Review paper

Significance of selected antioxidant enzymes in cancer cell progression

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Antioxidant enzymes (AOEs), including superoxide dismutase isoenzymes (SOD), catalase (CAT), glutathione peroxidase (GSH-Px) along with glutathione reductase (GR), reduced glutathione (GSH) and glutathione transferase (GST), are thought to be necessary for life processes in all oxygen-metabolizing cells by removing reactive oxygen species (ROS). The biological significance of AOEs in transformed cells is still unclear, but their capacity to survive may be affected by changes in cellular processes such as proliferation, invasiveness, migration, apoptosis and drug resistance. This review summarizes the significance of antioxidant enzymes in cancer cell progression mainly in an *in vitro* context.

Key words: antioxidant enzymes, reactive oxygen species, cancer progression, lipid peroxidation, ROS-mediated treatment of cancer.

Introduction

Reactive oxygen species (ROS) are chemically reactive molecules such as superoxide anions (O_2^{-*}), hydroxyl radicals (OH^{-*}) and hydrogen peroxide (H_2O_2) that have essential functions in living organisms. ROS are constitutively produced by the mitochondrial electron transport chain during the course of cellular respiration, by cytochrome P450-related components of microsomes, lipoxygenase, cyclooxygenase [1] and in many human tumors and normal counterparts by NADPH oxidase [2]. ROS production in cancer cells also usually occurs following exposure to chemotherapeutic drugs such as doxorubicin, vinblastine, paclitaxel and platinum compounds, leading to multiple cellular responses to oxidative stress [3–8].

Tumor cells have higher levels of ROS than normal cells. A moderate increase in ROS production in cancer cells can promote cell proliferation and differentiation [9, 10]. On the other hand, excessive amounts of ROS can cause oxidative damage to proteins, DNA and lipids, acting as toxic agents [11]. Cancer cells with increased levels of ROS are likely to be more vulnerable to damage by further ROS insults induced by exogenous agents such as chemotherapeutic drugs [12-14]. Nevertheless, cancer cells can also adapt to survive under certain levels of oxidative stress, mainly due to increased activity of antioxidant enzymes [12, 15]. This oxidative adaptation process may contribute to cancer cell progression via inducing proliferation of cancer cells in primary tumors, activating an invasion potential or drug resistance phenotype [16].

Living organisms possess antioxidant mechanisms, which protect normal and malignant cells from ROS levels under physiological conditions and which consist of enzymes and non-specific antioxidants. These

enzymes include copper- and zinc-containing superoxide dismutase (Cu/ZnSOD also termed Sod-1), manganese-dependent superoxide dismutase (MnSOD also known as Sod-2), glutathione peroxidase (GSH-Px), and catalase (CAT). Malondialdehyde (MDA) is a marker of lipid peroxidation [17-19]. Superoxide dismutase (SOD) catalyzes the conversion of O_2^- to H_2O_2, which can then be converted to water by catalase (CAT) or glutathione peroxidase (GSH-Px) coupled with glutathione reductase (GR) [17, 18]. There are two main forms of SOD in eukaryotic cells: Sod-1, also known as copper- and zinc-containing superoxide dismutase, primary located in the cytosol but also in the nucleus; and Sod-2, also named manganese-dependent superoxide dismutase (MnSOD), sited in the mitochondrial matrix [20]. The GSH system functions via glutathione peroxidase (GSH-Px) enzymes, which inactivate H_2O_2 and other hydroperoxides (including alkyl and lipid peroxides) by conversion of GSH to glutathione disulfide (GSSG), which is converted back to GSH by glutathione reductase (GR) using NADPH [21]. Substrate specific cooperation between various antioxidative enzymes and cofactors are presented in Fig. 1.

