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Review

Glutathione peroxidases in different stages of carcinogenesis ☆

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ABSTRACT

Cancer cells produce high amounts of reactive oxygen species (ROS) and evade apoptosis. Hydroperoxides support proliferation, invasion, migration and angiogenesis, but at higher levels induce apoptosis, thus being pro- and anti-carcinogenic. Accordingly, glutathione peroxidases (GPxs) regulating hydroperoxide levels might have dual roles too. GPx1, clearly an antioxidant enzyme, is down-regulated in many cancer cells. Its main role would be prevention of cancer initiation by ROS-mediated DNA damage. GPx2 is up-regulated in cancer cells. GPx1/GPx2 double knockout mice develop colitis and intestinal cancer. However, GPx2 knockdown cancer cells grow better *in vitro* and *in vivo* probably reflecting the physiological role of GPx2 in intestinal mucosa homeostasis. GPx2 counteracts COX-2 expression and PGE_2 production, which explains its potential to inhibit migration and invasion of cultured cancer cells. Overexpression of GPx3 inhibits tumor growth and metastasis. GPx4 is decreased in cancer tissues. GPx4-overexpressing cancer cells have low COX-2 activity and tumors derived therefrom are smaller than from control cells and do not metastasize. Collectively, GPxs prevent cancer initiation by removing hydroperoxides. GPx4 inhibits but GPx2 supports growth of established tumors. Metastasis, but also apoptosis, is inhibited by all GPxs. GPx-mediated regulation of COX/LOX activities may be relevant to early stages of inflammation-mediated carcinogenesis.

1. Introduction

A low intake of selenium has been shown to correlate with a higher incidence of cancer [1,2] and, therefore, chemopreventive functions have generally been attributed to selenium [3]. Cancer prevention by selenium supplementation has finally been demonstrated in a large controlled clinical trial [4]. A follow-up of the same trial revealed that only those participants entering the study with a low selenium status experienced reduction in total cancer incidence, whereas in those with a better selenium status the incidence was rather elevated [5]. A second follow-up analysis reported on a selenium-dependent increase in the risk of squamous cell carcinoma and total non-melanoma skin cancer in the subjects with a history of non-melanoma skin cancer [6]. The second large clinical trial, the Selenium and Vitamin E Cancer Prevention Trial (SELECT) did not reveal any benefit of selenium supplementation on prostate cancer or other cancers [7]. Selenium even could have adverse effects [7,8]. This shows that a benefit of selenium may depend on (1) the basal selenium status, (2) the type of cancer, and probably (3) the stage of the cancer at which up-regulation of certain selenoproteins starts.

In contrast, different forms of selenium at supranutritional dosages provided protection in a high number of animal studies [9,10] (reviewed in [11,12]). Underlying mechanisms are not well understood. It is not known whether individual selenoprotein(s) or all of them contribute to cancer prevention by selenium or whether particular selenium compounds act independently from selenoprotein biosynthesis. Discussed mechanisms such as alterations in gene expression and DNA damage and repair (reviewed in [13]) may depend on the chemical form of selenium and the time point of intervention [14,15]. Other discussed mechanisms such as dampened inflammatory response, induction of apoptosis, regulation of cell cycle control, or inhibition of tumor cell invasiveness in part at least might be influenced by selenoproteins [16–19].

The human genome contains 25 genes for selenoproteins, the mouse genome 24 [20]. The resulting number of selenoproteins might be higher, since splice variants are possible and the number of them will increase with time [21,22]. The function of most of these proteins is still unknown. Even the individual role of the so far 8 known glutathione peroxidases, of which five are selenoproteins in humans, is not entirely clear. They all might be able to reduce hydroperoxides:

$$ROOH + 2GSH \rightarrow ROH + H_2O + GSSG$$

Their function, therefore, should be related to the removal and/or metabolism of hydroperoxides. A role of GPxs in carcinogenesis seems straight forward, since many processes of cancer development depend on or are influenced by hydroperoxides. The review, therefore, will focus on the relevance of hydroperoxides to carcinogenesis and the role of individual glutathione peroxidases.

