Role of Glutathione in Cancer

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Molecular changes in the glutathione antioxidant system and disturbances in its homeostasis have been implicated in tumor initiation, progression, and treatment response with glutathione having both protective and pathogenic roles. Although in healthy cells it is crucial for the removal and detoxification of carcinogens, elevated glutathione levels in tumor cells are associated with tumor progression and increased resistance to chemotherapeutic drugs. Recently, several novel therapies have been developed to target the glutathione antioxidant system in tumors as a means for increased response and decreased drug resistance. In this comprehensive review we explore glutathione functionalities and different therapeutic approaches and their development through experimental and computational approaches.

Keywords: glutathione ; tumor therapy ; tumor metabolism ; nitrosoglutathione ; S-nitrosation ; ferroptosis ; reactive oxygen species ; metabolism modeling

1. Glutathione, Inflammation, and Cancer

Chronic infection, injury and inflammation have long been recognized as risk factors for the development and progression of various human cancers [1]. There is ample evidence to indicate that factors present in the tumor microenvironment (TME), such as products of inflammatory cells reactive nitrogen and oxygen species may cause DNA damage to the neighboring cells, thereby contributing to tumor progression [2]. A detailed review of many roles of the inflammation-driven changes in tumorigenesis has been recently provided [2]. It has been established that GSH deficiency or a change in GSH/GSSG ratio increases the vulnerability of cells to oxidative stress, inflammation, and tumor progression. However, elevated GSH levels increase antioxidant capacity and resistance to oxidative stress as is evident in many tumors. It has been demonstrated that exogenously added GSH inhibits the inflammatory response through regulation of ROS while endogenous GSH has been recently shown to have a role in fine-tuning the innate immune response to infection thereby regulating inflammation [3]. GSH therefore has a dual role in the inflammatory response as an antioxidant ROS scavenger in the oxidative stress as well as signaling molecule that regulates protein function through thiol–disulfide exchange reactions such as protein glutathionylation with many examples of regulation of oncogenes (e.g., p53, HIF-1, c-jun) presented in the database developed by Chen et al., [4] as well as in a recent review [5]. Here we will focus on the role of different antioxidant enzymes, specifically peroxiredoxin and glutathione peroxidases as well as the impact of NO-mediated post-translational modification of oncoproteins on tumor progression.

1.1. Role of Peroxiredoxin (PRDX)

PRDX is a family of ubiquitously expressed proteins that catalyzes reduction of H_2O_2, organic hydroperoxides and peroxynitrite [6]. In human cells there are six different isoforms of PRDX with specific localization in the cytosol, mitochondria, nuclei, peroxisomes, endoplasmic reticulum, as well as secretome. In all isoforms enzymatic function depends on the conserved peroxidatic cysteine residue contained within an active-site conserved across all isoforms as well as cysteine in the carboxy-terminal region functional in some of the PRDX isoforms. Antioxidant function of PRDX is typically performed through reaction between the main cysteine residue and the residue on the second subunit of the dimer. GSH together with thioredoxin can act as a physiological reductant in this process. Specific roles of different PRDX isoforms in tumor-promoting, tumor-suppression as well as tumor treatment resistance have been reviewed previously for different cancers [6] with a number of specific applications to different cancer types including for example breast [7], lung [8] or pancreatic cancers [9]. In addition to the direct role in redox processes, PRDX in relation to glutathione has a major link to inflammation leading to cancer.

Inflammatory stimuli cause release of oxidized peroxiredoxin-2 (PRDX2), a ubiquitous redox-active intracellular enzyme that extracellularly acts as a redox-dependent inflammatory mediator. The glutathionylation of GSH to PRDX2 cysteine residues has been shown to be central to the regulation of immunity. Additionally, several studies show presence of PRDXs in body fluids of cancer patients, suggesting that PRDXs and its glutathionylation may be a link between

inflammation and cancer [10]. Additionally, ROS-induced cysteine glutathionylation and its modulation have been shown to be the key regulators of IL-1beta bioactivity by preventing the irreversible ROS-elicited deactivation [11]. IL-1beta is increasingly understood as a major regulator of tumor promoting inflammation through its activity on different components of tumor microenvironment including recruitment of tumor infiltrating myeloid cells, angiogenesis, and suppression of anti-tumor immune response [12][13].

1.2. Role of Glutathione Peroxidases (GPXs)

Tumor cells have increased ROS levels and thus upregulate the antioxidant response, including glutathione peroxidases (GPXs). GPXs are members of a multi-enzyme family that catalyze the reduction of H_2O_2, organic hydroperoxides and/or lipid hydroperoxides to generate their corresponding lipid alcohols and water at the expense of oxidation of two molecules of GSH [14]. There are eight isozymes of GPXs (GPX 1–8) that have been identified in humans in different tissues, among these five are selenoproteins (GPXs 1–4 and GPX6) that play an important role in defense against oxidative stress.

