

Oxidative Stress in Carcinogenesis and Therapy

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Abstract

Oxidative stress results from a disequilibrium between production and their elimination by cellular antioxidant systems. This leads to the accumulation of ROS that have a deleterious effect on vital biological macromolecules. It is now well established that cancer cells exhibit a pro-oxidant state due to metabolic and genetic abnormalities. The disequilibrium in redox homeostasis in cancer cells promotes genomic instability leading to the activation of oncogenes, mitochondrial dysfunction and an alteration in antioxidants activities. All these events can further escalate ROS levels, causing more DNA damage and genetic instability. This vicious cycle is "beneficial" for the process of carcinogenesis and numerous reports have indeed proven the pivotal role of ROS in cancer initiation, cell migration, invasion and metastasis. In order to cope with this sustained redox deregulation, cancer cells, very likely, utilize the full antioxidant capacity of their enzymatic and non-enzymatic systems. Cancer cells are thus highly dependent on their antioxidant systems and especially antioxidant enzymes. Keeping with this, targeting the enzymatic antioxidant system could be an efficient strategy to preferentially kill cancer cells by increasing intracellular ROS levels beyond a certain "threshold" of tolerance eventually leading to specific cancer cell death. In this review we present an overview on ROS generation and focus on the implication of ROS in cancer initiation, epithelial-mesenchymal transition, cell migration, invasion and metastasis, as well as the cancer stem-like phenotype. We finally present different therapeutic approaches that target the enzymatic antioxidant system in order to selectively kill cancer cells.

Keywords: ROS; Carcinogenesis; Epithelial mesenchymal transition; Invasion; Metastasis; Cancer stem-like cells; Antioxidant enzymes; Therapy

Introduction

Cancer is a complex disease in which multiple genomic and metabolic alterations are implicated in the process of tumor initiation and development. The cancer research field have attracted many researchers over the years which lead to the development of numerous therapeutic strategies mostly based on targeting oncogenes or specific signaling pathways, since the disruption of one oncogene often leads to cancer cell arrest [1]. However recent studies have shed light on the metabolic abnormalities of cancer cells and highlighted the implication of reactive oxygen species (ROS) in the process of cancer initiation and progression. ROS is a term used to qualify chemical species that are derived from molecular oxygen (dioxygen; O₂) but are more reactive than the latter one. Aerobic organisms have developed different antioxidant systems in order to maintain ROS levels in a nontoxic range. Oxidative stress occurs when the antioxidants systems are overwhelmed by high ROS levels, originating from intracellular or extracellular sources. These reactive species can damage vital biological macromolecules like DNA causing genome alterations. In this review we will focus on ROS generation in mammalian cells and the implication of oxidative stress in cancer initiation, cell migration, and epithelial-mesenchymal transition (EMT) and the cancer stem-like cells (CSCs) phenotype. We will discuss signaling pathways that are

affected by ROS and implicated in the process of carcinogenesis. Finally, we will overview the recent use of an alternative and promising therapeutic strategy based on targeting the enzymatic antioxidant system to sensitize cancer cells.

ROS Generation

O₂ is vital for aerobic respiration and participates in numerous redox reactions. The chemical properties of O₂ dictate its reactivity towards organic molecules. O₂ is a stable diradical at a triplet state, meaning that the two unpaired electrons in its antibonding orbitals have the same spin. This feature renders O₂ a non-reactive molecule which protects organic macromolecules such as polysaccharides, lipids, proteins and nucleic acids from spontaneous oxidation [2]. However, the activation of O₂ through the excitation of one of the two unpaired electrons or via a mono-electronic reduction leads to the formation of either singlet oxygen ¹O₂ (a highly reactive form of oxygen with two electrons with opposing spins) or superoxide anion (O₂^{•-}) which is a precursor of a range of molecules and free radicals. The chemical species derived from oxygen are known as ROS and are capable of damaging biological macromolecules. We can distinguish an endogenous and an exogenous source of ROS. In mammalian cells ROS can be generated "inadvertently" during biochemical reactions or synthesized "deliberately" by different enzymes to serve a biological purpose. O₂^{•-}, the precursor anion of different types of endogenous ROS, is primarily generated in mitochondria after a mono-electronic reduction of O₂ due to electron leakage from different redox centers

(such as NADH-ubiquinone oxidoreductase (complex I) and ubiquinol-cytochrome c oxidoreductase (complex III)) of the mitochondrial respiratory chain during oxidative phosphorylation. This process accounts for the production of 160–320 mmol and 215–430 mmol of $O_2^{\bullet-}$ by the mitochondria of a 60 kg woman and 80 kg man respectively [3-5]. In a more deliberate way, $O_2^{\bullet-}$ is produced by different enzymes such as NADPH oxidases (NOX/DUOX) located on the cell membrane of phagocytes and different other cell types [6] and is a byproduct of the detoxifying activity of cytochrome P450 and b5 families of enzymes and of the catalytic activity of different soluble oxidases such as xanthine oxidase, aldehyde oxidase, dihydroorotate dehydrogenase, flavoprotein dehydrogenase and tryptophan dioxygenase [7].