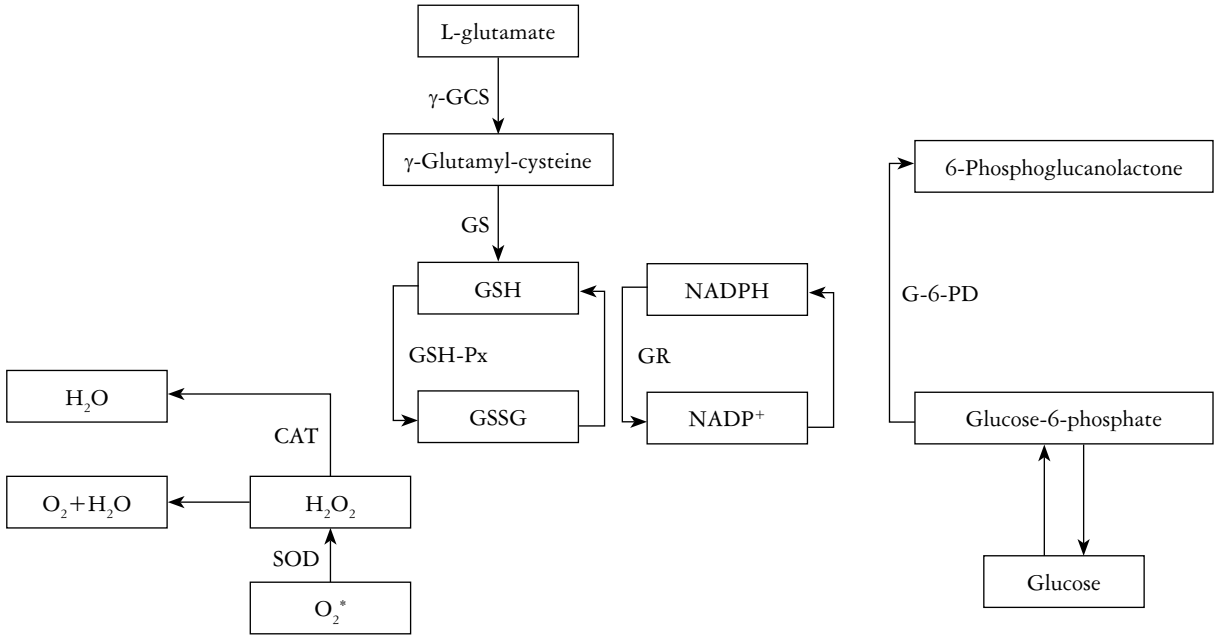
In this review, the effects of selected antioxidant enzymes activity on cancer cells proliferation, chemoresistance, invasine and migratory potential of this cells, are presented. Table I shows influence of antioxidant enzymes overexpression/activity on proliferation, invasion, metastasis and drug resistance phenotype of cancer cells an *in vitro.*

The correlation between AOE expression and the clinical outcome of cancer patients has been investigated using biopsy specimens. Studies have documented high levels of MnSOD in malignant tumors of the mesothelium, stomach, ovary, cervix brain, and thyroid [23-28]. Pancreatic cancer, however, has been demonstrated to have low activity of antioxidant enzymes [29]. Immunohistochemical studies demonstrated that MnSOD, Cu/ZnSOD, CAT and GSH-Px are decreased in human pancreatic ductal carcinoma specimens when compared to normal human pancreas. Similar findings are seen in primary pancreatic cancer cell lines, including pancreatic cancer cell line MIA, PaCa-2, which has decreased levels of MnSOD immunoreactivity and enzyme activity when compared to normal pancreas [30].

In malignant gliomas, high immunoreactivity of GST, but not that of Cu/ZnSOD, was related to the short survival time after recurrence in tumor-bearing patients [31]. In malignant mesothelioma, high MnSOD activity also reduced tumor progression [26]. In gastric cancers, elevated expression and activity of MnSOD in cancer cells were correlated with a poor overall survival rate in cancer patients [32].

SOD isoenzymes affect proliferation of cancer cells

SOD enzymes can affect tumor cell proliferation via their effects on peroxide levels. Decreased proliferation of cancer cells with high activity of MnSOD isoenzyme was observed in U118 and U118-9 human glioma cells [33] and MIA PaCa-2 pancreatic cell carcinoma



SOD – superoxide dismutase; CAT – catalase; GSH-Px – glutathione peroxidase; GR – glutathione reductase; γ-GCS – γ-glutamylocysteine synthetase; GS – glutamine synthetase; G-6-PD – glucose-6-phophate dehydrogenase

Fig. 1. Substrate specific cooperation between various antioxidative enzymes and cofactors; adapted from Polaniak *et al.* 2010

Table I. The influence of antioxidant enzymes overexpression/activity on proliferation, invasion, metastasis and drug resistance phenotype of cancer cells an *in vitro*

