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Chapter 5

GLUTATIONE AND GLUTATHIONE-DEPENDENT ENZYMES IN REDOX REGULATION OF CELLULAR PROCESSES

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ABSTRACT

The chapter summarizes the data on the role of glutathione, glutathione transferase, and glutaredoxin in the regulation of cellular redox-dependent processes and includes the following sections: glutathione and cellular redox homeostasis, the role of glutathione in the redox-dependent regulation of apoptosis, glutaredoxin and redox regulation of cellular viability, the family of glutathione transferases in the regulation of redox-dependent processes. The role of glutathione in redox-dependent processes in the cytoplasm, nucleus and mitochondria including its role in redox-dependent regulation of MAPK-induced apoptotic signaling is discussed. It is considered the important role of the superfamily of glutathione S-transferase isoenzymes in redox-dependent processes. A separate section is devoted to the value of glutaredoxin in redox regulation including S-glutathionylation and protein-protein interaction.

Keywords: glutathione, glutathione transferase, mitochondrial GSH, glutaredoxin, cellular redox-dependent processes

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GLUTATHIONE AND CELLULAR REDOX HOMEOSTASIS

The state of redox status determines the most vital functions of cells, including proliferation, differentiation, apoptosis and autophagy, and depends on the balance of oxidation–reduction processes. It is controlled by the redox-dependent regulation based, in great part, on the thiol-disulfide exchange, which is highly sensitive to changes in the ROS/antioxidants balance, providing a cellular response to a change in the ROS level [1-4].

Maintenance of the GSH/GSSG ratio is important for control of the thiol-disulfide exchange. Among the several functions of glutathione, its participation in the defense of the cell from products of oxidative stress should be mentioned as the most important one. Hydrogen peroxide is reduced by glutathione peroxidase using GSH as the cosubstrate. Organic hydroperoxides can be reduced to the corresponding alcohols in the reactions catalyzed by glutathione peroxidases as well as due to the peroxidase activity of Se-independent glutathione S-transferases that also use GSH as the cosubstrate. Glutathione can reduce oxidized glutaredoxin that is formed during reduction of disulfides [5].

Glutathione is a low molecular weight antioxidant that can also take part in the nonenzymatic antioxidant defense, playing a role as an efficient scavenger of free radicals [6]. Oxidative stress leads to damage of carbohydrates, lipids, and nucleic acids, resulting in cell dysfunctions and death. Oxidative stress and/or change in cellular redox status can affect the state of the nuclear chromatin and alter gene expressions. Progression of oxidative stress can result in single- or double-strand breaks in DNA molecules. Mitochondrial damage is followed by a decrease in the transmembrane potential, changes in membrane permeability, and accelerated release of apoptotic factors, which leads to cell death [7]. Under physiological conditions, reactive oxygen and nitrogen species (ROS and RNS, respectively) are involved in processes of redox-signaling, which are fast, specific, and reversible reactions regulating the activity of proteins that are important for cell functioning. The processes involved in redox-signaling can occur in various cell compartments at certain times with participation of different redox pairs, such as GSH/GSSG or $NADH(H^+)/NAD^+$ [8]. Special attention is paid to the GSH/GSSG ratio as the main marker of redox status and an important factor of signal transduction [9].

Functionally important cysteine residues of many proteins are subjected to posttranslational modifications including oxidation. Under physiological conditions, the thiol group of free cysteine is mainly protonated (pK_a = 8.5), which excludes its oxidative modification. Within proteins, however, cysteine can be activated, i.e., exists as the thiolate anion. This is due to numerous factors including hydrogen bonding, the impact of neighboring amino acid residues, the microenvironment of cysteine residues, and binding with substrates [10]. The cysteine residue of GSH can interact with cysteine residues of proteins yielding a disulfide bond (protein-SSG). This process called S-glutathionylation defends proteins from oxidative stress and makes a significant contribution to redox

signaling and regulation of protein activities [11]. S-glutathionylation can affect ability of the protein to form disulfide bonds and to correct its folding, which influences the functional state of the protein. Also, S-glutathionylation protects sulfenic acid derivatives (Cys-SOH), formed during oxidation of cysteine residues, from oxidation to sulfinic acid (Cys-SO_2H) and then further to sulfonic acid (Cys-SO_3H), which cannot be reduced under physiological conditions. The reversibility of oxidation of cysteine residues is of great importance for the normal functioning of proteins and their ability to participate in signal transduction cascades. Glutathione is the main substrate for the reduction of oxidized cysteine residues.

Overproduction of glutathionylated proteins indicates the progression of oxidative stress leading to cell death. Change in sulfhydryl homeostasis of the cell, especially the steady state of glutathionylation of specific regulatory proteins, modulates various pathways of signal transduction, shifting the cell state from survival to death. For example, the functioning of the cell actin is regulated by reversible S-glutathionylation, and disturbance in S-glutathionylation changes the structural organization of stress fibrils of the actin cytoskeleton [12]. Under normal conditions, modifications such as protein-SSG are transient and reversible. If the cysteine residue is essential, its S-glutathionylation can affect the functioning of the protein. For example, S-glutathionylation of subunits p65 and p50 of the transcriptional factor NF-κB inhibits their binding with DNA [13], while S-glutathionylation of the β-subunit of IκB kinase suppresses the activation of NF-κB [14].

The state of the thiol–disulfide system is determined by the cellular redox status, which is characterized by the GSH/GSSG ratio. Under physiological conditions, GSH/GSSG is about 100:1, which minimizes the oxidative action of ROS/RNS. Disturbance of this ratio significantly affects, in context of the redox regulation, processes of signal transduction, control of gene expression, cell proliferation, differentiation, state of cell metabolism, and vital functions overall [15, 16]. Glutathione is synthesized only in the cytoplasm and then transferred to the mitochondria, peroxisomes, endoplasmic reticulum, and nucleus. More than 70% of the total GSH remains in the cytoplasm, while the nucleus and mitochondria can accumulate up to 10% and 30% of the total intracellular GSH content, respectively [17].

The content of GSH in mitochondria (mGSH) is approximately the same as in the cytoplasm (10-14 mM) [18]. Glutathione is not synthesized in mitochondria, but it is transported from the cytoplasm. GSH easily penetrates through the porin channels of the outer mitochondrial membrane. Since under physiological conditions GSH exists as an anion, it is unable to diffuse into the matrix through the inner mitochondrial membrane that has a high negative transmembrane potential. GSH is transferred into the mitochondrial matrix by transporters in the inner mitochondrial membrane working against the electrochemical gradient. In mitochondria of liver and kidneys, this role is played by carriers of 2-oxoglutarate (OGC) and dicarboxylates (DIC), which transfer GSH into the mitochondrial matrix by the antiport mechanism in exchange with 2-oxoglutarate and

inorganic phosphate, respectively. Since these transporters provide liver mitochondria only 45-50% of their total GSH, some additional mechanism must exist.

GSSG does not come out from mitochondria to the cytoplasm, being instead reduced by glutathione reductase yielding GSH. This process depends on the presence of a sufficient amount of NAPDH(H^+). It should be noted that accumulation of GSSG affects glutathionylation of mitochondrial proteins, thus changing their functioning. For example, the activity of NADH-ubiquinone reductase (complex I of the mitochondrial respiratory chain) depends on the GSH/GSSG ratio [19].

In an experimental model with mitochondrial membranes from rat heart, it was found that addition of GSSG after the action of Grx2 resulted in glutathionylation of complex I. In contrast, addition of GSH and Grx2 caused its deglutathionylation [20]. For mitochondria from bovine heart, the sites of glutathionylation in complex I were found to be the cysteine residues Cys531 and Cys704 of the 75-kDa NDUSF1 subunit [21]. The role of S-glutathionylation is of importance for the defense of NADH-ubiquinone reductase from irreversible oxidation and for the control of ROS production by the mitochondria in response to changes in the local redox environment. In this case, the important condition is the simultaneous S-glutathionylation of complex I and the α-ketoglutarate dehydrogenase complex, since the latter supplies NADH(H^+) for oxidation by complex I, i.e., both protein complexes contribute to generation of ROS by the mitochondria [22]. Under conditions of oxidative stress, glutathionylation of these two enzyme complexes decreases their activities and production of ROS [23, 24]. After the return of $O_2^{\bullet-}$ and H_2O_2 to the normal level, the α-ketoglutarate dehydrogenase complex and NADH dehydrogenase are deglutathionylated by Grx2, and oxidative phosphorylation is restored.

The role of mGSH is of great interest. Functions of mitochondria are closely related to the maintenance of cellular redox balance. Mitochondria are the main consumers of oxygen and the main source of ROS, which are mainly generated during functioning of the electron transfer chain. Under physiological conditions, incomplete one-electron reduction of molecular oxygen results in formation of superoxide anion radical $O_2^{\bullet-}$, which gives rise to another ROS. The concentration of $O_2^{\bullet-}$ in the mitochondrial matrix under steady-state conditions exceeds 5-10-fold its concentration in the cytoplasm [25]. Besides, the action of various toxins and some pathological states affecting mitochondrial functions can increase production of ROS. The presence of the antioxidant defense system in mitochondria prevents disturbances in mitochondrial functioning. The main component of this system is mGSH, which prevents or repairs damage occurring under normal aerobic metabolism.

In mitochondria Mn-dependent superoxide dismutase converts $O_2^{\bullet-}$ to H_2O_2, which can be neutralized by GSH-dependent systems with the participation of glutathione transferase and glutathione peroxidase. Glutathione peroxidase GPx1, the most active isoform towards H_2O_2, is mostly localized in the cytoplasm, but a small amount is also present in the mitochondrial matrix [18]. In mitochondria, GST catalyzes formation of GSH-conjugates

and reduction of organic hydroperoxides using GSH as a cosubstrate. In contrast to Se-containing GPx, GST does not interact with H_2O_2, but it efficiently reduces hydroperoxides of polyunsaturated (linoleic and arachidonic) fatty acids, phospholipids, mononucleotides and DNA. The Se-independent GPx4 plays an important role in detoxication of lipid hydroperoxides in mitochondria. Recently, it was shown that GPx4 prevents development of cell apoptosis in presence of apoptosis inducing factor (AIF), and supports oxidative phosphorylation in the intestinal epithelial cells [26]. Due to its ability to reduce hydroperoxides of cardiolipin, GPx4 takes part in regulation of release of apoptogenic proteins from mitochondria [27]. In the mitochondria of human cells, peroxidase activity is exhibited by GST isoenzymes hGSTA4-4, hGSTA1-1, hGSTA2-2, and hGSTP1-1, among which isoform hGSTA4-4 is the most active [28].

Grx2 is the mitochondrial isoform of glutaredoxin which also occurs in the nucleus. Interestingly, the oxidized form of Grx2 can be reduced by both thioredoxin reductase (TrxR) and GSH, providing functional activity of Grx2 in the oxidized microenvironment that is common for mitochondria.