As a charged and short-lived anion, $O_2^{\bullet-}$ possesses low membrane permeability and oxidizes relatively few biological molecules. $O_2^{\bullet-}$ may react with other radicals including nitric oxide (NO^{\bullet}) which is generated by nitric oxide synthase (NOS) or even by mitochondrial cytochrome oxidase under hypoxic conditions [8]. The product, peroxynitrite ($ONOO^-$), and other oxidants derived from NO^{\bullet} are referred to as reactive nitrogen species (RNS) and their role in cellular signaling was reviewed by Poyton et al. [9]. A part from reacting with NO^{\bullet} , the majority of $O_2^{\bullet-}$ generated at the mitochondrial level is rapidly dismutated to an uncharged and more stable derivative, hydrogen peroxide (H_2O_2) [10]. The reaction is catalyzed by superoxide dismutases (SODs) with a rate constant estimated to be $3 \times 10^9 M^{-1}.s^{-1}$ [11,12]. Unlike $O_2^{\bullet-}$, H_2O_2 is a stable molecule capable of diffusing across biological membranes. This compound has thus a dual role, serving both as an oxidant and as an essential signaling molecule that regulates cellular biological processes [13]. Although dismutation of $O_2^{\bullet-}$ probably accounts for much of the H_2O_2 produced in eukaryotic cells, H_2O_2 can be directly produced in peroxisomes by H_2O_2 generating enzymes reviewed in [14,15]. Because of the stability of its oxygen-oxygen bond, H_2O_2 is a weaker oxidant than $O_2^{\bullet-}$. However, according to Fenton reaction, H_2O_2 can react with transition metals such as iron or copper leading to the formation of hydroxyl radical HO^{\bullet} . HO^{\bullet} can be also produced following Haber-Weiss reaction where $O_2^{\bullet-}$ reduces H_2O_2 to form O_2 , HO^{\bullet} and the hydroxide anion HO^- . With a life time in biological media of approximately 1 μs and a redox potential close to +2 V, HO^{\bullet} is a very powerful oxidant capable of oxidizing most organic molecules at diffusion-limited rates [16-18]. ROS can also be produced by different exogenous sources in cellular environment. Radiations (ionizing radiation and UVs) have a direct deleterious effect on biological macromolecules. However the damage caused can be mediated by ROS production. For instance UVA generates 1O_2 and a series of radical species leading to DNA damage [19] and ionizing radiation, through radiolysis of water, generates in few nanoseconds different radicals such as aqueous ion ($e-aq$), HO^{\bullet} and H^+ ion. Furthermore, ionizing radiation causes the decrease of NADH dehydrogenase (Complex I) activity which is responsible for mitochondrial dysfunction and persistent oxidative stress that promotes genome instability [20-22]. As mentioned earlier, transition metals play a cardinal role in Fenton and Haber-Weiss reactions and a variety of metals including cobalt (Co (II)) or nickel (Ni (II)) can enter these reactions to generate directly ROS. On the other hand heavy metals like cadmium, lead and mercury damage cellular components via indirect ROS production by affecting different enzymes activities or antioxidants pools [23,24]. Apart from metals, different carcinogenic molecules exert their deleterious action through ROS generation. This is the case of paraquat, a commonly used herbicide worldwide, which undergoes cyclic single-electron reduction/oxidation leading to ROS

formation [25]. Another example is asbestos that, when inhaled, causes inflammation in the lung a process linked with high ROS production. This inflammatory microenvironment promotes carcinogenesis and in this case enhances the risk of lung cancer after asbestos exposure [26].