I. AOEs overexpression affect cell proliferation		
Overexpression of MnSOD decreased proliferation rate		
Cell line	**Methods**	**Literature**
MIA, PaCa-2 pancreatic carcinoma cell lines	Transfection or transduction of Mn-SOD cDNA plasmid into cancer cells	Weydert *et al.* 2003 [34]
U-118 human glioma cell line		Zhong *et al.* 1997 [33]
A172R rat glioma		Zhong *et al.* 1996 [35]
SCC-25 human oral squamous carcinoma cell		Liu *et al.* 1997 [38]
MCF-7 human breast carcinoma cell line		Li *et al.* 1995 [39]
WI-38 human lung fibroblasts		Yan *et al.* 1996 [40]
Overexpression of MnSOD had marginal effect on cancer cell proliferation		
HeLa human cervical carcinoma cell line cultured in standard condition	Transfection or transduction of Mn-SOD cDNA plasmid into cancer cells	Palazzotti *et al.* 1999 [23]
HeLa human cervical carcinoma cell line cultured in serum deprivation; protection the cells from growth suppression and cell death		Palazzotti *et al.* 1999 [23]

II. Invasive and migratory potential of cancer cells depend on AOEs capacity			
Cell line	**Method**	**Effect**	**Literature**
HT-1080 fibro-sarcoma cell line overexpressing MnSOD isoen-zyme	Adenoviral transduction of MnSOD cDNA plasmid	Increased frequency of tumour invasion and metastasis *via* induction of MMPs enzyme activities	Connor *et al.* 2007 [47]
HT-1080 fibro-sarcoma cell line overexpressing MnSOD and CAT enzymes	Co-transduction of MnSOD and CAT cDNA plasmid	Catalase (CAT), attenuated the Mn-SOD-dependent increases in MMPs expression. Co-expression of CAT in the MnSOD-overexpressing cancer cell lines reversed the increase in invasion potential of these cells	Nelson *et al.* 2003 [49]

III. AOEs alters drug resistance phenotype and cell survival of some cancer cells			
Cell line	**Effects**		
MCF-7/ADR^R selected *in vitro* for doxorubicin resistance	Increased activity of GSH-Px, MDR phenotype	Decreased level of hydrogen peroxide due to GSH-Px activity contributes to cellular resistance to doxorubicin of these cells	Dusre *et al.* 1989 [50]
	Increased GSH level	Increased level of reduced glutathi-one, the co-factor of GSH-Px has been associated with a multidrug resistance phenotype (MDR) of some cancer cell lines	Kuo 2009 [56]

[34]. Indeed, up-regulation of SOD in human cancer cell lines increases H_2O_2 production and reduces tumor growth in the absence of anticancer agents [35]. Among the ROS, H_2O_2 is a good candidate for therapeutic challenge because of its cytotoxic manner. H_2O_2 readily crosses cellular membranes and causes oxidative damage to DNA, proteins and lipids by direct oxidation or via the transition metal driven Haber-Weiss reaction to the extremely reactive hydroxyl radical. It was also reported that H_2O_2 induces apoptosis of a wide range of cancer cells *in vitro* via activation of the caspase cascade [36]. Of greater importance, many anticancer drugs exhibit antitumor activity via H_2O_2-dependent activation of apoptotic cell death [17, 37] showed that enforced expression of MnSOD in pancreatic cell lines (MIA and PaCa-2) changes the *in vitro* biological characteristics of

pancreatic cancer, specifically increasing doubling time of cancer cells. The injection of the MnSOD plasmid into established tumors *in vivo* also demonstrated promising results. Tumors grew slower in nude mice injected with the adenoviral MnSOD construct compared with the parental cell line [34]. Moreover, transfection or transduction of MnSOD cDNA into U118 human glioma cells [33], A172R rat glioma [35], human pancreatic carcinoma cells [34], human oral squamous carcinoma cells SCC-25 [38], human breast carcinoma MCF-7 cells [39], and virally transformed WI-38 human lung fibroblast [40] also suppressed the malignant phenotype. In all these tumor types, overexpression of MnSOD led to suppression of at least part of the tumor cell phenotype. Thus, the evidence appears substantial that MnSOD elevation by cDNA transfection or adenoviral transduction can suppress the malignant phenotype in a great variety of tumors.