In mitochondria, Grx2 plays a significant role in the antioxidant defense system and in redox-dependent signaling *via* interaction of the GSH pool with thiol groups of proteins [28-30]. The thioredoxin-dependent system in mitochondria is represented by thioredoxin 2 (Trx2), thioredoxin reductase (TrxR2) and Prx3, the most important peroxiredoxin isoform. The mGSH/GPx and Prx3/Trx2 systems, that defend against H_2O_2, are interrelated. For example, it was shown that decrease in mGSH resulted in oxidation of Trx2 [31]. Thus, the role of the redox-cycle of mGSH in maintenance of an efficient antioxidant system and homeostasis of hydrogen peroxide in mitochondria is evident.

Some investigations have shown that GSH is accumulated in the nucleus at the beginning of the G1 phase [32], so it could play an important role in maintenance of the redox status of the nucleus during the cell cycle [33]. During mitosis, the nuclear membrane breaks down and appears again around the daughter DNA molecules packed into the chromosomes. During cell division, a high pool of GSH is maintained near the chromatin, which is consistent with reported data on high redox potential of the dividing cell. The nuclear glutathione (nGSH) pool is resistant to the factors decreasing the cellular and the mitochondrial GSH levels such as the action of the thiol-binding compounds N-ethylmaleimide and diethyl maleate, as well as that of the GSH synthesis inhibitor buthionine sulfoximine (BSO). It has been found that cells with a high level of nGSH are more resistant to apoptosis under oxidative stress conditions. However, the role of nGSH in oxidative stress has been little studied [33].

The mechanisms of transport and deposition of nGSH are still little studied. A significant amount of nGSH presumably comes from the parental nucleus to the daughter nuclei during the telophase due to the high concentration of GSH in proximity to the replicating genetic material [33].

Nuclear pores allow various ions and small molecules, including GSH, to penetrate the nucleus [34]. At the same time, an ATP-dependent mechanism of GSH transport into the nucleus was also demonstrated [35]. Currently, the role of protein Bcl-2 in transmembrane transport of GSH is being discussed. It has been found that the nGSH content is significantly increased in tumor cells exhibiting overexpression of the *bcl-2* gene [36]. It should be noted that the BH-3 domain of Bcl-2 protein can bind to GSH. Participation of Bcl-2 in the maintenance of GSH level in mitochondria has been reported [37]. These data together with the fact that the antiapoptotic protein Bcl-2 is involved in formation of pores in the membrane indicate the ability of members of the Bcl-2 protein family to serve as mediators of GSH translocation into the nucleus [32, 36].

Several studies on plant cells have demonstrated that gene expression is sensitive to accumulation of GSH in the nucleus and to its decrease in the cytoplasm [36]. Decrease in redox potential in the cytoplasm and its growth in the nucleus affects not only gene expressions, but the ability of proteins to bind with their targets in the nucleus as well. It was shown that at the beginning of the G1 phase of the cell cycle in animal cells, activation of oxidative processes in the cytoplasm caused by epidermal growth factor resulted in accumulation of ROS, which activated phosphorylation cascades and DNA replication, and induced cell division [38]. The decrease in the cytoplasmic GSH level in the G1 phase may cause accumulation of ROS. It is suggested that changes in the nuclear redox status may act as the trigger element for the other components that are essential for transcription. Such a mechanism was shown for the proteins NF-κB, AP-1, and p53 [39]. For example, for the interaction of NF-κB with DNA, the cysteine residue in the DNA-binding region of NF-κB must be reduced. Similar behavior was reported for the transcriptional factors Fos, Jun, and Nrf2 [36].

GSH content and its changes in the nucleus can modulate structural organization of chromatin [40]. The extent of glutathionylation of nuclear proteins increases at the beginning of cell proliferation [32]. In the animal cells, while going from proliferation through differentiation to cell death, the cellular redox status changes towards a more oxidized state. Thus, the GSH/GSSG ratio is a kind of switch from proliferation to differentiation and then to programmed cell death [41]. The nuclear GSH is related to the synthesis of DNA, presumably being the redox sensor for the beginning of DNA synthesis. GSH maintains the necessary organization of the nucleus at the expense of the optimal redox status for replication of DNA and maintenance of its intact structure. Also, it was found that nGSH affects the proteasomal degradation of nuclear proteins [32].

Activity of Se-dependent glutathione peroxidase was found in the nuclear fraction of Wistar rat hepatocytes [35]. The nucleus-specific isoform of glutathione peroxidase GPx4/snGPx was shown to maintain stability of the chromatin structure in sperm [42]. The nuclear localization of this enzyme emphasizes the important role of glutathione in the regulation of the cell cycle and the chromosomal organization, since nuclear proteins,

mainly histones and other chromatin-binding proteins, presumably should be maintained in the reduced state for optimal functioning [33].

The role of GSH in the repair of DNA damage should also be pointed out. Glutathione is not the most efficient protector of DNA from X-radiation, but it controls repair mechanisms of damaged DNA molecules [43]. An important component of the repair mechanism of oxidative damages of DNA is poly(ADP-ribose)polymerase (PARP), which catalyzes the growth of the polymer chains from the ADP-ribose molecules on target proteins (particularly on histones). This process proceeds virtually in all eukaryotic cells in response to DNA damage [44]. Expression of genes and activity of PARP proteins are related to nGSH level during the cell cycle. For plant cells, it was demonstrated that mRNA of PARP1 and PARP2 was accumulated with the growth of the GSH pool in the nucleus [45]. Similar character of changes in polyribosylation activity was observed in NIH3T3 fibroblast cells: polyribosylation of histones in their nuclei grew during proliferation, when the nGSH level was maximal [32, 46]. It should be mentioned, however, that several details concerning the mechanisms of GSH transport into the nucleus and the role of nGSH in various genetic and epigenetic processes remain unclear [32].

ROLE OF GLUTATHIONE IN REDOX-DEPENDENT REGULATION OF APOPTOSIS

The protective role of GSH in the mechanism of apoptosis is widely considered. According to a contemporary concept, decrease of GSH level below a critical value results in appearance of a signal for apoptosis, which is initiated by activation of the death receptor or by mitochondrial apoptotic signaling. In contrast, an increase in the GSH level provides defense of cells from Fas-induced apoptosis [47]. Numerous data indicate the crucial role of GSH in cell defense from various apoptotic stimuli, since disturbances of redox homeostasis of the cell caused by GSH oxidation or GSH export facilitate development of apoptosis [48, 49]. In studies with various types of cells, it has been shown that disturbance of the GSH/GSSG balance caused by an oxidant precedes induction of mitochondrial apoptotic signaling [50, 51]. Restoration of the GSH/GSSG ratio to the normal level after the action of the oxidant does not prevent progress of the apoptosis, suggesting that it is induced in the early stage of the GSH/GSSG disbalance. Use of the thiol antioxidant N-acetylcysteine before the action of the compounds resulting in the oxidative (*tert*-butyl hydroperoxide) or carbonyl (methylglyoxal) stress prevents induction of apoptosis. These data are consistent with other reports and indicate that the signal for apoptotic death is triggered at the very beginning of the decrease of the GSH/GSSG ratio [50-52].

Special attention has been given to investigation of the mitochondrion as the cell organelle involved in activation of apoptosis. The GSH/GSSG ratio is considered to be the

main redox system maintaining redox homeostasis of the mitochondrial matrix and defending mitochondrial proteins and DNA from the action of ROS. Using various cell models, it has been shown that selective decrease in the mGSH content resulted in a reduction in activity of the respiratory chain complexes, growth in production of ROS, decrease in transmembrane potential ($\Delta\Psi$), and release of apoptogenic factors from mitochondria. For example, in diabetic cardiomyocytes, stress-induced oxidation of mitochondrial, but not cytoplasmic, GSH resulted in a decrease in the $\Delta\Psi$ value and activation of caspase-9 and caspase-3 [53, 3]. In human B-cell lymphoma cells, ROS-induced decrease in mGSH level initiated apoptosis that was accompanied by drop in the $\Delta\Psi$ value, release of cytochrome *c*, and activation of caspase-3 [54]. A direct relation between decrease in the mGSH content and activation of apoptosis has been demonstrated in different cell types. In hepatocytes, decrease in mGSH content was a necessary condition for TNF-α-induced apoptosis, which was preceded by tBid/Bax-initiated permeabilization of the mitochondrial membrane, release of cytochrome *c*, assembly of apoptosomes, and activation of caspase-3 [55]. In large intestine cells, oxidation of mGSH was the main factor in development of menadione-induced mitochondrial dysfunction and cytochrome *c*-dependent activation of apoptosis [56].

The precise mechanism of mitochondrial dysfunction caused by a decrease in the mGSH content is not completely understood. However, it was found that cisplatin-induced apoptosis is related to disbalance in the mGSH/GSSG ratio, decrease in NADPH(H^+) content, and oxidative damage of cardiolipin and aconitase, which disturbs functioning of mitochondria and activates caspase-3 [57]. Later works demonstrated that a sharp drop in the mGSH content induced generation of ROS/RNS, leading to apoptosis in HL-60 and Raji cells. In this case, apoptosis was caused by breakdown of complex I of the respiratory chain due to destabilization of the Fe-S cluster of the NDUGS3 subunit of the complex, resulting in inhibition of respiration and drop in the $\Delta\Psi$ value [58]. Noteworthy, a slight decrease in the mGSH content of hepatocytes caused by moderate hypoxia did not lead to apoptosis. This fact demonstrates that the content of mGSH must drop to a critical level to induce apoptosis [17].

Permeability of the mitochondrial membrane may be controlled by the mGSH content. In early works, decrease in mGSH was attributed to changes in mitochondrial permeability that were caused by redox modulation of adenine nucleotide translocase and subsequent release of apoptogenic factors such as cytochrome *c* and AIF from the mitochondria to the cytoplasm [59, 60]. Later investigations showed that change in redox balance of mGSH was a crucial factor in control of mitochondrial membrane permeability [61]. Drop in the mGSH/GSSG ratio from 300:1 to 20:1 leads to the opening of the anion channels in the inner mitochondrial membrane and the mitochondrial pores. If the mGSH/GSSG ratio lies in the region from 150:1 to 100:1, instability of the $\Delta\Psi$ value is observed. In the case of pronounced oxidation, when the ratio is less than 50:1, irreversible depolarization of the mitochondrial membrane takes place, accompanied by opening channels and breakdown

of the mitochondria [61]. Accelerated transport of glutathione into mitochondria suppressed the menadione-induced increase of the mGSSG level, preventing decrease in the ATP content, drop in the $\Delta\Psi$ value, release of cytochrome *c* into the cytoplasm, and activation of caspase-3 and caspase-9 [56].