Antioxidants in Mammalian Cells

An antioxidant is a compound capable of either delaying or preventing the oxidation of the substrate [27]. In mammalian cells antioxidants are the main defense against ROS and include both enzymatic and non-enzymatic compounds. The different enzymatic systems form the “first line of defense” against ROS and the enzymes implicated can be separated, according to their mechanism of detoxification, into thiol/selenothiol independent and thiol/selenothiol dependent enzymes. SODs and catalase are thiol/selenothiol independent enzymes which antioxidant activity is supported by a metallic ion. In humans, three isoforms of SODs exist and catalyze the dismutation of $O_2^{\bullet-}$ to H_2O_2 and O_2 . SOD1 is a copper and zinc containing enzyme (Cu/Zn-SOD), primarily localized in the cytosol but has been detected in the nucleus, peroxisomes and mitochondrial intermembrane space (IMS). SOD2 is a manganese SOD localized to the mitochondrial matrix and SOD3 is an extracellular Cu/Zn-SOD (EC SOD) detected in plasma, lymph and synovial fluids. [28-31]. The H_2O_2 produced by intracellular SODs is eliminated by catalase and thiol/selenothiol peroxidases. Catalase is a heme containing enzyme, mainly found in peroxisomes of mammalian cells, capable of eliminating one million molecule of H_2O_2 per sec. However, the catalytic cycle of catalase requires the interaction of two molecules of H_2O_2 with a single active site which renders the enzyme very efficient, preferentially at high levels than low levels of H_2O_2 [32-34]. In these conditions, thiol/selenothiol peroxidases play an instrumental role in eliminating not only H_2O_2 but also a range of organic peroxides. Thiol/selenothiol peroxidases belong either to the glutathione (a highly abundant thiol antioxidant present at millimolar range in eukaryotic cells) [35] or thioredoxin systems which both use NADPH as the final electron donor. Glutathione peroxidases (GPx) are the main peroxidases of the glutathione dependent redox network and use glutathione for the reduction of H_2O_2 or organic hydroperoxides to water or the corresponding alcohols. Eight human GPx have been described with GPx 1 (cytosolic and mitochondrial), 2 (present in the intestinal epithelium), 3 (in the plasma), 4 (membranic) and 6 (present in the olfactory epithelium) being selenoproteins with a selenocysteine (Sec) in the catalytic center while GPx5 (secreted in the epididymis), 7 (present in the lumen of the endoplasmic reticulum) and 8 (present in the membrane of the endoplasmic reticulum) are reported to have a cysteine instead of a Sec in the active center and have low GPx activity [36]. GPx1 was the first selenoprotein identified in mammals and is one of the most abundant members of the GPx family. GPx1 has also been reported to be more effective than catalase at removing intracellular peroxides under many physiological conditions [37]. Controversially, only GPx1 relies exclusively on glutathione as an electron donor while other GPxs such as GPx3 and GPx4 can be maintained in a reduced state by thioredoxins [36,38,39]. The glutathione system is complemented by the thioredoxin system in which peroxiredoxins (Prx) are responsible for the reduction of H_2O_2 , organic peroxides, $ONOO^-$ and different radicals using the reducing equivalents provided mainly by thioredoxins [40,41]. Mammalian cells express six isoforms of Prx divided in three subclasses based on the location and presence or absence of one (resolving cysteine) of the two conserved redox active cysteine residues in the catalytic center that is responsible for the peroxidase activity of the enzymes. Prx I (cytosolic),

II (cytosolic), III (mitochondrial) and IV (endoplasmic reticulum) are typical two-cysteine Prxs (an intermolecular disulfide is formed between the peroxidatic cysteine that reacts with the peroxide and the resolving cysteine) Prx V (cytosolic, mitochondrial, and peroxisomal) is an atypical two-cysteine Prxs (an intramolecular disulfide is formed between the peroxidatic cysteine that reacts with the peroxide and the resolving cysteine) and Prx VI (mainly cytosolic) is a one-cysteine Prxs (the resolving cysteine is absent and glutathione reduces the oxidized peroxidatic cysteine) [42]. Prxs are highly reactive towards low levels of H_2O_2 which makes these enzymes ideal H_2O_2 sensors. It is interesting to note that two-cysteine Prxs are inactivated by high levels of H_2O_2 . This is suggested to act as "floodgates", keeping resting levels of H_2O_2 low, while permitting higher levels during signal transduction [43].

The enzymatic system is assisted by a non-enzymatic system constituted of antioxidant molecules such as vitamin E, vitamin C, β -carotene, alpha-lipoic acid, reduced coenzyme Q, glutathione, ubiquinone, uric acid, bilirubin, melatonin etc. the mechanism of action of each antioxidant is different from one another but mainly antioxidants directly scavenge ROS or lead to their decomposition into less reactive species [27].

Oxidative Stress and Cancer Initiation

ROS and RNS generated endogenously or exogenously are counteracted by cellular antioxidant defenses. Under normal growth conditions, there is a balance between ROS/RNS generation and their detoxification by antioxidants systems which maintain redox homeostasis and protect vital macromolecules from irreversible oxidation. An enhancement in ROS/RNS production or a defect in the cellular antioxidant capacity shifts the balance towards higher ROS/RNS levels which exerts a deleterious effect on biological macromolecules. This situation is defined as oxidative stress and has been detected in numerous diseases especially cancer. In fact one of the significant features of cancer cells, when compared to the normal ones, is a persistent pro-oxidant state [44-46]. Despite the lack of a clear description of the biological pathways leading to ROS generation in cancer cells, several extrinsic and intrinsic mechanisms are believed to cause oxidative stress during cancer development and progression. Chronic inflammation is a clear example on how cellular microenvironment plays a role in neoplastic transformation. During inflammation processes, neutrophils and macrophages have been shown to release large quantities of $O_2^{\bullet-}$, H_2O_2 , and HO^{\bullet} [47] and different cell lines co-cultured with activated neutrophils had an elevated rate of DNA strand breaks, sister chromatid exchange and mutations [48-51]. These genomic alterations can cause the activation of oncogenes or the inactivation of tumor suppressor genes and can also alter nuclear DNA repair mechanisms, resulting in the generation of tumor-initiating cells. On the other hand, mitochondrial DNA is more prone to oxidation than nuclear DNA due to the absence of histones and a decreased activity of DNA repair enzymes in this cellular compartment [52,53]. This being said, mutations in mitochondrial genes encoding for proteins implicated in oxidative phosphorylation have been reported in several cancers [54,55] and almost all neoplastic cells show a deep alteration of their metabolic status. Early studies reported that initiated cells had pronounced decrease in oxidative phosphorylation in favor of aerobic glycolysis.