On the other hand, it is difficult to predict how tumors will respond to increases in steady-state production of H_2O_2 due to their heterogeneous makeup. The above-mentioned findings contrast with other data presented by Palazzotti *et al.* [23]. This research group revealed that MnSOD overexpression had marginal effects on the growth of human cervical carcinoma HeLa cell line in standard medium but markedly protected the cells from growth suppression and cell death in conditions of serum deprivation. These observation might be due to the fact that HeLa cells express high levels of SOD and CAT enzymes and would therefore be able to counteract the cytotoxic effects of peroxide. The outcome of increased SOD activity would more likely reflect the capacity of SOD to reduce levels of oxygen radicals (superoxide anions O_2^{*-}) [23]. These data are still not irreconcilable, since the biological response to MnSOD is likely to be influenced by multiple factors, including cell type used in experiments and the constitutive abundance of the protein. This contention is consistent with the wide variation in MnSOD expression observed in tested cancers.

Our previous findings suggest that fat derived adipokine such as: visfatin triggers a redox adaptation response, leading to an up-regulation of SOD isoenzymes, GSH-Px and CAT enzymes in Me45 human malignant melanoma cells *in vitro*. Increased antioxidant enzymes activity induced by visfatin led to a significantly increased proliferation rate in the study using the [3H] thymidine incorporation method in these cells. Unlike insulin, visfatin-induced melanoma cell proliferation was not mediated by an insulin receptor [41, 42].

Invasive and migratory potential of cancer cells depend on AOE capacity

Recent data indicated that primary tumors and metastatic lesions are associated with changes in the content and activity of antioxidant enzymes with an associated change in growth characteristics depending on the H_2O_2 concentrations. *In vitro* studies have shown that a number of cancer cell lines contain elevated levels of mitochondrial manganese-containing superoxide dismutase (MnSOD) and decreased activity of CAT, and that this change in steady state levels of H_2O_2 correlates with increased metastasis and resistance to apoptosis [43, 44]. Epidemiologic evidence has also linked a single nucleotide polymorphism in the MnSOD gene, which increases its activity, to risk of developing breast [45] and prostate [46] cancers in populations with a poor dietary antioxidant status.

Connor *et al.* [47], using the adenoviral transduction method, reported that up-regulation of MnSOD is associated with an increased frequency of tumor invasion and metastasis in certain cancers. Overexpression and increased activity of MnSOD isoenzyme in HT-1080 fibrosarcoma cells significantly enhanced their migration 2-fold in a wound healing assay and their invasive potential 3-fold in a transwell invasion assay [47]. This study also showed that the MnSOD-dependent production of H_2O_2 leads to increased expression of matrix metalloproteinase (MMP) family members and that there is a strong correlation between this increase in MMP levels and enhanced metastasis. An essential and rate limiting step in metastasis is the remodeling and degradation of the extracellular matrix and basement membrane by MMP enzymes. These enzymes are major contributors of stromal degradation and are vital to the process of cellular invasion [48]. Another study performed by Nelson *et al.* [49] also revealed that the MnSOD-overexpressing HT-1080 cell line displayed increased invasive potential by enhanced MMP-1 expression and activity. They also found that the H_2O_2-detoxifying enzyme catalase (CAT) attenuated the MnSOD-dependent increases in MMPs expression. Co-expression of CAT in the MnSOD-overexpressing cancer cell lines reversed the increase in invasive potential of these cells [49].

Both studies clearly demonstrated that the metastatic potential of HT-1080 fibrosarcoma cell lines is enhanced in response to MnSOD overexpression in a H_2O_2-dependent manner.

Increased antioxidant enzyme activities alter drug resistance phenotype

Increased GSH-Px and cofactor GSH, CAT and Trx enzyme metabolism have been known for years to be correlated with high tumor aggression and resistance to chemotherapy [50-55]. The development of drug resistance to cancer chemotherapy is a major obstacle to the effective treatment of human malignancies. It has been established that membrane proteins, notably multidrug resistance protein (MRP), play important roles in the development of multidrug resistance (MDR). Moreover, ROS and redox adaptation to oxidative stress can affect the efficacy of cancer

treatment by multiple mechanisms, including chemosensitivity of cancer cells to anticancer drugs [56].