Induction of apoptosis under oxidative stress is caused by activation of mitogen-activated protein kinases (MAPKs) [62]. There are three classes of MAPKs: ERK (extracellular signal-regulated kinase), JNK (c-Jun N-terminal kinase), and p38. The signal transduction cascade includes consecutive phosphorylation steps resulting in activation of a specific MAPKKK (MAP3K, kinase of MAPK kinase) that activates MAPK kinase (MAP2K) which in turn, activates MAPK. Stress-induced apoptosis is related to activation of JNK and p38 MAPK, and this can be triggered by the kinase cascade involving apoptosis signal-regulating kinase 1 (ASK1), MAPK kinase 4/7 (MEK4/7), and JNK, or through the cascade of ASK1, MAPK kinase-3/6 (MEK 3/6), and p38 [62, 1, 63].

The role of GSH in redox-dependent regulation of MAPK-induced apoptotic signaling has been little studied to date. Since GSH is a key factor in maintenance of the intracellular redox homeostasis and plays an important role in antioxidant defense of the cell, it might be a modulator of MAPK-dependent signaling pathways. In some cell models it was shown that disbalance of GSH/GSSG activated the MAPK-signaling pathway and facilitated apoptosis. For example, induction of ROS formation by aloe-emodin resulted in disbalance of the GSH/GSSG ratio and redox-dependent activation of the GSTP1/JNK-signaling pathway in hepatoma cells [64].

Other works report that inhibition of the *de novo* GSH synthesis by BSO promotes redox-activation of MAPK and the apoptotic signaling pathway. Treatment of breast cancer cells with the antitumor agent aplidine after treatment with BSO, resulted in activation of the JNK- and p38-dependent signaling pathways and development of apoptosis [65]. Apoptosis of HepG2 cells treated with BSO was induced by andrographolide through activation of the ASK1/MEK4/JNK signaling cascade [66]. Addition of thiols (N-acetyl cysteine and GSH) prevented toxin-induced activations of MAPK, which suggested participation of GSH in functioning of MAPK and cellular response to stress [65].

While investigating the role of GSSG in initiation of apoptosis, it was found that extracellular GSSG can selectively activate the MAP kinase cascade ASK1/MEK3/6/p38 through the mechanism of GSSG-induced thiol-disulfide exchange on the cellular membrane and formation of mixed protein disulfides. Such redox stress can in turn result in breakdown of the Trx1/ASK1 complex and activation of the p38-dependent pathway of apoptosis [67]. The fact that each of these events can be prevented by GSH is consistent with the idea of the protective role of glutathione [67]. In this connection, it should be noted that in SH-SY5Y neuroblastoma cells, that are resistant to GSSG-induced apoptosis, apoptosis was activated after preliminary treatment with BSO, which was accompanied by increased ROS production and activation of JNK [68]. Such data point to the assumption that drop in GSH concentration below a critical level is a necessary condition for the

activating effect of GSSG on the MAPK-signaling pathway and induction of apoptosis. GSH regulates the redox state of Trx1 and the Trx1-dependent ASK1 signaling cascade inducing apoptosis. For example, the action of agents oxidizing GSH (diamide and dithionitrobenzoate) on stomach adenocarcinoma cells initiated the mitochondrial pathway of apoptosis [69]. In this case, redox activation of the Trx1/ASK1/p38 signaling cascade was triggered by increase in the GSSG content. Resistance of the cells towards systems (paraquat and xanthine/xanthine oxidase) producing H_2O_2 and ROS correlated with the Nrf2-dependent increase of the GSH content and protein S-glutathionylation [69]. Another response was observed in SH-SY5Y neuroblastoma cells, where H_2O_2 activated the Trx1/p38/p53 cascade and cellular apoptosis, while diamide activated the ERK-signaling pathway, Nrf2-dependent increase in GSH content, and expression of the heme oxygenase-1 gene, which assisted cell survival.

Concerning regulation of the GSH-dependent post-translational modifications of cysteine residues of the proteins involved in the MAPK-signaling pathways, it can be noted that under the oxidative stress caused by menadione (2-methyl-1,4-naphthoquinone), S-glutathionylation of Cys1238 in the ATP-binding domain of MEKK1 inhibits activity of the kinase [70]. However, a question is still open concerning the specific relation between the oxidative stress and S-glutathionylation in terms of activation/inactivation of specific MAPK-dependent signaling pathways and induction of apoptosis.

The presented data show that GSH plays an important role in the redox regulation of MAPK-dependent pathways of signal transduction. However, the difference between the induction of different signaling pathways that are responsible for cell death or survival is likely determined by not only the cellular content of GSH, but also by the cell type [69, 71].

GLUTAREDOXIN AND REDOX REGULATION OF CELLULAR VIABILITY

Glutaredoxin (Grx, EC 1.20.4.1) is one of the most important enzymes involved in processes of disulfide reduction and deglutathionylation. Isoenzymes of Grx are thermoresistant and low molecular weight (10-16 kDa) proteins functioning as GSH-dependent oxidoreductases. According to their structure, they belong to the thioredoxin superfamily, and together with Trx they play an important role in redox-dependent processes in cells. The active site sequence Cys-X-X-Cys/Ser is located in the N-terminal region of Grx, while the conserved glutathione-binding domain is at the C-terminal part of the molecule. Isoenzymes of Grx are found in virtually all living organisms except for some types of bacteria and archaea [72, 73].

Grx isoenzymes are classified in terms of presence of a cysteine residue in the second position of the active site sequence. Isoenzymes with the sequence Cys-Cys-X-Cys/Ser in the active site are called Grx of C-C type. Originally, it was suggested that the main

functions of Grx are reduction of disulfide bonds and deglutathionylation of proteins. However, later it was found that certain Grx isoenzymes rather serve as transfer proteins for iron-sulfur clusters [FeS] using GSH as ligand [74]. The oxidized form of Grx formed after the reduction of protein disulfides and glutathionylated thiols is reduced by GSH. However, some Grx isoenzymes are reduced by ferredoxin- or NADPH-dependent thioredoxin reductase, for example Grx4 of *E. coli* and human Grx2 [29]. Depending on the active site structure, Grx isoenzymes can be dithiol or monothiol (active site sequences Cys-X-X-Cys and Cys-X-X-Ser, respectively) [75]. The binding of the Fe-S clusters can lead to formation of dimers and tetramers. In these interactions, alternative protein-protein contact sites are possible in mono- and dithiol Grx isoenzymes, providing existence of both mono- and multidomain forms of Grx [76].

Concerning classification of the Grx isoenzymes, it should be noted that since bacteria, yeast, and mammals have a limited number of these proteins, their classification into mono- and dithiol isoenzymes is sufficient. For photosynthesizing organisms containing a wide range of Grx isoenzymes, a new classification is used [77] dividing the isoenzymes into six classes based the homology of their amino acid sequences. Dithiol Grx isoenzymes belong to class I, monothiol Grx isoenzymes occur in both classes I and II, and glutaredoxins of the C-C type belong to class III. Grx isoenzymes of classes I and II are found virtually in all organisms. Isoenzymes of class III are present in higher plants, where they control the functional activity of plants, for example, flowering [5, 78]. Isoenzymes of class IV are found in photosynthesizing eukaryotes. Glutaredoxins of class V occur in cyanobacteria and proteobacteria, while Grx of class VI are present only in cyanobacteria [79].

Grx isoenzymes use two catalytic mechanisms: monothiol and dithiol. The monothiol mechanism is characteristic for the reactions of deglutathionylation. In this case, only the catalytic cysteine residue (the first of two active site cysteines at the N-terminus) participates in the catalysis. Reduction of a glutathionylated substrate starts from nucleophilic attack of the thiol group of the Grx CysA residue. The substrate is released with formation of the intermediate glutathionylated product Grx-SSG. Further, glutaredoxin is regenerated by GSH, yielding $Grx(SH)_2$ and GSSG. The monothiol mechanism is used by both monothiol and dithiol Grx isoenzymes [5]. The dithiol mechanism, besides the catalytic cysteine residue, requires another cysteine residue (so-called recycling cysteine) that can be either the second cysteine residue of the Grx active site (CysB) or a cysteine residue apart from the active site (CysC). If the substrate is deglutathionylated, the first stage proceeds by the monothiol mechanism, but the glutathionylated intermediate Grx-SSG then releases GSH yielding the intramolecular disulfide bond $Grx(S_2)$ between the catalytic cysteine residue and one of the recycling cysteines. Furthermore, the disulfide bond is reduced using two GSH molecules or by thioredoxin reductase. If the substrate of Grx requires the reduction of an intra- or intermolecular disulfide bond, the CysA residue of Grx forms a transient disulfide bond

with one of the substrate cysteines, and then the reduced substrate is released, while the disulfide bond is formed between the CysA and CysB or CysC residues of Grx. Finally, the disulfide bond in Grx(S_2) is reduced by two molecules of GSH or by thioredoxin reductase [80]. All dithiol isoforms of Grx investigated so far are capable of functioning by the monothiol mechanism, but not all of them have been tested for their ability for dithiol catalysis. However, all dithiol Grx isoforms that have the ability for dithiol catalysis can also function using the monothiol mechanism.

Four Grx isoenzymes have been found in mammals: Grx1, Grx2, Grx3 (also known as protein interacting cousin of Trx (PICOT)), and Grx5 [81]. The dithiol isoenzyme Grx1 is localized mainly in the cytoplasm, but also can be translocated into the nucleus, secreted from the cell, and localized in the intermembrane space of mitochondria [75]. The dithiol Grx2 was originally found in mitochondria, but later it was found in the cytoplasm and nucleus of testes and in a number of tumor cells [82]. The monothiol isoenzyme Grx3 is a multidomain protein that is present in the nucleus as well as in the cytoplasm, while the monothiol Grx5 is found in mitochondria [81].

Although the amino acid sequences of Grx1 and Grx2 exhibit only 34% homology, these isoenzymes use the same catalytic mechanism [83, 84]. Grx1 is approximately 10-fold more active than Grx2, but the content of Grx2 in the intermembrane space of mitochondria is higher than that of Grx1, which presumably compensates the difference in their catalytic activity. Oxidized Grx1 is only reduced by GSH, while Grx2 can be reactivated using either glutathione or thioredoxin reductase, which suggests that this protein exhibits properties of both Grx and Trx [29] and implies the connection between the metabolic pathways controlled by glutathione and thioredoxin in mitochondria. Besides, the possibility of reduction of Grx2 by thioredoxin reductase allows Grx2 to function over a wide range of GSH/GSSG values and rather strong oxidative stress in mitochondria [20].