This phenomenon was defined as the "Warburg effect" first described by Otto Heinrich Warburg in 1927 [56]. In cancer cells, genes for glycolysis have been reported to be overexpressed which supports the idea that an increase in glycolysis is a compensatory mechanism for an impaired or damaged respiration [57,58]. Despite the metabolic shift towards aerobic glycolysis mitochondria still remain active in cancer cells and oxidative phosphorylation can even account for 80% of the total cellular energy. Matoba et al. presented a possible explanation for the "Warburg effect" by demonstrating that p53, one of the most frequently mutated genes in cancers, can modulate the expression of Synthesis of Cytochrome c Oxidase 2 (SCO2) which is required for the assembly of mitochondrial DNA-encoded COX II subunit into the COX (cytochrome c oxidase) complex. The disruption of p53 lead to a decrease in SCO2 protein expression, a reduced O_2 consumption and an increase in lactate production thus indicating a change in the mode of energy production to one favoring glycolysis [59]. The gain behind using glycolysis to produce cellular energy resides in the fact that this metabolic process lowers cellular dependency on oxygen. This plays a role in promoting tumorigenesis by allowing growth in hypoxic environment present inside solid tumors. Under these conditions glycolysis is sustained by hypoxia-inducible factor-1 α (HIF-1 α) that induces the expression of pyruvate dehydrogenase kinase 1 and most of the genes implicated in glucose uptake, glycolysis, and lactic acid production [60].

Oxidative Stress in Cell Migration, Invasion and Metastasis

Cell migration is a key process in development but also in pathological conditions such as tumor invasion and metastasis. To move and migrate, cells undergo complex changes involving cytoskeleton dynamics and modulation of adhesion molecules [61]. It is now well admitted that oxidative stress plays a crucial role in cell adhesion, migration and invasion. In this context, integrin activation directs changes in mitochondrial metabolism [62-64] and activates many oxidases including NOX and COX-2. It has been shown that Rac1 acts upstream of NOX, and elicits complex signaling axis to fine tune ROS production [65]. Rac1-induced ROS are involved in VE-cadherin cell-cell junction control [66]. Indeed, ROS triggers a signaling route leading to the dismantlement of cell-cell interaction in endothelial cells. This phenomenon is associated to vascular dysfunctions such as elevated permeability, endothelial migration and angiogenesis [66,67] (Figure 1). Moreover Rac1-ROS signaling nexus is involved in the phosphorylation of β -catenin and p120-catenin by Src and Pyk2 kinases [68,69]. Accordingly, antioxidants compounds were shown to prune VEGF-mediated angiogenesis, by stabilizing VE-cadherin surface expression, and preventing its phosphorylation and subsequent internalization [69].

ROS were also shown to promote aberrant matrix metalloproteinases (MMPs) activation in several cancer cells including breast cancer [70], glioblastoma [71], and pancreatic cancer [72]. These are proteases that degrade the extracellular matrix (ECM), favoring thus cell migration and metastasis (figure 1). In this context, blocking ROS activity using the ROS scavenger N-acetylcysteine abrogates membrane-type 1 matrix metalloproteinase (MT1-MMP)-mediated increase in cell migration and invasion [73].

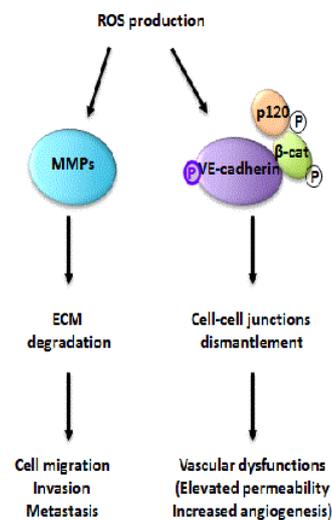


Figure 1 : ROS signaling in cell migration and invasion

Figure 1: **ROS signaling in cell migration and invasion.** ROS signaling nexus plays a role in β -catenin and p120-catenin phosphorylation, as well as the destabilization of cell-cell junctions. ROS were shown also to promote aberrant MMPs activation, resulting in ECM degradation and subsequent cell migration and invasion.