Increased levels of ROS in cancer cells may lead to the development of redox adaptation by increasing activity of antioxidant enzymes such as GSH-Px and CAT. Elevation of antioxidant enzyme activities and survival signals as a result of redox adaptation probably explains the drug resistance phenotype of some cancers cells [16]. For example, the human breast cancer cell line MCF-7/ADR^R, selected *in vitro* for doxorubicin resistance, has been shown to display the MDR phenotype. This resistance may also be due in part to elevated levels of glutathione-dependent peroxidase activity. Peroxidase activity in these cells is due mainly to increases in selenium dependent GSH-Px with minor increases in non-selenium dependent peroxidase. Moreover, decreased hydroxyl radical formation was demonstrated in resistant MCF-7/ADR^R cells after anticancer agent exposure, when compared with the parental strain. Thus, at least *in vitro*, increased activity of GSH-Px contributes to cellular resistance to doxorubicin [50]. Moreover, high levels of reduced glutathione (GSH), the co-factor of GSH-Px, have been associated with a multidrug resistance phenotype of some cancer cell lines [56]. From these studies, subsequent decomposition of hydrogen peroxide by CAT and GSH-Px appears to be critical in the resistance of several cancer cells to various ROS-generating agents.

Several members of the MRP family require GSH for transport activities. GSH is the most abundant antioxidant, underscoring the roles of redox regulation of multidrug resistance mediated by this group of ABC transporters. The role of GSH in MRP-1-mediated drug sensitivity in cultured cells was demonstrated in MRP-1-overproducing cells that effectively efflux daunorubicin outside the cells. This effect was partially reversed by exposing these cells to buthionine sulfoximine (BSO), an inhibitor of GSH synthesis. The influence of BSO on drug resistance was associated with decreased GSH content and increased intracellular accumulation of daunorubicin owing to inhibition of the enhanced drug efflux [52]. Sobhakumari *et al.* [57] reported that inhibition of GSH and Trx metabolism enhanced cell killing of human head and neck squamous cell carcinoma (HNSCC) cells by a mechanism involving oxidative stress. Inhibition of GSH and Trx metabolism with buthionine sulfoximine (BSO) and auranofin (AUR) (inhibitor of TR enzyme), respectively, induced significant decreases in clonogenic survival compared to either drug alone in FaDu, Cal-27 and SCC-25 HNSCC cells *in vitro* and *in vivo* in Cal-27 xenografts [57].

AOEs activity and cancer cell survival and resistance to chemotherapeutic drugs

Increased GSH-Px activity also enables cells to survive with a high level of ROS and maintain cellular viability. Furthermore, the increase in glutathione during the adaptation process can enhance the export of anticancer drugs and their inactivation. This altered drug metabolism together with enhanced cell survival may render cancer cells more resistant to chemotherapeutic agents [16]. Increased activity of GSH-Px or CAT in cancer cells can make tumor cells less susceptible to the effects of anticancer drugs, such as doxorubicin-mediated damage. It has been demonstrated that the addition of radical scavengers and compounds with peroxide activity can reduce the cytotoxic effect of anticancer drugs *in vitro* [7]. Samuels *et al.* [58] demonstrated increased doxorubicin sensitivity in the STSAR90 sarcoma tumor cell line in comparison to the STSAR11 wild cell line. Total GSH-Px activity in STSAR90 cells was approximately 6-fold higher than in STSAR11 cells. These results indicate that multidrug resistance due to P-glycoprotein-mediated drug efflux is not the only mechanism of doxorubicin resistance that occurs in sarcomas and that GSH-Px-dependent detoxification of doxorubicin-induced oxygen radicals may contribute to clinical doxorubicin resistance [58]. Likewise, several studies suggest that the resistance to agents that induce intracellular ROS production, such as paclitaxel, doxorubicin and platinum compounds, is correlated with increased antioxidant capacity [12, 16]. Moreover, H-*Ras* transformed cells which exhibited increased hydrogen peroxide and superoxide levels were shown to express higher levels of antioxidant enzyme such as thioredoxin peroxidase. Their enhanced antioxidant defense system is likely to serve as a key mechanism to evade ROS-induced apoptosis. *Ras*-transformed cells were also found to be more sensitive to depletion of glutathione (GSH), leading to ROS accumulation and cell death [59], suggesting a crucial role of antioxidant enzyme activities in cancer cell survival. Studies using inducible c-*Myc* in melanoma cells showed that c-*Myc* controlled the expression of the GSH synthesis enzyme. Apoptosis induced by downregulation of c-*Myc* was associated with cellular depletion of reduced GSH [60]. These data suggest that cells with active c-*Myc* may survive ROS stress by up-regulating GSH synthesis.

Thus it is conceivable that during malignant transformation the oncogenic signals both induce ROS generation to stimulate cell proliferation through redox-sensitive transcriptional factors and promote oxidative adaptation to minimize cellular ROS damage.