The thiol groups of the active site of some Grx isoenzymes can form complexes with iron-sulfur clusters. These enzymes include a limited number of dithiol Grx isoenzymes of humans, plants, and trypanosomes and virtually all monothiol Grx isoenzymes [85-87,161-163]. Most of such complexes were found in mitochondria. The cluster $[2Fe2S]^{2+}$ is located between two monomers of Grx, forming coordinate bonds with two active site cysteine residues at the N-termini and with two non-covalently bound GSH molecules. The GSH comes from the free GSH pool, indicating the important role of GSH in stabilizing Fe-S clusters [88]. Since the cofactor $[2Fe2S]^{2+}$within the holo-Grx complex interacts with the cysteine residues involved in the catalysis, such complex is catalytically inactive [85]. Degradation of the cluster and dissociation of the holo-complex restore activity of Grx. Slow degradation of the complex under aerobic conditions is efficiently prevented by GSH. In contrast, GSSG facilitates degradation of the cluster and activation of Grx [85]. Two GSH molecules in the complex successfully screen the iron atoms from the environment. Thus, the iron of the $[2Fe2S]^{2+}$ cluster has no possibility to interact with oxidants that

require direct molecular interaction, particularly H_2O_2. It has been shown that release of Grx monomers is caused by $O_2^{\bullet-}$ [89]. Presumably, the breakdown of the cluster as a response to the action of the oxidant, is related to formation of GSSG. It is suggested that the human Grx2/Fe-S complex is a kind of a redox sensor: under high GSH/GSSG values, Grx is bound to the complex in the inactive state, while changes in the cellular redox status result in release of the catalytically active Grx [85]. Besides, Grx2 is resistant to oxidative inactivation [90] and successfully functions as an alternative reducing system of Trx1 in the cytosol and Trx2 in mitochondria, under the action of inhibitors of thioredoxin reductase. Overexpression of the Grx2 gene in mitochondria protects Trx2 against oxidation, significantly decreasing development of apoptosis caused by the production of ROS in mitochondria [90].

Some studies have shown importance of the yeast cytosolic multidomain monothiol isoenzymes Grx3 and Grx4 in the intracellular distribution of iron [91]. A simultaneous decrease in Grx3 and Grx4 decelerates all iron-dependent processes in the cytoplasm, mitochondria, and nucleus, which is caused by iron deficiency in the organelles and insufficiency of its incorporation into proteins despite sufficient amount of iron in the cytoplasm. The ability of Grx to bind Fe-S complexes is necessary for bioavailability of iron in the cell [91]. For a better understanding functions of various human Grx isoenzymes, their specificity towards different disulfides must be investigated considering different cellular localization. Besides, their contribution to the processes of iron transport and maintenance of Fe-S clusters are also of importance. In this connection, recent investigation of Grx5 seems to be interesting. It was demonstrated that the gene of Grx5 had high expression level in bone tissue and played an antiapoptotic regulatory role in osteoblasts [92]. However, it remains unclear, what precisely affected development of the oxidative stress-induced apoptosis while changing the level of Grx5 (overexpression or knockout of the gene): alterations of the thiol–disulfide homeostasis or homeostasis of the iron–sulfur clusters.

Thiol–disulfide exchange influences not only the substrate structure, but also the structure of Grx. Comparison of the oxidized and reduced forms of Grx1 from *E. coli* and T4 bacteriophage demonstrated that the structures of the two forms of the isoenzymes are very similar, although there are some differences [93]. In the presence of mixed disulfide bond with GSH, the Grx1 of *E. coli* exhibits properties of the oxidized protein [94]. The structural changes involve the region of the active site, increasing its flexibility in the reduced form of the enzyme. Besides, in the oxidized Grx, the surface of the molecule involved in protein–protein interactions is masked. Therefore, affinity of Grx to the substrate decreases as soon as the substrate is reduced, resulting in dissociation of the Grx–substrate complex.

Grx1 and monomeric Grx2 can catalyze both deglutathionylation and the reverse reaction of S-glutathionylation. The direction of the reaction depends on the relative concentrations of the participants protein-SSG, protein-SH, GSH, and GSSG. The redox

potential of the GSH/GSSG pair is most important for determining the cellular redox potential. The value of the GSH/GSSG redox potential substantially depends on the functional state of the cell. During cell proliferation, this value is approximately –240 mV, during cell differentiation it reaches – 200 mV, and apoptosis results in further growth of this value to – 170 mV [95]. It has been ascertained that Grx acts as the GSH-dependent reductase at – 240 mV, while at – 170 mV it acts as the GSSG-dependent oxidase [96]. Under conditions when GSH/GSSG value is decreased, i.e., under the action of oxidizing factors, Grx can catalyze the S-glutathionylation reaction, while under weakening oxidative stress, Grx catalyzes deglutathionylation [97]. Grx facilitates S-glutathionylation of proteins *via* reaction of the disulfide bond with the GS· radical yielding the intermediate anion radical Grx-SSG, which then gives the mixed disulfide P-SSG. Reversion of S-glutathionylation depends on the extent and duration of the initiating stress, the removal of which usually results in deglutathionylation. The half-reduction time of the glutathionylated bonds is 2-3 h [98]. Evidently, Grx contributes to the control of signal transduction, regulating the processes of glutathionylation and deglutathionylation.

Glutathionylation in cells of humans and other mammals participates in regulation of several key proteins and processes in response to redox signals. More than 200 mammalian proteins are known to be involved in thiol–disulfide exchange. For example, S-glutathionylation has been shown to inhibit phosphofructokinase, carboanhydrase III, nuclear factor NF1, glyceraldehyde-3-phosphate dehydrogenase, protein tyrosine phosphatase 1B, protein kinase Cα, creatine kinase, actin, protein phosphatase 2A, protein kinase A, tyrosine hydroxylase, complex I of the mitochondrial respiratory chain, NR-κB transcription factor, and IκB kinase (IKK). In contrast, such proteins as microsomal S-glutathione transferase (GST), phosphatase of carbonic anhydrase III, HIV-1 protease, matrix metalloproteinase, HRAS GTPase, sarcoplasmic calcium ATPase, and complex II of the mitochondrial respiratory chain are activated by S-glutathionylation. Progression of oxidative stress and change in functioning of Grx disturb regulation of S-glutathionylation, which may facilitate several pathophysiological changes observed in diabetes, disorders of the lungs and heart, oncological diseases, and different neurodegenerative processes. For example, disturbance of glutathionylation of cytoskeletal elements promotes pathological changes in heart and skeletal muscles in ischemia and in neurons in Friedreich's ataxia [99]. Glutathionylation of actin prevents its polymerization, so the redox-dependent reversible glutathionylation of actin regulates the cytoskeleton structure, which is of special importance for functioning of such cells as thrombocytes, in which actin is the main protein [100]. Besides, glutathionylation of actin was shown to be necessary for dissociation of the actin–myosin complexes during cell adhesion [101].

The specific regulation of protein activities through glutathionylation/deglutathionylation processes are important for many aspects of cell functioning including regulation of the apoptotic signaling cascades. It was found that apoptosis induced by TNF-α and FasL is highly sensitive to S-glutathionylation. Thus, in epithelial lung cells, the Fas receptor is

glutathionylated on Cys294 during degradation of Grx1 involving caspase-8 and/or caspase-3, this resulting in the acceleration of apoptosis [102]. The results indicate existence of feedback between caspase-3 and Grx, since Grx activates procaspase-3, which causes degradation of Grx. At the same time, *in vitro* studies demonstrated inactivation of caspase-3 by glutathionylation [103]. For a better understanding of the interactions between Grx and caspase-3, it is necessary to determine the specific cysteine residues of caspase-3 that are glutathionylated/deglutathionylated. This is of importance for ascertaining the relation between the redox status of caspase-3 and the mechanism of activation of apoptosis. It is assumed that the deglutathionylation catalyzed by Grx can play a crucial role in the mechanism of the redox regulation of the processes from proliferation to apoptosis that is specific for different cell types [104].

In Alzheimer's disease, change in metabolism is partially associated with a decrease in the activity of α-ketoglutarate dehydrogenase. Activity of this enzyme decreased with glutathionylation under conditions of oxidative stress, which may take place in brain cells in Alzheimer's disease [105]. Besides, selective glutathionylation of protein p53 in brain cells was found in Alzheimer's disease, which also may facilitate progression of the oxidative stress [106]. In patients with type 2 diabetes, glutathionylated hemoglobin (HbSSG) was found, and its level correlated with development of diabetic microangiopathy [107]. At the same time, in a rat diabetes model an increased expression of Grx1 gene was revealed, facilitating translocation of NF-κB into the nucleus and activation of the cell adhesion molecules ICAM-1. Both processes make a significant contribution to the development of retinopathy. Disturbance in their regulation, in which glutathionylation plays an important role, is observed under the activation of Grx1 [108].

The crystalline lens contains high concentrations of GSH (6 mM) that acts as an antioxidant, maintaining transparency of the lens [109]. During progression of cataract, the GSH/GSSG ratio decreases and the lens proteins undergo structural changes resulting in unfolding of the protein globules and exposure of the buried cysteine residues, which increases disulfide bonding and S-glutathionylation [109]. Presumably, the maintenance of proper GSH level prevents or decelerates the progression of cataract. In the rat diabetes model, N-acetylcysteine and glutathione ethyl ester that are easily converted into GSH *in vivo*, successfully suppressed development of cataract in early stages [110].

Glutathionylation of the transcription factor p53 significantly decreased its ability to bind to DNA. Consequently, glutathionylation inhibits p53 that suppresses development of malignant tumors, which may influence oncogenesis [111]. It is supposed that inactivation of p53 through its glutathionylation provides the mechanism for cell adaptation that suppresses development of the apoptotic response in the early stage of oxidative stress and allows the cell to avoid immediate death.

Of note is that age is a risk factor for many diseases, since different damages accumulate with age, while repair systems slow down their activity. With age, mitochondrial functions can also be affected by negative changes facilitating ROS

production, which is observed simultaneously with a decrease in activity of the antioxidant enzymes. Such disorders in redox-regulation affect the S-glutathionylation of proteins, which makes the cell more sensitive to apoptosis and promotes development of pathologic changes [104].

It should be noted that Grx is capable of protein–protein interactions. For example, Grx participates in regulation of ASK1 protein kinase activating JNK1- and p38-dependent signaling pathways of apoptosis [112]. Using different cell lines, it was shown that ASK1 is activated by ROS, by H_2O_2 in particular, due to the breakdown of the complex with Grx1. The reduced Grx1 binds to the C-terminal domain of ASK1, resulting in inactivation of the kinase. In contrast, oxidation of Grx1 leads to dissociation of the complex, activation of ASK1, and induction of apoptosis [113]. This dissociation is prevented by catalase or N-acetylcysteine. The decrease in GSH content using BSO inhibits binding of Grx1 to ASK1. Presumably, GSH is necessary for reduction of the intermolecular disulfide bonds between the adjacent cysteine residues in Grx1, which enables the protein to bind ASK1 [113]. These data suggest that Grx1 can be considered as a redox-sensitive factor involved in regulation of signaling cascades of JNK1 and p38 MAP-kinases.