Oxidative Stress and EMT

Epithelial-mesenchymal transition (EMT) is another key step in tumor progression. EMT is a process during which epithelial cells lose their cell polarity and adhesion, and acquire mesenchymal, fibroblast-like properties. Even though EMT was first described to play an important role during embryonic development, it is now well admitted that EMT is instrumental in tumor progression, invasion and metastasis [74,75]. Mounting evidence have highlighted the possible involvement of ROS in controlling EMT in cancer cells. In this context, it has been described that elevated cellular ROS level drives metastatic skin squamous cell carcinoma into EMT [76]. In mammary carcinoma, miR-373 promotes EMT, migration, invasion and metastasis in a ROS dependent manner [77]. Moreover, numerous studies have linked ROS and TGF- β signaling in EMT. TGF- β is a key factor of the tumor microenvironment and plays an important role in cancer progression and EMT. It has been shown, that intracellular ROS production is increased upon TGF- β stimulation. Released ROS trigger Smad2, p38 and ERK1/2 phosphorylation, together with α -SMA and fibronectin upregulation, and E-cadherin repression, favoring thus tumor cells to undergo epithelial mesenchymal transition [78]. In keeping with this, blocking the NADPH oxidase 4 (Nox4) in pancreatic cancer cells abolished TGF- β -induced EMT phenotype [79]. TGF- β can also affect iron homeostasis, leading indirectly to elevated ROS levels. In murine hepatocytes, TGF- β signaling decreases ferritin heavy chain (FHC) levels, translating into an increased intracellular labile iron pool (LIP), which in turn elevates the production of ROS, ultimately engaging cells in the EMT process [80]. Accordingly, both ROS elimination and FHC overexpression resulted in EMT blockade.

ROS are also involved in hypoxia-induced EMT. Indeed, ROS were shown to stabilize HIF-1 α , which results in the activation of the downstream transcription factors, such as the E-cadherin repressor Snail [81]. In mammary epithelial cells, MMP-3 drives Snail activation and EMT in a NF-B/ROS dependent manner [82]. Interestingly, Snail was shown to elevate ROS levels [83], enabling thus a positive feedback loop driving mesenchymal phenotype in epithelial cells.

Oxidative stress and Cancer Stem like Cells

Cancer stem-like cells (CSCs) or cancer initiating cells constitute a subpopulation of cells able to self-renew, sustain and recapitulate the tumor mass. Such cells have been isolated and characterized in hematopoietic and solid tumors [84-86]. It has been proposed that CSCs drive cancer aggressiveness, invasion and represent the main cause of relapse [87,88]. In this context, novel therapeutic strategies aim at eliminating CSCs in hope of a better outcome for the patients. CSCs are very resistant to conventional radio and chemotherapy [89], which basically function by elevating ROS levels, which ultimately result in cell death. Indeed, CSCs possess effective protection mechanism against ROS-induced stress. Accordingly, it has been proposed that CSCs exhibit low ROS levels when compared to the tumor bulk [90]. Moreover, pharmacological depletion of ROS scavengers in CSCs markedly decreases their clonogenicity and results in radio-sensitization [91], suggesting that ROS levels supervise CSCs identity and fate [92].

Among the numerous weapons employed by CSCs to maintain low ROS levels (summarized in Figure 2), the detoxifying enzyme Aldehyde dehydrogenase 1 (ALDH1) arose as a general marker for stemness phenotype [93]. ALDH1 was proposed to protect CSCs

against alkylating agents such as cyclophosphamide. Indeed ALDH1 inhibition, results in heightened ROS levels which causes DNA damage, leading thus to cell death [94].