The mechanism of the redox adaptation process may involve multiple pathways to activate redox-sensitive transcription factors such as nuclear factor-κβ, Nrf2, c-Jun and HIF-1α, which lead to increased expression of antioxidant molecules such as SOD, catalase and GSH-Px, and the GSH antioxidant system [16]. These redox sensitive TFs also regulate the expression of proteins that are involved in proliferation,

immortalization, angiogenesis and metastasis, thus providing a further survival advantage [61].

GSH-Px and lipid peroxidation

Lipid peroxidation is one of the most investigated consequences of ROS' actions on membrane structure and function. It has been shown that lipid hydroperoxides and oxygenated products of lipid peroxidation degradation participate in the signal transduction cascade [62], the control of cell proliferation, and the induction of differentiation, maturation, and apoptosis [63, 64]. It has been shown that lipid peroxidation and ROS are triggers and essential mediators of apoptosis, which eliminates precancerous and cancerous, virus-infected and otherwise damaged cells that threaten our health. ROS react with polyunsaturated fatty acid residues in phospholipids, resulting in the production of a plethora of products, many of them reactive toward protein and DNA [65].

One of the most abundant carbonyl products of lipid peroxidation is malondialdehyde (MDA), which also reacts with DNA to form adducts to deoxyguanosine, deoxyadenosine, and deoxycytidine [66].

GSH-Px is an enzyme which reduces not only hydrogen peroxide but also organic superoxides. In such reactions an organic superoxide (ROOH) becomes reduced to an appropriate alcohol (ROH). In the case of lipid superoxide, this means that it cannot become an initiator of lipid peroxidation, and therefore glutathione peroxidase inhibits lipid peroxidation [67].

Lipid peroxidation appears to be a major source of endogenous DNA damage in humans that may contribute significantly to cancer.

ROS-mediated treatment of cancer as a therapeutic strategy

To exploit the ROS mediated cell-death mechanism as a therapeutic strategy, it is possible to combine drugs that induce ROS production with compounds that suppress the cellular antioxidant capacity. This approach might be particularly useful in drug-resistant cancer cells. For example, buthionine sulfoximine (BSO), a glutathione synthesis inhibitor, can increase the cytotoxicity of melphalan by preventing glutathione peroxidase activity and increasing H_2O_2 levels [68].

Alexandre *et al.* examined the effect of mangafodipir on the growth inhibiting properties of chemotherapeutic agents against mouse colon cancer cells and on their hematologic toxicity of paclitaxel in a murine model. Mangafodipir is a contrast agent used clinically for magnetic resonance imaging and possesses antioxidant (specifically, O_2^{-*} and H_2O_2-detoxifying) properties. This study revealed that mangafodipir is protective against the hematological toxicity of paclitaxel in a murine model. Moreover, the glutathi-