THE FAMILY OF GLUTATHIONE TRANSFERASES IN THE REGULATION OF REDODOX-DEPENDENT PROCESSES

Glutathione S-transferase (GST, EC 2.5.1.18) is represented by a superfamily of isoenzymes catalyzing conjugation of glutathione (GSH) with a wide range of nonpolar compounds of exogenous and endogenous origin containing electrophilic atoms of carbon, sulfur, nitrogen, and phosphorous, which assists in defense of the cell against possible toxic action of these compounds [114-117]. The GST superfamily includes three subfamilies of isoenzymes: cytosolic, mitochondrial, and microsomal. Isoenzymes of GST have now been found in most living organisms including aerobic bacteria, yeast, plants, insects, and vertebrates. In mammals, GST is present in virtually all organs and tissues, but the highest content of the enzyme is found in the liver.

The cytosolic isoenzymes of GST account for approximately 90% of the total GST activity in the cell. Based on the amino acid sequence homology, mammalian cytosolic GST isoenzymes are grouped into seven classes (α, μ, π, θ, ζ, ω, and σ) that comprise 17 isoenzymes [114, 115]. In humans and rodents, the cytosolic isoenzymes within the same class exhibit more than 40% homology (sometimes more than 90%), while the homology between the enzymes in different classes is less than 25%. Special attention in the contemporary classification is given to the primary structure of the conserved N-terminus of the polypeptide chain containing catalytic residues of tyrosine, cysteine, or serine [115].

In species other than mammals, GST isoenzymes of β, δ, ε, ϕ, λ, τ, and ν classes have been found [118].

A mitochondrial isoenzyme of human GST is GSTK1-1, which belongs to the κ-class [119]. The same isoenzyme was found in human peroxisomes. GSTK1-1 of rodents and humans is active towards several traditional GST substrates, 1-chloro-2,4-dinitrobenzol in particular. In *Caenorhabditis elegans*, GST is involved in metabolism of lipids [120].

Microsomal GSTs are integral membrane proteins that are now called membrane-associated proteins in eicosanoid and glutathione metabolism (MAPEG) [121, 122]. Isoenzymes of the MAPEG microsomal subfamily are divided into four subgroups (I-IV), the amino acid sequence homology between the subgroups constituting less than 20%. In humans, six isoenzymes have been found that belong to subgroups I, II, and IV [121]. Likewise the cytosolic and mitochondrial GST isoenzymes, the microsomal isoenzymes also catalyze conjugation of GSH with electrophilic compounds, but they also participate in reactions of isomerization of unsaturated compounds and biosynthesis of leukotrienes and prostaglandins [121, 123].

Cytosolic and mitochondrial isoforms of GST are homo- or heterodimers, and the subunits within heterodimers belong to the same class. Although the cytosolic GSTs are dimers, representatives of the microsomal subfamily can be mono-, di-, and trimers, and multienzyme complexes also occur [115]. Each subunit is composed of two domains linked with a small irregular region.

The N-terminal domain is the GSH-binding site (G-site). It exhibits topology that is similar to thioredoxin and contains four β-sheets (β1, β2, β3, and β4), three of which are antiparallel, and three α-helices. These elements of the secondary structure are composed into the sequences βαβαββα. The C-terminal domain is the cosubstrate-binding site (H-site), a completely α-helical region composed of either five or six α-helices (α4-8 or α4-9). In contrast to other classes, the isoenzymes of the α, θ, and ω classes have an additional α9 helix. The isoenzymes of the μ-class have a unique μ-loop at the C-terminus, while the isoenzymes of the ω-class contain an additional 19 amino acid sequence at the N-terminus. The θ-isoenzymes have a large loop between the α4- and α5-helices [114, 124, 125]. The differences in the structures of the representatives of various GST isoenzymes provide their wide substrate specificity and diversity of functions. Detailed comparative analysis of the amino acid sequence and structure of cytosolic GST isoenzymes considering the presence of certain amino acid residues in their active site allowed their division into two subgroups: Y-GST, isoenzymes using a tyrosine residue for the activation of GSH (α, μ, π, and σ classes), and S/C-GST, isoenzymes binding GSH through a serine (ϕ, τ, θ, and ζclasses) or a cysteine (β and ω classes) residue [125]. In GST of both subgroups and in mitochondrial GST, these amino acid residues being essential for GSH activation are relocated in the so-called catalytic loop that is next to the first β-sheet in the thioredoxin-like domain. As mentioned above, structure of the H-site exhibits significant variability in representatives of different classes. While forming the dimeric structure, domain I of one

of the subunits and domain II of the other interact with each other by the lock-and-key principle. Certain aromatic amino acid residues of the loop between the α3-helix and β2-sheet of the first monomer play a role as the key. They are located in the hydrophobic "lock" formed by the cavity between the α4- and α5-helices of the second monomer.

The main function of GST is its participation in the antioxidant system through its ability to reduce organic hydroperoxides to alcohols using GSH as the co-substrate [115]. *Via* Se-independent glutathione-peroxidase activity, GST reduces hydroperoxides of polyunsaturated higher fatty acids, phospholipids, and cholesterol [126]. Among the substrates of GSTA4-4 are the lipid peroxidation pruducts acrolein and 4-hydroxynonenal (4-HNE) [115, 127]. Conjugation of these compounds with glutathione protects proteins and DNA against covalent modification. As a result of oxidative stress, nucleotides can be oxidized to propenals and hydroperoxides, which are substrates of GSTP1-1. Oxidation of catecholamines also results in formation of compounds (aminochrome, dopachrome, noradrenochrome, and adrenochrome) that are substrates of GST isoforms. Conjugation of such compounds with GSH contributes to the cellular antioxidant defense system, since they contain a quinone structure producing $O_2^{\bullet-}$, and, consequently, promoting oxidative stress [115]. The cytosolic GSTM2-2 was shown to detoxify *o*-quinones of dopamine, which can protect dopaminergic systems of the brain against degenerative processes [115]. GSTP1-1 mediates defense against oxidative stress, recovering the peroxidase activity of the oxidized peroxiredoxin Prx6 [128].

As discussed above, GST was shown to be involved in the process of S-glutathionylation. Originally, it was considered that growth in ROS production leads to S-glutathionylation to prevent irreversible oxidation of protein cysteine residues and disturbance of protein functions [11]. Later it was found that S-glutathionylation plays an important role in the mechanisms of the cell signaling, changing the sensitivity of cysteine residues towards redox modification. The list of proteins whose structure and functions are modulated by S-glutathionylation is large: proteins involved in metabolism, proteins forming the cytoskeleton and ion channels, signal proteins (kinases and phosphatases), transcription factors, ras-proteins, and heat shock proteins [129]. The process of S-glutathionylation can proceed both non-enzymatically and with participation of enzymes, one of which is GSTP1-1. The ability of GSTP1-1 for S-glutathionylation is based on the catalytic activity of the enzyme. Under oxidative stress, GSTP1-1 is auto-S-glutathionylated at residues Cys47 and Cys101, each of which affects the catalytic activity of the enzyme and its ability to bind target proteins. Besides, specific S-glutathionylation causes oligomerization of GSTP1-1, which presumably has significant consequences for other components of the cellular stress response.

S-Glutathionylation of the GSTP1-1 monomer decreases the number of α-helical regions, i.e., alters the secondary structure of the enzyme, which subsequently leads to a change in the tertiary and quaternary structures [98], affecting ability of the GSTP1-1 to interact with proteins. An example of such regulation is the complex between GSTP1-1

and JNK1. S-glutathionylation of GSTP1-1 at residues Cys47 and/or Cys101 results in dissociation of the complex between GSTP1-1 and JNK1, activation of JNK1, and aggregation of GSTP1-1 [98, 130].

Of special importance is the role of GST in regulation of cell signaling due to the protein–protein interactions with kinases that are activated by oxidative stress. Under physiological conditions, some GSTP1-1 is bound to kinase JNK1, resulting in its inactivation, which regulates the level of active JNK1. Under conditions increasing ROS level, which is observed, for example, under the action of a number of antitumor drugs, the complex between GSTP1-1 and JNK1 dissociates, and GSTP1-1 associates into oligomers. The release of JNK1 induces a cascade of processes, starting from the phosphorylation of Jun-c and resulting in apoptosis. The enhanced expression of the GSTP1-1 gene observed in some tumors can significantly inhibit JNK1, and consequently, suppress the signaling pathway leading to apoptosis, which contributes to formation of drug resistance of the tumor cells [131, 132]. A similar interaction of GSTP1-1 with TRAF2 (factor 2 bound to the TNF-α receptor) blocks action of the kinases JNK1, p38, and ASK1 in the case of the signaling cascade induced by TNF-α. Dissociation of the complex between GSTP1-1 and TRAF2 activates proliferation under gentle oxidative stress, while a prolonged and strong oxidative stress leads to apoptosis [133]. It should be noted that catalytic activity of GSTP1-1 does not change during protein–protein interactions, suggesting that the active sites of the enzyme are not involved in this process [116].

The GSTA1-1 isoenzyme also participates in regulation of apoptotic signaling pathways through protein–protein interactions with JNK1. Enhanced expression of the GSTA1-1 gene significantly decreases the number of cells subjected to apoptosis due to inhibition of JNK1-dependent phosphorylation of Jun-c and activation of caspase-3 [134]. GSTM1-1 exhibits regulatory functions that are similar to those of GSTP1-1. The complex between GSTM1-1 and ASK1 is important for maintenance of the normal level of phosphorylation of p38. Under stress conditions of heat shock or increased ROS level, the complex dissociates, and the GSTM1-1 associates into oligomers, while ASK1 is activated [135]. Since ASK1 is a kinase of MAPK kinase activating the JNK1- and p38-dependent signaling pathways, dissociation of this complex results in cytokine- and stress-induced apoptosis [136].

