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 AOE s 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.
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 \( \text{O}_2^- \) to \( \text{H}_2\text{O}_2 \), 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 \( \text{H}_2\text{O}_2 \) 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, invasive 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

---

**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

<table>
<thead>
<tr>
<th>CELL LINE</th>
<th>METHODS</th>
<th>LITERATURE</th>
</tr>
</thead>
<tbody>
<tr>
<td>MIA, PaCa-2 pancreatic carcinoma cell lines</td>
<td>Transfection or transduction of Mn-SOD cDNA plasmid into cancer cells</td>
<td>Weydert et al. 2003 [34]</td>
</tr>
<tr>
<td>U-118 human glioma cell line</td>
<td></td>
<td>Zhong et al. 1997 [33]</td>
</tr>
<tr>
<td>A172R rat glioma</td>
<td></td>
<td>Zhong et al. 1996 [35]</td>
</tr>
<tr>
<td>SCC-25 human oral squamous carcinoma cell</td>
<td></td>
<td>Liu et al. 1997 [38]</td>
</tr>
<tr>
<td>MCF-7 human breast carcinoma cell line</td>
<td></td>
<td>Li et al. 1995 [39]</td>
</tr>
<tr>
<td>WI-38 human lung fibroblasts</td>
<td></td>
<td>Yan et al. 1996 [40]</td>
</tr>
</tbody>
</table>

II. INVASIVE AND MIGRATORY POTENTIAL OF CANCER CELLS DEPEND ON AOE CAPACITY

<table>
<thead>
<tr>
<th>CELL LINE</th>
<th>METHOD</th>
<th>EFFECT</th>
<th>LITERATURE</th>
</tr>
</thead>
<tbody>
<tr>
<td>HT-1080 fibrosarcoma cell line overexpressing MnSOD isoenzyme</td>
<td>Adenoviral transduction of MnSOD cDNA plasmid</td>
<td>Increased frequency of tumour invasion and metastasis via induction of MMPs enzyme activities</td>
<td>Connor et al. 2007 [47]</td>
</tr>
<tr>
<td>HT-1080 fibrosarcoma cell line overexpressing MnSOD and CAT enzymes</td>
<td>Co-transduction of MnSOD and CAT cDNA plasmid</td>
<td>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 invasion potential of these cells</td>
<td>Nelson et al. 2003 [49]</td>
</tr>
</tbody>
</table>

III. AOE ALTERS DRUG RESISTANCE PHENOTYPE AND CELL SURVIVAL OF SOME CANCER CELLS

<table>
<thead>
<tr>
<th>CELL LINE</th>
<th>EFFECT</th>
<th>LITERATURE</th>
</tr>
</thead>
<tbody>
<tr>
<td>MCF-7/ADR³ selected in vitro for doxorubicin resistance</td>
<td>Increased activity of GSH-Px, MDR phenotype</td>
<td>Dusre et al. 1989 [50]</td>
</tr>
<tr>
<td></td>
<td>Decreased level of hydrogen peroxide due to GSH-Px activity contributes to cellular resistance to doxorubicin of these cells</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Increased level of reduced glutathione, the co-factor of GSH-Px has been associated with a multidrug resistance phenotype (MDR) of some cancer cell lines</td>
<td>Kuo 2009 [56]</td>
</tr>
</tbody>
</table>

[34]. Indeed, up-regulation of SOD in human cancer cell lines increases \( \text{H}_2\text{O}_2 \) production and reduces tumor growth in the absence of anticancer agents [35]. Among the ROS, \( \text{H}_2\text{O}_2 \) is a good candidate for therapeutic challenge because of its cytotoxic manner. \( \text{H}_2\text{O}_2 \) 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 \( \text{H}_2\text{O}_2 \) 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 \( \text{H}_2\text{O}_2 \) -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 \( \text{H}_2\text{O}_2 \) 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 \( \text{O}_2^{-*} \)) [23]. These data are still not reconcilable, 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 \( \text{H}_2\text{O}_2 \) 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 \( \text{H}_2\text{O}_2 \) 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 isozyme 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 \( \text{H}_2\text{O}_2 \) 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 \( \text{H}_2\text{O}_2 \)-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 \( \text{H}_2\text{O}_2 \)-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<sup>a</sup>, 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<sup>a</sup> 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 <i>et al.</i> [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 <i>et al.</i> [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<sup>+</sup>-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-κB, 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$_2$O$_2$ 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$_2$O$_2$-detoxifying) properties. This study revealed that mangafodipir is protective against the hematological toxicity of paclitaxel in a murine model. Moreover, the glutathi-
one precursor N-acetylcysteine (NAC), which can function directly in the detoxification of $H_2O_2$ and as a thiol donor to protect critical sulphydryl 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 vitro. These chemical compounds also protected normal leukocytes from the toxic effects of oxaliplatin and 5-fluouracil 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 antimutagenic substrate, but 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 antimutagen treatment. Fang et al. [73] reported that GO, which generates $H_2O_2$, during oxidation of glucose, showed antimutagenic 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. Moreover 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.

Grant sponsor: Medical University of Silesia in Katowice. Grant number: KNW-2-009/N/3/N.

References


Address for correspondence
Dr Rafał Jakub Buldak
Medical University of Silesia, Katowice
School of Medicine with the Division of Dentistry
Department of Physiology in Zabrze
Jordana 19,
41-808 Zabrze, Poland
tel./fax +48 32 272 23 62
e-mail: rbuldak@sum.edu.pl