Cellular models and transgenic mice deficient in individual *GPX* genes have been utilized to study the potential role of individual GPXs in malignancy. Several in-depth reviews have been published on the molecular mechanisms of GPXs regulation in tumors and their function in different stages of tumor progression [15][16][17]. GPX1 and 2 are highly expressed in the gastrointestinal epithelium and have been implicated in the development and promotion of colorectal cancer. For instance, mice deficient in both *GPX1* and *GPX2* genes, spontaneously developed ileocolitis at a young age. The ileum of these mice lost GSH-dependent activity to reduce peroxides and had increased expression of *NOX1* mRNA and elevated levels of TNF, suggesting that NOX1 is the major source of ROS and further supporting the anti-inflammatory function of GPX1 and GPX2 [18]. Using GPX1 and GPX2-deficient mice, a recent study has shown that loss of GPX2 resulted in a robust NFkB activation and release of IL-1beta as compared to GPX1 mice. However, loss of GPX1 induced the expression of cyclooxygenase-2 and release of prostaglandins [19]. Taken together, these studies demonstrate that GPX1 and 2 have important roles in regulating the synthesis and release of proinflammatory mediators.

GPX3 is the only extracellular GPX that detoxifies lipid hydroperoxides at the expense of GSH. GPX3 mRNA is expressed in the kidney, pancreas, lung, breast, brain and gastrointestinal tract, but the majority of GPX3 is expressed in the plasma which is kidney-derived. It has been reported that GPX3 can act both as a tumor suppressor and a pro-survival protein. Lower GPX3 levels in the plasma and tumor tissue of non-small-cell lung cancer, glioblastoma, hepatocellular carcinoma and colorectal carcinoma have been linked to selenium deficiency and increased lipid peroxidation, supporting the notion that loss of GPX3 contributes to oxidative stress [20]. GPX3 loss in the plasma of cancer patients is often associated with poor patient outcome. The exact mechanism of GPX3 loss is not known but might be associated with enhanced utilization of selenium and glutathione by tumor cells. Systemic GPX3 loss in mice was shown to promote tumor initiation and accelerate inflammatory colonic tumorigenesis [21]. It is noteworthy that GPX3 loss was not associated with increased ROS levels or DNA damage, however exposure to carcinogens resulted in an increase in M2 macrophages, elevated proinflammatory mediators and increased DNA damage in tumor cells.

There are also studies demonstrating that GPX3 expression is increased in certain cancers, including clear cell adenocarcinoma, colorectal carcinoma, ovarian cancer and leukemia [20]. Hence the role of GPX3 in tumor progression is controversial and further mechanistic studies are needed to achieve specific targeting.

GPX4 is also an important enzyme that protects membranes against lipid peroxidation [22]. It has emerged as a central regulator of a recently discovered form of cell death known as ferroptosis which is induced by cytosolic and lipid ROS. Depletion of *GPX4* in cells and mice resulted in increased lipid-based oxidative stress and induction of 12/15-lipoxygenase, lipid peroxidation and apoptosis-inducing factor-mediated cell death [23]. GPX4 dampens inflammation by decreasing lipid-based oxidative stress produced by the arachidonic acid metabolites and NFkB pathway [24]. While the role of GPXs 1,2,3 and 4 in tumorigenesis is established, the knowledge on GPXs 6–8 is limited and should be systematically investigated.

1.3. Role of S-Nitrosation

NO is produced enzymatically by nitric oxide synthases (NOS) not only by tumor cells but also by infiltrating inflammatory immune cells and stromal cells [25][26]. As mentioned previously, NO carries out its biological function mainly through protein S-nitrosation, a redox modification in which NO is covalently attached to the thiol group in cysteine residues of almost all functional classes of proteins. Increasing evidence suggests that dysregulated S-nitrosation, which could result from alterations in the expression or activity of NOS and denitrosylases, including GSNO reductase has emerged as an important mechanism promoting tumor progression. Several human tumors, including breast, ovarian,

pancreatic, liver, lung, glioblastoma, prostate, melanoma and colorectal cancer often express increased eNOS, nNOS and iNOS, however, tumor-infiltrating immune cells mainly express iNOS [27]. A large number of oncogenic proteins, including epidermal growth factor (EGFR), mitogen-activated protein kinase phosphatase-1, tumor necrosis factor receptor associated protein 1, phosphatase and tensin homolog (PTEN), p53, ras and src tyrosine kinase have been shown to be directly activated by S-nitrosation, which might have diverse functions in different stages of tumor progression [28]. S-nitrosation of EGFR and src resulted in activation of oncogenic signal transduction pathways, including c-Myc, Akt, and β-catenin in human basal-like breast cancer [29]. These signaling pathways are abnormally activated in various types of breast cancers and may offer survival advantage and promote invasion and metastasis. It was found that aberrant S-nitrosylation increased survival of HER^{2+} breast tumors as well as resistance to trastuzumab [30]. More recently, Ehrenfeld et al., demonstrated that S-nitrosation of endothelial cell proteins might be an important mediator of tumor angiogenesis, invasion and metastasis [31]. Therefore, novel therapeutic development is focused on promoting or inhibiting S-nitrosation or restoring a balance between S-nitrosation and denitrosation in tumor cells, thus specifically targeting tumor progression.

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