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2. Redox state in tumor cells

Two characteristics of tumor cells compared to normal cells are an increased generation of reactive oxygen species (ROS) [23] and a decreased capacity to eliminate ROS. In addition, a high number of tumor cells have acquired resistance to drug therapy by the up-regulation of Phase II and Phase III enzymes/proteins that eliminate anti-cancer drugs. Up-regulation most probably occurs via the activation of the Nrf2/Keap1 system which, apart from a high number of thiol modifying compounds is activated by hydroperoxides (for a recent review see [24]). Support for a persistent oxidative stress in cancer cells comes from elevated oxidative DNA lesions, as evidenced by 8-hydroxy-2′-deoxyguanosine (8-OH-dG) in various tumors [25–27]. Cancer cells use ROS to stimulate proliferation, invasion, migration and angiogenesis, but most importantly have also developed mechanisms to evade apoptosis [28]. Several sources of ROS in cancer cells have been discussed: (1) enhanced release of $O_2^{\cdot-}$ and/or H_2O_2 from mitochondria [29], (2) production of $O_2^{\cdot-}$ and subsequently H_2O_2 by activated NADPH oxidase (NOX) systems [30,31]; (3) suppression of antioxidant enzymes such as MnSOD, GPx1 [32,33] and, in some cases, catalase [34,35], whereas CuZnSOD has been reported to be increased or decreased [36]; (4) exposure to ROS and pro-inflammatory cytokines released by inflammatory cells surrounding the tumor [37–39]; or (5) a combination of those.

ROS-driven growth stimulation is obtained by alterations in the activity of protein kinases, phosphatases, and nuclear transcription factors (see below). Pathways and systems are regulated in a way that low or moderate levels of ROS activate them, whereas they are rather inhibited at high levels. To cope with both situations, tumor cells obviously have acquired the ability to adjust oxidative stress to a level which is sufficient to maintain their survival but not to initiate their elimination by apoptosis [40]. This growth advantage of tumor cells was the rationale for the attempt to prevent carcinogenesis by antioxidants. Interference with the redox balance of tumor cells, however, did not result in a beneficial outcome in all situations (see above). Whereas removal of ROS would inhibit DNA damage and cancer cell proliferation, it would at the same time inhibit ROS-induced apoptosis. Thus, hydroperoxides appear to have a dual role in carcinogenesis which might similarly hold true for GPxs. We, therefore, summarize reports undertaken to modulate GPx activities by alterations in the selenium status or by genetic intervention to figure out their putative role in tumor initiation, proliferation, and metastasis.

3. Production of hydroperoxides

3.1. Mitochondria

Respiratory complexes and enzymes are mitochondrial sources of $O_2^{\cdot-}$ [41] (reviewed in [42–44]). Complex I appears to be the primary source in the brain under basal and pathological conditions, whereas complex III is responsible for $O_2^{\cdot-}$ produced in mitochondria from heart and lung. Superoxide formation occurs on the outer mitochondrial membrane, in the matrix and on both sides of the inner membrane and accounts for 1–2% of the overall oxygen consumption. Whereas $O_2^{\cdot-}$ generated in the matrix is dismutated into H_2O_2 by MnSOD present in that compartment, $O_2^{\cdot-}$ produced in the intermembrane space can be exported via voltage-dependent anion channels [45] or is dismutated by CuZnSOD that is transiently activated by H_2O_2-mediated modification of critical thiol groups [46]. The main task of mitochondria, however, is energy production. A decrease in mitochondrial energy metabolism was hypothesized to be the reason for the development of cancer already by Warburg in 1926 when he observed that cancer cells produce most of their ATP through glycolysis and not through the respiratory chain. Later studies challenged this idea and revealed that tumor mitochondria are able to produce ATP. However, glycolysis is indeed up-regulated by hypoxia which prevails in cancer cells. Up-regulation is mediated by the activation of hypoxia-inducible factors (HIF) that are activated and/or induced by ROS (reviewed in [47]).