Ability of the homodimers of GSTP1-1 (sometimes GSTM1-1) to dissociate and form heterodimers with other monomeric proteins underlies its ability to provide these proteins with glutathione [137]. The cytosolic isoforms of GST are catalytically active in the dimeric form, the surface of the dimer being the site of non-catalytic binding of ligands. Several studies report that the isoforms of mammalian GSTP1-1 and GSTM1-1 in the monomeric form can interact with ASK1, JNK1, or with peroxiredoxin 6 (Prx6) [128, 133, 135]. Investigations of the GSTP1-1 molecule have shown that structural features of its C-terminus promote dissociation of the homodimer into monomers. At the same time, the Trx-like domain at the N-terminus promotes formation of heterodimers between GSTP1-1

monomers with other proteins, especially with those containing a Trx-like domain [138]. Recovery of the peroxidase activity of Prx6 can be an example of the protein–protein interaction of GSTP1-1 with simultaneous reduction of the protein by glutathione. The Prx6 molecule has one catalytically active cysteine residue, Cys47, at the N-terminus. Oxidation of this residue yields a sulfenic acid derivative, inactivating peroxidase activity of Prx6 towards H_2O_2 and hydroperoxides of phospholipids. It has been found that GSTP1-1, as a complex with GSH, forms a heterodimer with Prx6 and reduces the Cys47 residue. Binding of GSH induces conformational changes allowing formation of the heterodimer between GSTP1-1 and Prx6 [139]. Then Prx6 is S-glutathionylated at the oxidized Cys47 residue with subsequent disulfide bonding between Cys47 of Prx6 and Cys47 of GSTP1-1, followed by reduction of the disulfide bond by GSH.

GST takes part in regulation of the serine/threonine AMP-activated protein kinase (AMPK), which controls the energy balance of the cell [140]. A diversity of AMPK functions are involved in the control of various metabolic pathways and physiological processes such as proliferation and cell motility. AMPK is activated by ROS and RNS through AMP-dependent and AMP-independent mechanisms and can be involved in cellular redox regulation [141]. *In vitro* studies demonstrated that, under close to physiological conditions, the mammalian isoenzymes GSTM1-1 and GSTP1-1 promoted S-glutathionylation of AMPK at the same cysteine residues that were glutathionylated during the nonenzymatic H_2O_2-dependent process, which also increased the kinase activity [142, 143]. The interaction with AMPK activates GSTM1-1 and GSTP1-1, which in turn results in the S-glutathionylation and activation of AMPK. These data illustrate well the role of AMPK as an important element in redox-dependent signal transduction [144-146]. The activated AMPK activates the transcriptional factor FOXO3 that affects such processes as cell proliferation, gluconeogenesis, and defense against oxidative stress through the activation of the PI3K/AKT signaling pathway [141, 147]. The contribution of FOXO3 to antioxidant defense is accounted for the enhanced expression of Mn-superoxide dismutase, catalase, thioredoxin [148, 149], metallothioneins [150], mitochondrial uncoupling protein UCP2 [151], γ-glutamylcysteine synthetase [148], glutathione peroxidase [149], and GSTM1-1 [150]. The GST-mediated S-glutathionylation and activation of AMPK can be considered as an additional mechanism of regulation of AMPK as a redox sensor of energetic stress and antioxidant defense [142].

As mentioned above, one of the main products of lipid peroxidation is 4-hydroxynonenal (4-HNE), which forms adducts with proteins and nucleic acids. 4-HNE is involved in the MAPK-dependent signaling pathways of cellular stress response, particularly by facilitation of phosphorylation of JNK and p38, which results in their activation [152]. A dose-dependent regulation of cellular signaling pathways by 4-HNE has been demonstrated: at concentrations above 10 μM, 4-HNE exerted cytotoxic effect, while at concentrations below 10 μM (physiological range) 4-HNE modulated cell growth, i.e., affected cell proliferation [153]. Besides, 4-HNE inhibits expression of cyclins D1,

D2, and A and, consequently, activity of the cyclin-dependent kinases 4/6 (Cdk4/6) and Cdk2 [154]. Furthermore, it increases expression of p21waf1, which inhibits the functioning of some cyclin-dependent complexes [155]. Thus, 4-HNE can simultaneously affect the expression of different genes involved in the control of cell proliferation. Undoubtedly, the intracellular content of 4-HNE must be regulated to protect the cell from damage and/or to control stress-dependent signaling pathways. 4-HNE is metabolized by GST, forming the HNE-GSH conjugates. The most specific isoform towards 4-HNE is GSTA4-4 [156].

It has been found that under conditions of oxidative stress, phosphorylation of GSTA4 subunits in the cytoplasm increases, facilitating their binding to the Hsp70 protein, fast dimerization, and subsequent translocation into mitochondria. If the subunits are not hyperphosphorylated, they do not exhibit high affinity to Hsp70. In this case, the formed dimers remain in the cytoplasm [157]. Thus, the oxidative stress-activated import to mitochondria of the GSTA4-4 isoform exhibiting high specificity towards 4-HNE protects mitochondria from oxidative stress and modulates signaling pathways that are affected by 4-hydroxynonenal [157]. It should be noted that TNFα, IL-6, and epidermal growth factor enhance the GSTA4-4 content in mitochondria *in vivo* [158]. Decrease in the GSTA4-4 level results in an increase of ROS production and disturbs mitochondrial functions, which promotes development of the insulin resistance and type 2 diabetes [159]. In whole, different studies show that concentration of 4-HNE in the cell is important for activation of the cell cycle and the signaling cascades regulating cell differentiation, proliferation, and apoptosis. The level of 4-HNE is strongly dependent on the activity of GSTA4-4 both in the cytoplasm and the mitochondria.

There are several specific features of the impact of GSTP1-1 on the redox-dependent pathways regulating cell signaling and metabolism. It is supposed that some of the numerous changes of regulatory proteins observed under acute or chronic cocaine injections could be related to S-glutathionylation catalyzed by GSTP1-1. For example, actin, JNK and AMP-dependent protein kinase are regulated through S-glutathionylation under the action of cocaine [98, 160]. Presumably, it is the enhanced S-glutathionylation that results in neuroadaptation under cocaine-induced oxidative stress. GSTP1-1 was shown to directly inhibit the cyclin-dependent kinase Cdk5, interacting with its regulatory p25/p35 subunit [160]. Stimulation of Cdk5 results in generation of ROS, which leads to cell death due to a feedback mechanism. Under neurotoxic conditions, introduction of the GSTP1-1 gene provides successful neuroprotection due to the ability of GSTP1-1 to modulate the Cdk5-dependent signaling, which protects the cell from oxidative stress and prevents neurodegeneration [161].

GSTP1-1 was shown to prevent the origin and progression of Parkinson's disease, suppressing activation of Jun-c [162]. GSTP1-1 gene knockout mice were more sensitive to the neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine, which led to the early degeneration of dopaminergic neurons and corpus striatum fibers.

Expression levels of GST isoforms differ in normal and tumor tissues. High expression of the GSTP1-1 gene often correlates with drug resistance that is observed in tumor tissues of the ovaries, lungs, mammary glands, large intestine, and in some oncological diseases of the blood [163, 164]. Ability of GSTP1-1 as the inhibitor of JNK1, ASK1, and TRAF2 to regulate kinase signaling pathways determining the cell fate can provide the resistance of tumor cells to antitumor drugs including those with prooxidant action [165, 166]. Transfection of the GSTA1-1 gene into H69 cells (small-cell lung cancer) led to resistance towards doxorubicin, exhibiting prooxidant action [134]. Overexpression of the GSTA1-1 gene protected the cells from the decrease in GSH level caused by doxorubicin, decelerating lipid peroxide oxidation. Besides, overexpression of the GSTA1-1 gene significantly decreased the number of apoptotic cells due to the inhibition of JNK1-dependent phosphorylation of both Jun-c and caspase-3 [134].

The mechanisms regulating the work of genes of the GST isoforms are still not completely understood. It was shown that exogenous or endogenous compounds of different structure are inducers of GST. Some of them activate transcription of the GST genes, acting on the antioxidant-responsive (ARE), xenobiotic-responsive (XRE), or glucocorticoid-responsive (GRE) elements of the promoter region [132, 167, 168]. The presence of ARE in the promoter region is characteristic for genes whose products are involved in the defense of the cell from oxidative stress or xenobiotics. GST isoforms are also encoded by genes that are often called ARE genes, and the corresponding proteins are called ARE proteins. Expression of ARE genes is controlled by the transcription factor Nrf2. Normally, Nrf2 is located in the cytoplasm in a complex with Keap1 protein, which provides ubiquitinylation of Nrf2 and its proteasomal degradation. The mechanism that was supposed to be responsible for activation of Nrf2 involves oxidation of cysteine residues of Keap1 under oxidative stress, resulting in the dissociation of the Keap1–Nrf2 complex and translocation of Nrf2 to the nucleus, where it forms a dimeric complex with small Maf protein. This complex activates expression of genes whose products are involved in cell defense [169]. However, some data suggest that the idea of direct dissociation of the Keap1–Nrf2 complex is incorrect, since the affinity of this interaction is rather high. It is suggested that stress conditions do not affect affinity of the complex, but rather decrease the ability of Keap1 to ubiquitinylate Nrf2, which finally allows the transcription factor to be accumulated in the nucleus and to stimulate expression of the ARE genes [170].

CONCLUSION

The data, which have been reviewed in the chapter, show significance of GSH and GSH-related enzymes (glutathione transferase and glutaredoxin) in supporting redox homeostasis and consequently, in redox regulation of vital cellular functions such as proliferation, apoptosis, protein folding, and cell signaling. A special role in maintenance

of the cellular redox status due to participation in thiol–disulfide exchange and antioxidant defense belongs to reduced glutathione through the suppression of free radical damage as well as regulation of numerous cellular redox-dependent processes from enzyme activity to gene expression. The crucial conditions for cellular viability are supporting the optimal level in GSH/GSSG ratio. Its decrease is related to pathological anomalies including cardiovascular diseases and cancer. Disruption of GSH/GSSG level causes an alteration in the including cardiovascular mechanism of cellular redox signaling that is controlled both nonenzymatically and enzymatically with participation of glutathione transferases and glutaredoxins

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REFERENCES

[1] Sies, H; Berndt, C; Jones, DP. Oxidative Stress. *Ann. Rev. Biochem.* 2017, 86, 715-748.

[2] Yamamoto, M; Kensler, TW; Motohashi, H. The KEAP1-NRF2 System: A thiol-based sensor-effector apparatus for maintaining redox homeostasis. *Physiol. Rev.* 2018, 98, 1169-1203.

[3] Go, YM; Fernandes, J; Hu, X; Uppal, K; Jones, DP. Mitochondrial network responses in oxidative physiology and disease. *Free Rad. Biol. Med.* 2018, 116, 31-40.

[4] Ursini, F; Maiorino, M; Forman, HJ. Redox homeostasis: The golden mean of healthy living. *Redox Biol.* 2016, 8, 205-215.

[5] Deponte, M. Glutathione catalysis and the reaction mechanisms of glutathione-dependent enzymes. *Biochim. Biophys. Acta*. 2013, 1830, 3217-3266.

[6] Galano, A; Alvarez-Idaboy, JR. Glutathione: mechanism and kinetics of its non-enzymatic defense action against free radicals. *RSC Adv*. 2011, 1, 1763-1771.

[7] Ortega, AL; Mena, S; Estrela, JM. Glutathione in cancer cell death. *Cancers (Basel)*. 2011, 3, 1285-1310.