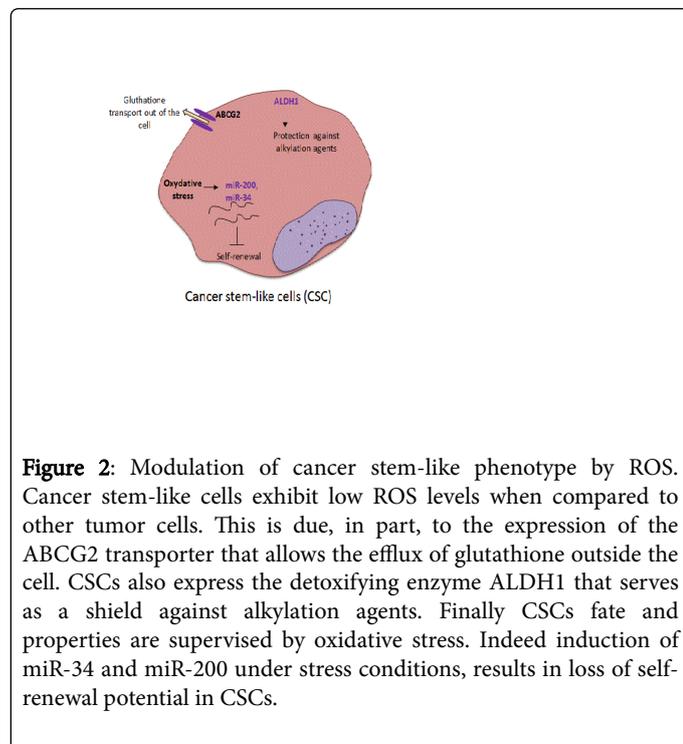


Figure 2: Modulation of cancer stem-like phenotype by ROS. Cancer stem-like cells exhibit low ROS levels when compared to other tumor cells. This is due, in part, to the expression of the ABCG2 transporter that allows the efflux of glutathione outside the cell. CSCs also express the detoxifying enzyme ALDH1 that serves as a shield against alkylation agents. Finally CSCs fate and properties are supervised by oxidative stress. Indeed induction of miR-34 and miR-200 under stress conditions, results in loss of self-renewal potential in CSCs.

Cancer Stem-like Cells are also characterized by the expression of ATP-binding cassette 2 of the subfamily G (ABCG2), which plays an important role in the maintenance of their phenotype [95]. Indeed, ABCG2 functions as an efflux pump transporting glutathione out of the cells, contributing to alleviate intracellular ROS levels [96]. Furthermore, ABCG2 expression was shown to protect cells from ROS-induced death and to inhibit ROS-induced expression of inflammatory genes as well as the activation of NF- κ B pathway in Alzheimer disease [97].

Many studies have highlighted the role of miRNAs in controlling CSCs fate and phenotype in a ROS-dependent fashion. For instance, oxidative stress was shown to increase the expression on miR-34, which in turn reduces self-renewal potential of pancreatic and glioma stem cells [98,99]. Keeping with this, oxidative stress also enhances the expression of two members of miR-200s family, miR-200a and miR-141. Indeed, it has been reported that miR-200 decreases the expression of Bmi1, Suz12, and Notch-1, key regulators of stem cells phenotype, consistent with the inhibition of CSC self-renewal capacity [100-102].

Targeting the Enzymatic Antioxidant System as Anti-Cancer Therapeutic Strategy

As we mentioned earlier, one of the significant features of cancer cells is a persistent pro-oxidant state. In cancer cells, the events such as activation of oncogenes, mitochondrial dysfunction and redox deregulation can increase ROS levels, leading to intrinsic oxidative stress. In order to cope with this sustained redox deregulation, cancer cells, very likely, utilizes the full antioxidant capacity of their enzymatic and non-enzymatic systems. Cancer cells are thus highly dependent on

their antioxidant systems and especially antioxidant enzymes such as manganese SOD and Prxs (especially Prx I and III) that have been suggested to play an instrumental role in carcinogenesis [103-105]. Thus targeting the enzymatic antioxidant system could be an efficient strategy to preferentially kill cancer cells by increasing intracellular ROS levels. In fact it has been hypothesized that a further oxidative stress induced for example by ROS generating anticancer drugs could increase the intracellular ROS levels beyond a certain “threshold” of tolerance and eventually lead to cell death [106]. In this scope, different molecules have been developed and as shown in Figure 3 we will focus on those directed against the enzymatic antioxidant systems.

Glutathione is the central molecule in the glutathione antioxidant system and many molecules have been developed with the purpose of altering intracellular glutathione levels or redox homeostasis. Since glutathione is enzymatically formed from cysteine, glutamate, and glycine, altering the cellular availability of these amino acids especially glutamate and cysteine will lead to the diminishment of glutathione intracellular levels. Sulphasalazine is an inhibitor of xCT, a transporter subunit of the xC(-) system, which is responsible for the import of cystine, the precursor of cysteine, into the cell [107]. The use of sulphasalazine as an anticancer drug has been reported in several studies where sulphasalazine has been shown to decrease glutathione levels and reduces the growth and viability of human breast and pancreatic cancer cells [108,109]. On the other hand, glutamine is the precursor of glutamate that is converted subsequently into glutathione and drugs affecting glutamine metabolism such as L-asparaginase or molecule 968 that targets kidney-type glutaminase also referred to as glutaminase 1 (GLS), respectively show an anti-leukemic and antiproliferative effect [110,111]. Although it was not assessed by the authors, we can speculate that the use of L-asparaginase and 968 could alter glutathione intracellular levels and thus affect cellular redox homeostasis. On the other hand inhibiting the enzyme responsible for glutathione synthesis is a more direct way to decrease glutathione intracellular levels. Buthionine sulfoximine (BSO) is an inhibitor of glutamate cysteine ligase (GCL), the rate-limiting enzyme for glutathione synthesis [112]. This compound has been shown to cause glutathione depletion and exhibits anticancer activity in various types of cancer cells [113,114]. The depletion of glutathione can be also achieved by β -phenylethyl isothiocyanate (PEITC) or imexon two compounds that conjugate with glutathione via electrophile-nucleophile interactions [115,116]. Furthermore glutathione depletion by PEITC was also reported to inhibit GPx activity leading to ROS increase in oncogenically transformed ovarian epithelial cells [117]. Another strategy to hamper the glutathione antioxidant system is to affect glutathione redox state. This is the mechanism of action of NOV-002, a glutathione disulfide mimetic that alters reduced/oxidized glutathione levels and ratio thus increasing oxidative stress [118].