The impact of mitochondria-derived ROS in cancer-relevant processes has been demonstrated by numerous mutations in mitochondrial DNA of tumors but not in surrounding tissues of the same individuals [48]. In MnSOD overexpressing cells, mitogen-activated kinase (MAPK) pathways are activated and subsequently matrix metalloproteinase-1 (MMP-1) is induced [49]. The main function of mitochondria-derived ROS, however, clearly is the regulation of cell death pathways [47,50], whereas those derived from NADPH oxidase are more likely involved in signalling processes [50].

3.2. NADPH oxidase systems

NADPH oxidases (NOXs) are not restricted to phagocytic leukocytes, but are also expressed in a variety of cell and tissue types to produce H_2O_2 as signalling molecule. In fact, various growth factors and cytokines including PDGF, EGF, insulin, and TNFα signal via NOX-derived H_2O_2 [51]. The first detected NOX [52], now termed NOX-2, consists of the membrane-bound cytochrome b_{558} comprising the catalytic flavin- and heme-binding glycoprotein $gp91^{phox}$ and the smaller $p22^{phox}$ subunit. Cytosolic components, $p47^{phox}$, $p67^{phox}$, $p40^{phox}$ and the small GTPase Rac are recruited to the membrane upon stimulation to form the active complex. Up to now 4 more NOXs (NOX-1, NOX-3 to 5) and two dual oxidases (DUOX 1 and 2) with different tissue localization [51] and subcellular compartmentation [53] have been described. The expression in tumor tissues does not always correlate with normal tissue, with NOX-4 being the isoform most frequently expressed in the tumor cells investigated [54]. This shows that NOX expression is dysregulated during carcinogenesis. The involvement of NOXs in promoting cell survival over apoptosis has been demonstrated in cells and tumors. NOX-1-derived ROS are potent triggers of the angiogenic switch and increase molecular markers of angiogenesis, such as vascular endothelial growth factor (VEGF), VEGF receptors, and matrix metalloproteinases [55,56]. Inhibition of NOX-4 in pancreatic cancer cells led to apoptosis and to inhibition of survival signals and tumor cell growth [57], whereas NOX-5 appeared to contribute to the protein tyrosine phosphatase (PTP)-dependent maturation of B cells into malignant hairy cells [58]. Furthermore, the metastatic potential of fibrosarcoma cells was decreased when they were injected into $gp91^{phox\,-/-}$ mice [59].

4. Systems for hydroperoxide removal

4.1. Glutathione peroxidases

So far 5 selenium-containing glutathione peroxidases (GPx) have been identified in humans, i.e. GPx 1–4 and GPx6. They all can react with H_2O_2 and soluble fatty acid hydroperoxides. GPx4 is the only one that also reacts with complex lipid hydroperoxides (reviewed in [60]). H_2O_2 oxidizes the selenol (−SeH) group of selenocysteine (SeCys) in the active centre of GPxs to the selenenic acid (−SeOH) which then is reduced stepwise by two molecules of glutathione. The reaction requires the deprotonation of SeCys ($-Se^-$) which easily occurs at physiological pH, since the pK of −SeH is low (pK = around 5.2) compared to the pK of cysteines (pK = around 8.2). The reaction of GPx with H_2O_2 is extremely fast ($k'_A = 5\times10^7\ M^{-1}\ s^{-1}$) which guarantees fast removal of H_2O_2, especially when concentrations become high.

4.2. Peroxiredoxins

A novel family of enzymes also able to reduce hydroperoxides is the family of peroxiredoxins (Prx) [61]. So far six members, peroxiredoxin I–VI are known. Prx I–IV are classified as typical 2-Cys