[8] Go, YM; Jones, DP. Redox compartmentalization in eukaryotic cells. *Biochim. Biophys. Acta.* 2008, 1780, 1273-1290.

[9] Jones, DP. Redefining oxidative stress. *Antiox. Redox Signal*. 2006, 8, 1865-1879.

[10] Cai, Z; Yan, LJ. Protein oxidative modifications: Beneficial roles in disease and health. *J. Biochem. Pharmacol. Res.* 2013, 1, 15-26.

[11] Zhang, J; Ye, ZW; Singh, S1; Townsend, DM; Tew, KD. An evolving understanding of the S-glutathionylation cycle in pathways of redox regulation. *Free Rad. Biol. Med.* 2018, 120, 204-216.

[12] Fiaschi, T; Cozzi, G; Raugei, G; Formigli, L; Ramponi, G; Chiarugi, P. Redox regulation of beta-actin during integrin-mediated cell adhesion. *J. Biol. Chem*. 2006, 281, 22983-22991.

[13] Qanungo, S; Starke, DW; Pai, HV; Mieyal, JJ; Nieminen, AL. Glutathione supplementation potentiates hypoxic apoptosis by S-glutathionylation of p65-NFkappaB. *J. Biol. Chem.* 2007, 282, 18427-18436.

[14] Reynaert, NL; Van der Vliet, A; Guala, AS; McGovern, T; Hristova, M; Pantano, C; Heintz, NH; Heim, J; Ho, YS; Matthews, DE; Wouters, EF; Janssen-Heininger, YM. Dynamic redox control of NF-kappaB through glutaredoxin-regulated S-glutathionylation of inhibitory kappaB kinase beta. *Proc. Natl. Acad. Sci. USA.* 2006, 103, 13086-13091.

[15] Fitzpatrick, AM; Jones, DP; Brown, LA. Glutathione redox control of asthma: from molecular mechanisms to therapeutic opportunities. *Antiox. Redox Signal*. 2012, 17, 375-408.

[16] He, L; He, T; Farrar, S; Ji, L; Liu, T; Ma, X. Antioxidants maintain cellular redox homeostasis by elimination of reactive oxygen species. *Cell. Physiol. Biochem.* 2017, 44, 532-553.

[17] Lluis, JM; Morales, A; Blasco, C; Colell, A; Mari, M; Garcia-Ruiz, C; Fernandez-Checa, JC. Critical role of mitochondrial glutathione in the survival of hepatocytes during hypoxia. *J. Biol. Chem*. 2005, 280, 3224-3232.

[18] Mari, M; Morales, A; Colell, A; Garcia-Ruiz, C; Fernandez-Checa, JC. Mitochondrial glutathione, a key survival antioxidant. *Antiox. Redox Signal.* 2009, 11, 2685-2700.

[19] Taylor, ER; Hurrell, F; Shannon, RJ; Lin, TK; Hirst, J; Murphy, MP. Reversible glutathionylation of complex I increases mitochondrial superoxide formation. *J. Biol. Chem*. 2003, 278, 19603-19610.

[20] Beer, SM; Taylor, ER; Brown, SE; Dahm, CC; Costa, NJ; Runswick, MJ; Murphy, MP. Glutaredoxin 2 catalyzes the reversible oxidation and glutathionylation of mitochondrial membrane thiol proteins: implications for mitochondrial redox regulation and antioxidant defence. *J. Biol. Chem.* 2004, 279, 47939-47951.

[21] Hurd, TR; Requejo, R; Filipovska, A; Brown, S; Prime, TA; Robinson, AJ; Fearnley, IM; Murphy, MP. Complex I within oxidatively stressed bovine heart mitochondria is glutathionylated on Cys-531 and Cys-704 of the 75-kDa subunit: potential role of CYS residues in decreasing oxidative damage. *J. Biol. Chem*. 2008, 283, 24801-24815.

[22] Mailloux, RJ; Jin, X; Willmore, WG. Redox regulation of mitochondrial function with emphasis on cysteine oxidation reactions. *Redox Biol*. 2014, 2, 123-139.

[23] Tretter, L; Adam-Vizi, V. Inhibition of Krebs cycle enzymes by hydrogen peroxide: A key role of [alpha]-ketoglutarate dehydrogenase in limiting NADH production under oxidative stress. *J. Neurosci*. 2000, 20, 8972-8979.

[24] Gibson, GE; Blass, JP; Beal, MF; Bunik, V. The alpha-ketoglutarate-dehydrogenase complex: a mediator between mitochondria and oxidative stress in neurodegeneration. *Mol. Neurobiol.* 2005, 31, 43-63.

[25] Cadenas, E; Davies, KJ. Mitochondrial free radical generation, oxidative stress, and aging. *Free Rad. Biol. Med.* 2000, 29, 222-230.

[26] Cole-Ezea, P; Swan, D; Shanley, D; Hesketh, J. Glutathione peroxidase 4 has a major role in protecting mitochondria from oxidative damage and maintaining oxidative phosphorylation complexes in gut epithelial cells. *Free Rad. Biol. Med*. 2012, 53, 488-497.

[27] Liang, H; Ran, Q; Jang, YC; Holstein, D; Lechleiter, J; McDonald-Marsh, T; Musatov, A; Song, W; Van Remmen, H; Richardson, A. Glutathione peroxidase 4 differentially regulates the release of apoptogenic proteins from mitochondria. *Free Rad. Biol. Med.* 2009, 47, 312-320.

[28] Mari, M; Morales, A; Colell, A; Garcia-Ruiz, C; Kaplowitz, N; Fernandez-Checa, JC. Mitochondrial glutathione: features, regulation and role in disease. *Biochim. Biophys. Acta*. 2013, 1830, 3317-3328.

[29] Johansson, C; Lillig, CH; Holmgren, A. Human mitochondrial glutaredoxin reduces S-glutathionylated proteins with high affinity accepting electrons from either glutathione or thioredoxin reductase. *J. Biol. Chem*. 2004, 279, 7537-7543.

[30] Knoops, B; Goemaere, J; Van der Eecken, V; Declercq, JP. Peroxiredoxin 5: structure, mechanism, and function of the mammalian atypical 2-Cys peroxiredoxin. *Antiox. Redox Signal*. 2011, 15, 817-829.

[31] Zhang, H; Go, YM; Jones, DP. Mitochondrial thioredoxin-2/peroxiredoxin-3 system functions in parallel with mitochondrial GSH system in protection against oxidative stress. *Arch. Biochem. Biophys.* 2007, 465, 119-126.

[32] Markovic, J; Borras, C; Ortega, A; Sastre, J; Vina, J; Pallardo, FV. Glutathione is recruited into the nucleus in early phases of cell proliferation. *J. Biol. Chem.* 2007, 282, 20416-20424.

[33] Garcia-Gimenez, JL; Markovic, J; Dasi, F; Queval, G; Schnaubelt, D; Foyer, CH; Pallardo, FV. Nuclear glutathione. *Biochim. Biophys. Acta.* 2013, 1830, 3304-3316.

[34] Bellomo, G; Palladini, G; Vairetti, M. Intranuclear distribution, function and fate of glutathione and glutathione-S-conjugate in living rat hepatocytes studied by fluorescence microscopy. *Microsc. Res. Tech*. 1997, 36, 243-252.

[35] Ho, YF; Guenthner, TM. Uptake and biosynthesis of glutathione by isolated hepatic nuclei. *Toxicologist*. 1994, 14, 178.

[36] Voehringer, DW; McConkey, DJ; McDonnell, TJ; Brisbay, S; Meyn, RE. Bcl-2 expression causes redistribution of glutathione to the nucleus. *Proc. Natl. Acad. Sci. USA*. 1998, 95, 2956-2960.

[37] Zimmermann, AK; Loucks, FA; Schroeder, EK; Bouchard, RJ; Tyler, KL; Linseman, DA. Glutathione binding to the Bcl-2 homology-3 domain groove: a molecular basis for Bcl-2 antioxidant function at mitochondria. *J. Biol. Chem.* 2007, 282, 29296-29304.

[38] Carpenter, G; Cohen, S. Epidermal growth factor. *J. Biol. Chem*. 1990, 265, 7709-7712.

[39] Jang, JH; Surh, YJ. Potentiation of cellular antioxidant capacity by Bcl-2: implications for its antiapoptotic function. *Biochem. Pharmacol*. 2003, 66, 1371-1379.

[40] Gadea, J; Gumbao, D; Matas, C; Romar, R. Supplementation of the thawing media with reduced glutathione improves function and the *in vitro* fertilizing ability of boar spermatozoa after cryopreservation. *J. Androl.* 2005, 26, 749-756.

[41] Schafer, FQ; Buettner, GR. Redox environment of the cell as viewed through the redox state of the glutathione disulfide/glutathione couple. *Free Rad. Biol. Med*. 2001, 30, 1191-1212.

[42] Conrad, M; Moreno, SG; Sinowatz, F; Ursini, F; Kolle, S; Roveri, A; Brielmeier, M; Wurst, W; Maiorino, M; Bornkamm, GW. The nuclear form of phospholipid hydroperoxide glutathione peroxidase is a protein thiol peroxidase contributing to sperm chromatin stability. *Mol. Cell. Biol.* 2005, 25, 7637-7644.

[43] Pujari, G; Berni, A; Palitti, F; Chatterjee, A. Influence of glutathione levels on radiation-induced chromosomal DNA damage and repair in human peripheral lymphocytes. *Mutat. Res.* 2009, 675, 23-28.

[44] Berger, F; Ramirez-Hernandez, MH; Ziegler, M. The new life of a centenarian: signalling functions of NAD(P). *Trends Biochem. Sci.* 2004, 29, 111-118.

[45] Pellny, TK; Locato, V; Vivancos, PD; Markovic, J; De Gara, L; Pallardo, FV; Foyer, CH. Pyridine nucleotide cycling and control of intracellular redox state in relation to poly(ADP-ribose) polymerase activity and nuclear localization of glutathione during exponential growth of Arabidopsis cells in culture. *Mol. Plant*. 2009, 2, 442-456.

[46] Garcia-Gimenez, JL; Ledesma, AM; Esmoris, I; Roma-Mateo, C; Sanz, P; Vina, J; Pallardo, FV. Histone carbonylation occurs in proliferating cells. *Free Rad. Biol. Med.* 2012, 52, 1453-1464.

[47] Cazanave, S; Berson, A; Haouzi, D; Vadrot, N; Fau, D; Grodet, A; Letteron, P; Feldmann, G; El-Benna, J; Fromenty, B; Robin, MA; Pessayre, D. High hepatic glutathione stores alleviate Fas-induced apoptosis in mice. *J. Hepatol*. 2007, 46, 858-868.