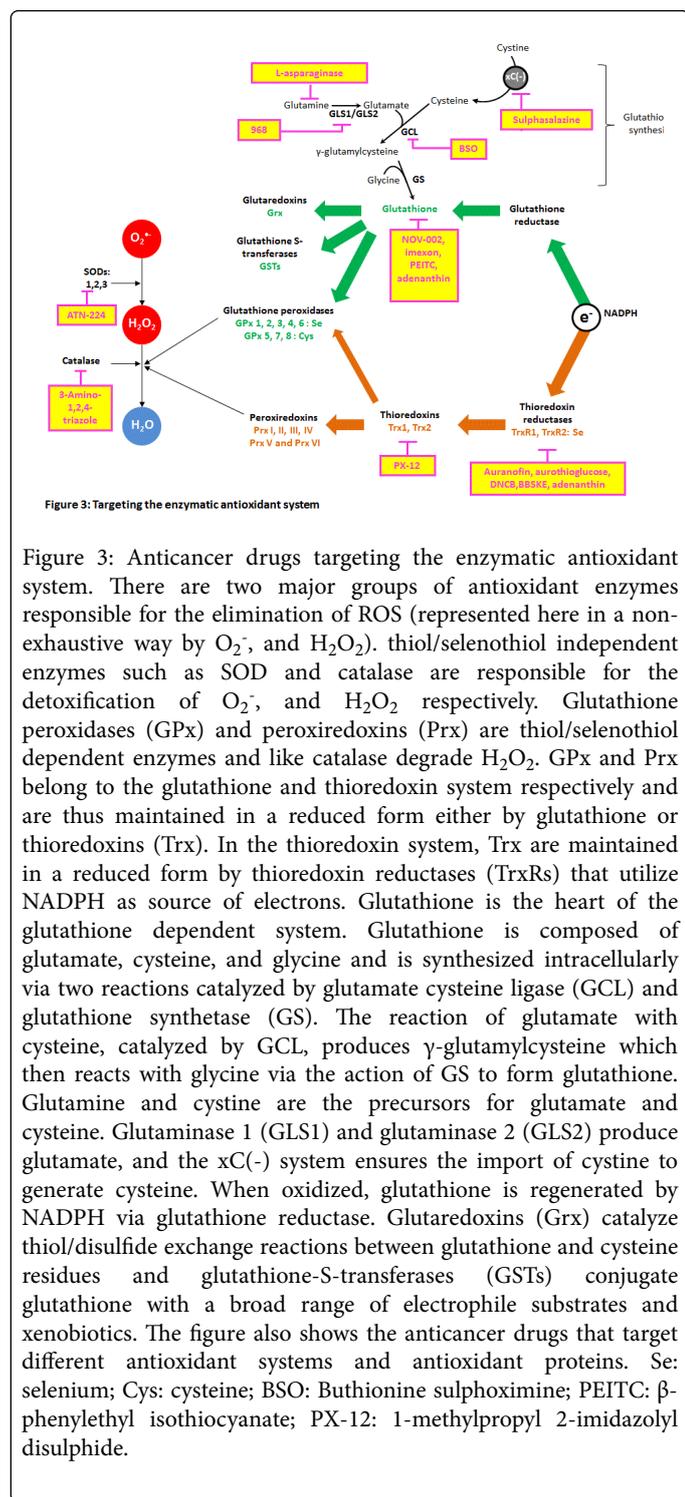


Figure 3: Anticancer drugs targeting the enzymatic antioxidant system. There are two major groups of antioxidant enzymes responsible for the elimination of ROS (represented here in a non-exhaustive way by O_2^- and H_2O_2). thiol/selenothiol independent enzymes such as SOD and catalase are responsible for the detoxification of O_2^- and H_2O_2 respectively. Glutathione peroxidases (GPx) and peroxidoredoxins (Prx) are thiol/selenothiol dependent enzymes and like catalase degrade H_2O_2 . GPx and Prx belong to the glutathione and thioredoxin system respectively and are thus maintained in a reduced form either by glutathione or thioredoxins (Trx). In the thioredoxin system, Trx are maintained in a reduced form by thioredoxin reductases (TrxRs) that utilize NADPH as source of electrons. Glutathione is the heart of the glutathione dependent system. Glutathione is composed of glutamate, cysteine, and glycine and is synthesized intracellularly via two reactions catalyzed by glutamate cysteine ligase (GCL) and glutathione synthetase (GS). The reaction of glutamate with cysteine, catalyzed by GCL, produces γ -glutamylcysteine which then reacts with glycine via the action of GS to form glutathione. Glutamine and cysteine are the precursors for glutamate and cysteine. Glutaminase 1 (GLS1) and glutaminase 2 (GLS2) produce glutamate, and the xC(-) system ensures the import of cysteine to generate cysteine. When oxidized, glutathione is regenerated by NADPH via glutathione reductase. Glutaredoxins (Grx) catalyze thiol/disulfide exchange reactions between glutathione and cysteine residues and glutathione-S-transferases (GSTs) conjugate glutathione with a broad range of electrophile substrates and xenobiotics. The figure also shows the anticancer drugs that target different antioxidant systems and antioxidant proteins. Se: selenium; Cys: cysteine; BSO: Buthionine sulphoximine; PEITC: β -phenylethyl isothiocyanate; PX-12: 1-methylpropyl 2-imidazolyl disulphide.