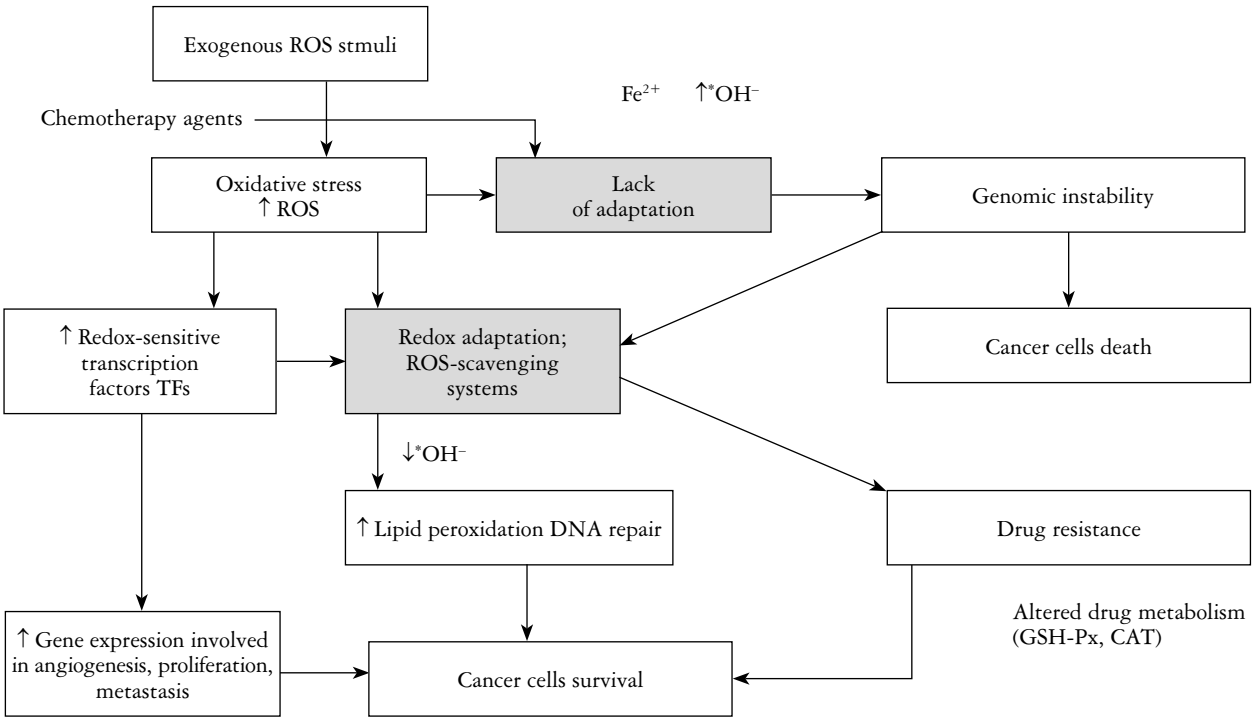


Fig. 2. Redox adaptation to oxidative stress in cancer cells. (A) Severe accumulation of cellular ROS under exogenous stress stimuli such as chemotherapeutic agents may induce lethal damage in cancer cells (via increased level of ROS such as OH*. (B) In certain cancer cells, persistent ROS stress may induce adaptive stress responses leading to an increase in the expression/activation of ROS-scavenging enzymes such as SOD, GSH-Px, CAT. Adapted with modification from [8]

one precursor N-acetylcysteine (NAC), which can function directly in the detoxification of H_2O_2 and as a thiol donor to protect critical sulfhydryl groups in cell proteins, also prevented the hematological toxicity of paclitaxel. On the other hand, mangafodipir, but not NAC appears to improve the therapeutic activity of this chemotherapeutic agent against CT26 mouse colon cancer cells *in vivo*. These chemical compounds also protected normal leukocytes from the toxic effects of oxaliplatin and 5-fluorouracil *in vitro*. Because mangafodipir is widely used, this drug might be appropriate for study as a chemoprotective compound in human trials [12, 69, 70].

On the other hand, hydrogen peroxide is also known as a strong oxidant that induces apoptosis of tumor cells *in vitro* [3, 71]. Perhaps H_2O_2 alone is relative unstable and is a small water-soluble molecule. These characteristics hamper the utility of H_2O_2 as an antitumor agent that might be selectively delivered to the tumor. In fact, H_2O_2 used alone was ineffective when injected into a tumor or into the circulation [72, 73], perhaps because of its rapid clearance and decomposition by catalase in erythrocytes. Use of an H_2O_2-generating enzyme has been proposed as an alternative approach to developing an H_2O_2-dependent antitumor treatment. Fang *et al.* [73] reported that GO, which generates H_2O_2 during oxidation of glucose, showed antitumor activity in solid tumor models. However, regulation of H_2O_2 production by exogenously administered GO in tumor-bearing hosts is problematic because the availability of its substrates, oxygen and glucose, cannot be significantly modulated with the possible induction of severe systemic side effects due to systemic H_2O_2 production. In fact, GO administration to produce H_2O_2 required injection of antioxidants to minimize systemic toxicity. Morover Fang and co-workers delivered to tumor-bearing mice polyethylene glycol conjugated with D-amino acid oxidase (PEG-DAO). DAO is a flavoprotein that catalyzes the stereoselective oxidative deamination of D-amino acids to the corresponding alpha keto acids. During this oxidation reaction, molecular oxygen is used as an electron acceptor, and H_2O_2 is generated. DAO activity and hence generation of H_2O_2 was regulated by exogenous administration of D-amino acids. Fang and co-workers in first time treatment administered PEG-DAO *i.v.* to tumor-bearing mice. After an adequate lag time, the substrate of DAO, D-proline, was injected *i.p.* This treatment resulted in significant suppression of tumor growth compared with tumor growth in control animals [73].

In conclusions: Modulation of ROS production might be a promising approach to increase anticancer agents cytotoxicity.

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