms involved in Wnt signaling. Scheme was drawn with ScienceSlides Suite 2105 (Visiscience).

8. Natural compounds as pharmacological pro-oxidants

As discussed earlier, cancer cells produce high levels of ROS that allow these cells to maintain a state of increased basal oxidative stress. The increased state of oxidative stress promotes survival but on the other hand makes the cancer cells vulnerable to further increase in ROS levels over a cancer-specific threshold. Accordingly, pro-oxidant agents and increased oxidative stress levels could then selectively target cancer cells. Different compounds of natural origins modulate the intracellular ROS levels and induce both chemopreventive and anticancer effect in different cancer types.

Polyphenolic extracts from artichokes (*Cynara cardunculus*) at high doses induce apoptosis and decrease the invasive potential of human metastatic breast cancer. Apoptosis was regulated in a caspase-independent manner. Additionally, sublethal concentrations of artichoke increased ROS and induced significant increase in senescence-associated β-galactosidase along with upregulation of tumor suppressor genes p16[INK4] and p21[Cip1//Waf1]. Altogether, NAC attenuated the antiproliferative effect induced by artichoke extracts, which suggests that induction of premature senescence and apoptosis is regulated in a ROS-dependent manner [120].

20(S)-ginsenoside Rg3 [20(S)-Rg3], a chemical compound extracted from *Panax ginseng*, induced senescence in glioma cells at sublethal concentrations, which was abrogated by NAC treatment suggesting involvement of ROS. Moreover, depletion of Akt and inactivation of the p53/p21 pathway attenuated the compound-induced senescence. These results suggest that ROS is playing a role in activation of Akt and p53/p21, which leads to growth arrest in human glioma cancer [121].
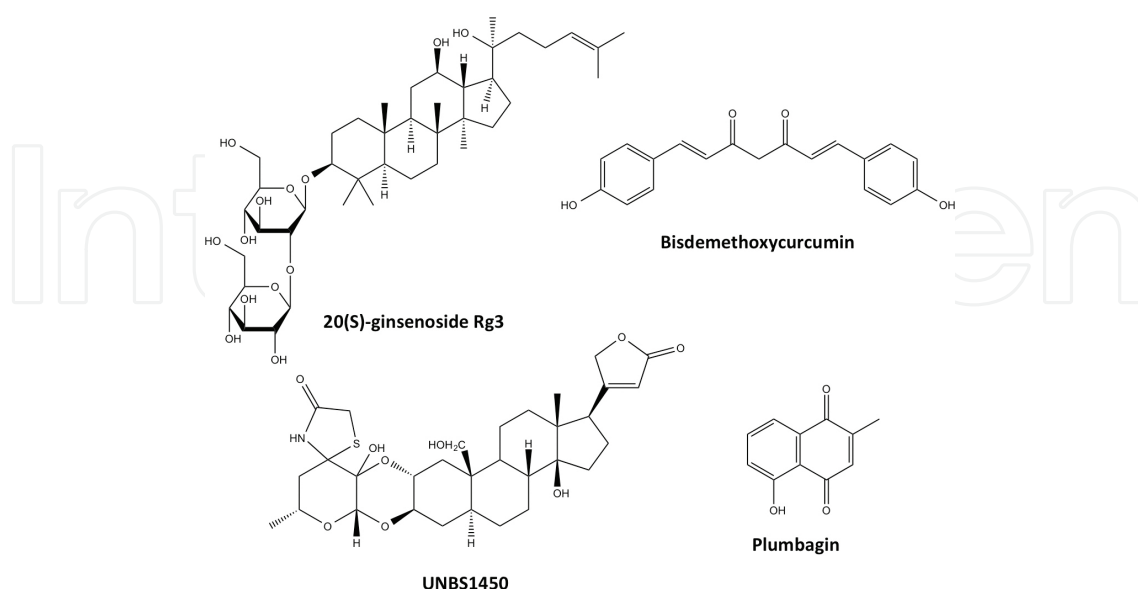
Figure 10. Molecular scaffolds involved ROS generation. Molecular structures were drawn with ChemDraw13 (Perkin Elmer).

Bisdemethoxycurcumin, a curcuminoid from turmeric, demonstrated potential chemotherapeutic activities by inhibiting proliferation and decreasing the cell viability of hormone-dependent breast cancer. Bisdemethoxycurcumin treatment leads to increased ROS production, which disrupted mitochondrial membrane potential assessed using mitochondrial potential sensor JC-1. Moreover, the compound induced increased expression of pro-apoptotic protein p53 and its downstream effector p21 along with cell cycle regulator p16 and its downstream regulator retinoblastoma protein (pRb). The results overall suggested bisdemethoxycurcumin-induced ROS accumulation, which leads to inhibition of hormone-dependent breast cancer [122].

We have previously reported that garlic-derived organosulfur compounds including diallyltetrasulfide induce growth arrest and apoptosis in colon cancer cells by disrupting the redox status in the cells. Drug-induced cell cycle arrest in G2/M phase followed by apoptosis was further associated with decreased Cdc25c expression, one of the key enzymes responsible for G2/M transition [32]. Moreover, we have also shown that plumbagin, a plant naphtoquinone, reduces cell viability and induces apoptosis in a series of hematopoietic cancer cell lines including HL-60, Jurkat, K562, Raji, and U937 with a most pronounced effect on AML U937 cells by 10-fold increase in ROS production. This was followed by decreased expression of anti-apoptotic proteins Mcl-1 and Bcl-2 along with activation of caspases-8, caspases-9, caspases-7, and caspases-3 [123]. Recently, we have also demonstrated ROS induction in neuroblastic and stromal neuroblastoma cells by hemisynthetic cardenolide UNBS1450. ROS induction was followed by autophagic response eventually leading to apoptosis or necroptosis. Time-dependent increase in ROS affected lysosomal integrity of the cells inducing lysosome-associated membrane protein (LAMP)-2 degradation leading to cathepsin B and L activation [124] (**Figure 10**).

9. Conclusion

Natural compounds or their derivatives comprise of more than 50% of cancer chemotherapeutic agents available in the clinics. Information encoded by the human genome project would definitely lead to identification of several gene products, which could potentially be targeted by novel anticancer drugs. Due to various advantages associated with the use of natural compounds such as high availability and reduced toxicity, it is likely that the natural products templates combined with chemistry will allow the generation of novel analogues with enhanced pharmacological benefits to enter clinics.

Malignant cells, which often exhibit increased ROS generation that is associated with tumor proliferation and drug resistance, highlight the crucial role of ROS stress in cancer. Therefore, targeting the redox-modulated biochemical properties of cancer cell may allow to develop a feasible therapeutic approach to overcome challenges associated with cancer treatment. Furthermore, not critically explored unique redox biology of cancer stem cells suggests the use of redox modulating strategies to eradicate these cells.

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