Parallel to the glutathione antioxidant system the thioredoxin system is one of the main thiol-dependent electron donor systems and participates actively in ROS detoxification. Thioredoxin reductase (TrxR) is a major actor of this system. This seleno-protein catalysis the NADPH dependent reduction of disulfide in oxidized thioredoxins (Trx) and some other proteins [119]. Furthermore, growing evidence indicates that TrxR is over-expressed or constitutively active in many tumor cells and exhibits protective effects against multiple cellular

stresses and chemotherapeutic agents [120,121] Different electrophilic drugs such as 1-chloro-2,4-dinitrobenzene (DNCB) and 1,2-[bis (1,2-Benzisoselenazolone-3 (2H) -ketone)]ethane (BBSKE) have been identified as capable of inhibiting TrxR both *in vitro* and *in vivo* [122-124]. But the most well-known inhibitors of TrxR are gold compounds aurothioglucose and auranofin that are commonly used in the treatment of rheumatoid arthritis [125]. Recently, different groups showed an interest in repurposing auranofin to treat cancer through ROS modulation. In fact recent data demonstrated that auranofin induces lethal oxidative stress in chronic lymphocytic leukemia (CLL) [126] and chronic myeloid leukemia (CML) cells resistant to imatinib [127]. Furthermore, auranofin was found to be effective against various types of drug-resistant cancer cells such as human ovarian cells [128]. Concerning breast cancer cell models, auranofin has been recently reported to inhibit the proliferative growth of MDA-MB-231 cells [129] and combining selenocystine with auranofin induces apoptosis in MCF-7 cells [130]. Similar results were obtained in A549 human lung adenocarcinoma cells where selenocystine was used to enhance auranofin induced A549 apoptosis *in vitro* and *in vivo* through synergistic inhibition of TrxR1 [131]. Besides from targeting TrxR, specific inhibitors have been developed to target other antioxidant proteins of the thioredoxin system. This is the case for 1-methylpropyl 2-imidazolyl disulphide (PX-12) an inhibitor of Trx1 that was shown to induce ROS-mediated apoptosis in drug resistant multiple myeloma. [132]. Adenanthin is a diterpenoid isolated from the leaves of *Rabdosia adenantha* and was first reported by Liu et al. to induce differentiation of acute promyelocytic leukemia (APL) cells [133]. Initially described as a specific Prx I and II inhibitor adenanthin was recently reported to form stable adducts with glutathione and to inhibit TrxR activity [134].

Least but not last, specific inhibitors have been developed to target SODs and catalase. ATN-224 is a copper chelator that targets SOD1 leading to the inhibition of endothelial cell proliferation *in vitro*, attenuation of angiogenesis *in vivo* and apoptotic death of multiple myeloma cells [135]. On the other hand 3-amino-1,2,4-triazole is a catalase inhibitor reported to cause the reduction of catalase activity by 75% in rat 36B10 glioma cell which sensitizes them to further oxidative stress [136].

Conclusion

Continuous ROS production due to an oxidative environment and the saturation of the intracellular detoxification system leads to oxidative stress. This will damage biological macromolecules, eventually causing genomic instability as well as mitochondrial dysfunction. Maintaining high ROS levels favors cancer initiation and progression since ROS are implicated in EMT, cell migration, invasion, metastasis and CSCs phenotype. Although no specific gene mutation or chromosomal abnormality is common to all cancers [137], nearly all cancer cells exhibit high ROS levels and are thus highly dependent on the antioxidant systems in order to cope with this intrinsic oxidative state. Hampering the activity of antioxidant enzymes, especially those that are overexpressed in cancers, could lead to specific cancer cells death. Furthermore combining this approach with ROS generating drugs can be an efficient strategy to eliminate highly resistant cancer cells and eventually CSCs. ROS based anti-cancer approaches have flourished these past 10 years and repurposing U.S. Food and Drug Administration (FDA) approved drugs as anticancer agents, based on ROS modulation mechanisms, will most probably lead to a fast emergence of new anticancer therapies in the years to come.

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