[48] Aoyama, K; Watabe, M; Nakaki, T. Modulation of neuronal glutathione synthesis by EAAC1 and its interacting protein GTRAP3-18. *Amino Acids*. 2012, 42, 163-169.

[49] Thompson, JA; Franklin, CC. Enhanced glutathione biosynthetic capacity promotes resistance to As^{3+}-induced apoptosis. *Toxicol. Lett*. 2010, 193, 33-40.

[50] Wang, TG; Gotoh, Y; Jennings, MH; Rhoads, CA; Aw, TY. Lipid hydroperoxide-induced apoptosis in human colonic CaCo-2 cells is associated with an early loss of cellular redox balance. *FASEB J*. 2000, 14, 1567-1576.

[51] Ekshyyan, O; Aw, TY. Decreased susceptibility of differentiated PC12 cells to oxidative challenge: relationship to cellular redox and expression of apoptotic protease activator factor-1. *Cell. Death Differ*. 2005, 12, 1066-1077.

[52] Okouchi, M; Okayama, N; Aw, TY. Differential susceptibility of naive and differentiated PC-12 cells to methylglyoxal-induced apoptosis: influence of cellular redox. *Curr. Neurovasc. Res.* 2005, 2, 13-22.

[53] Ghosh, S; Pulinilkunnil, T; Yuen, G; Kewalramani, G; An, D; Qi, D; Abrahani, A; Rodrigues, B. Cardiomyocyte apoptosis induced by short-term diabetes requires mitochondrial GSH depletion. *Am. J. Physiol. Heart Circ. Physiol*. 2005, 289, H768-776.

[54] Armstrong, JS; Steinauer, KK; Hornung, B; Irish, JM; Lecane, P; Birrell, GW; Peehl, DM; Knox, SJ. Role of glutathione depletion and reactive oxygen species generation in apoptotic signaling in a human B lymphoma cell line. *Cell. Death Differ*. 2002, 9, 252-263.

[55] Mari, M; Colell, A; Morales, A; Caballero, F; Moles, A; Fernandez, A; Terrones, O; Basanez, G; Antonsson, B; Garcia-Ruiz, C; Fernandez-Checa, JC. Mechanism of mitochondrial glutathione-dependent hepatocellular susceptibility to TNF despite NF-kappaB activation. *Gastroenterology*. 2008, 134, 1507-1520.

[56] Circu, ML; Rodriguez, C; Maloney, R; Moyer, MP; Aw, TY. Contribution of mitochondrial GSH transport to matrix GSH status and colonic epithelial cell apoptosis. *Free Rad. Biol. Med.* 2008, 44, 768-778.

[57] Martins, NM; Santos, NA; Curti, C; Bianchi, ML; Santos, AC. Cisplatin induces mitochondrial oxidative stress with resultant energetic metabolism impairment, membrane rigidification and apoptosis in rat liver. *J. Appl. Toxicol*. 2008, 28, 337-344.

[58] Chen, G; Chen, Z; Hu, Y; Huang, P. Inhibition of mitochondrial respiration and rapid depletion of mitochondrial glutathione by beta-phenethyl isothiocyanate: mechanisms for anti-leukemia activity. *Antiox. Redox Signal*. 2011, 15, 2911-2921.

[59] Chernyak, BV; Bernardi, P. The mitochondrial permeability transition pore is modulated by oxidative agents through both pyridine nucleotides and glutathione at two separate sites. *Eur. J. Biochem.* 1996, 238, 623-630.

[60] Costantini, P; Chernyak, BV; Petronilli, V; Bernardi, P. Modulation of the mitochondrial permeability transition pore by pyridine nucleotides and dithiol oxidation at two separate sites. *J. Biol. Chem.* 1996, 271, 6746-6751.

[61] Aon, MA; Cortassa, S; Maack, C; O'Rourke, B. Sequential opening of mitochondrial ion channels as a function of glutathione redox thiol status. *J. Biol. Chem*. 2007, 282, 21889-21900.

[62] Son, Y; Cheong, YK; Kim, NH; Chung, HT; Kang, DG; Pae, HO. Mitogen-activated protein kinases and reactive oxygen species: How can ROS activate MAPK pathways? *J. Signal Transduct.* 2011, 2011, 792639.

[63] Circu, ML; Aw, TY. Reactive oxygen species, cellular redox systems, and apoptosis. *Free Rad. Biol. Med*. 2010, 48, 749-762.

[64] Lu, GD; Shen, HM; Chung, MC; Ong, CN. Critical role of oxidative stress and sustained JNK activation in aloe-emodin-mediated apoptotic cell death in human hepatoma cells. *Carcinogenesis*. 2007, 28, 1937-1945.

[65] Cuadrado, A; Garcia-Fernandez, LF; Gonzalez, L; Suarez, Y; Losada, A; Alcaide, V; Martinez, T; Fernandez-Sousa, JM; Sanchez-Puelles, JM; Munoz, A. Aplidin induces apoptosis in human cancer cells via glutathione depletion and sustained activation of the epidermal growth factor receptor, Src, JNK, and p38 MAPK. *J. Biol. Chem*. 2003, 278, 241-250.

[66] Ji, L; Shen, K; Jiang, P; Morahan, G; Wang, Z. Critical roles of cellular glutathione homeostasis and jnk activation in andrographolide-mediated apoptotic cell death in human hepatoma cells. *Mol. Carcinog.* 2011, 50, 580-591.

[67] Filomeni, G; Rotilio, G; Ciriolo, MR. Glutathione disulfide induces apoptosis in U937 cells by a redox-mediated p38 MAP kinase pathway. *FASEB J.* 2003, 17, 64-66.

[68] Filomeni, G; Aquilano, K; Civitareale, P; Rotilio, G; Ciriolo, MR. Activation of c-Jun-N-terminal kinase is required for apoptosis triggered by glutathione disulfide in neuroblastoma cells. *Free Rad. Biol. Med.* 2005, 39, 345-354.

[69] Piccirillo, S; Filomeni, G; Brune, B; Rotilio, G; Ciriolo, MR. Redox mechanisms involved in the selective activation of Nrf2-mediated resistance versus p53-dependent apoptosis in adenocarcinoma cells. *J. Biol. Chem.* 2009, 284, 27721-27733.

[70] Cross, JV; Templeton, DJ. Oxidative stress inhibits MEKK1 by site-specific glutathionylation in the ATP-binding domain. *Biochem. J*. 2004, 381, 675-683.

[71] Filomeni, G; Piccirillo, S; Rotilio, G; Ciriolo, MR. p38(MAPK) and ERK1/2 dictate cell death/survival response to different pro-oxidant stimuli via p53 and Nrf2 in neuroblastoma cells SH-SY5Y. *Biochem. Pharmacol.* 2012, 83, 1349-1357.

[72] Fernandes, AP; Holmgren, A. Glutaredoxins: glutathione-dependent redox enzymes with functions far beyond a simple thioredoxin backup system. *Antiox. Redox Signal*. 2004, 6, 63-74.

[73] Berndt, C; Lillig, CH. Glutathione, Glutaredoxins, and Iron. *Antiox. Redox Signal.* 2017, 27, 1235-1251.

[74] Rouhier, N. Plant glutaredoxins: pivotal players in redox biology and iron-sulphur centre assembly. *New Phytol*. 2010, 186, 365-372.

[75] Lillig, CH; Berndt, C; Holmgren, A. Glutaredoxin systems. *Biochim. Biophys. Acta*. 2008, 1780, 1304-1317.

[76] Johansson, C; Roos, AK; Montano, SJ; Sengupta, R; Filippakopoulos, P; Guo, K; von Delft, F; Holmgren, A; Oppermann, U; Kavanagh, KL. The crystal structure of human GLRX5: iron-sulfur cluster co-ordination, tetrameric assembly and monomer activity. *Biochem. J.* 2011, 433, 303-311.

[77] Couturier, J; Jacquot, JP; Rouhier, N. Evolution and diversity of glutaredoxins in photosynthetic organisms. *Cell. Mol. Life Sci*. 2009, 66, 2539-2557.

[78] Hofmann, NR. Glutaredoxin functions in floral development. *Plant Cell*. 2009, 21, 363.

[79] Benyamina, SM; Baldacci-Cresp, F; Couturier, J; Chibani, K; Hopkins, J; Bekki, A; de Lajudie, P; Rouhier, N; Jacquot, JP; Alloing, G; Puppo, A; Frendo, P. Two Sinorhizobium meliloti glutaredoxins regulate iron metabolism and symbiotic bacteroid differentiation. *Environ. Microbiol.* 2013, 15, 795-810.

[80] Xing, S; Lauri, A; Zachgo, S. Redox regulation and flower development: a novel function for glutaredoxins. *Plant Biol (Stuttg)*. 2006, 8, 547-555.

[81] Hanschmann, EM; Godoy, JR; Berndt, C; Hudemann, C; Lillig, CH. Thioredoxins, glutaredoxins, and peroxiredoxins--molecular mechanisms and health significance: from cofactors to antioxidants to redox signaling. *Antiox. Redox Signal*. 2013, 19, 1539-1605.

[82] Lonn, ME; Hudemann, C; Berndt, C; Cherkasov, V; Capani, F; Holmgren, A; Lillig, CH. Expression pattern of human glutaredoxin 2 isoforms: identification and characterization of two testis/cancer cell-specific isoforms. *Antiox. Redox Signal*. 2008, 10, 547-557.

[83] Gallogly, MM; Starke, DW; Mieyal, JJ. Mechanistic and kinetic details of catalysis of thiol-disulfide exchange by glutaredoxins and potential mechanisms of regulation. *Antiox. Redox Signal.* 2009, 11, 1059-1081.

[84] Stroher, E; Millar, AH. The biological roles of glutaredoxins. *Biochem. J*. 2012, 446, 333-348.

[85] Lillig, CH; Berndt, C; Vergnolle, O; Lonn, ME; Hudemann, C; Bill, E; Holmgren, A. Characterization of human glutaredoxin 2 as iron-sulfur protein: a possible role as redox sensor. *Proc. Natl. Acad. Sci. USA*. 2005, 102, 8168-8173.

[86] Riondet, C; Desouris, JP; Montoya, JG; Chartier, Y; Meyer, Y; Reichheld, JP. A dicotyledon-specific glutaredoxin GRXC1 family with dimer-dependent redox regulation is functionally redundant with GRXC2. *Plant Cell. Environ.* 2012, 35, 360-373.

[87] Feng, Y; Zhong, N; Rouhier, N; Hase, T; Kusunoki, M; Jacquot, JP; Jin, C; Xia, B. Structural insight into poplar glutaredoxin C1 with a bridging iron-sulfur cluster at the active site. *Biochemistry*. 2006, 45, 7998-8008.

[88] Berndt, C; Hudemann, C; Hanschmann, EM; Axelsson, R; Holmgren, A; Lillig, CH. How does iron-sulfur cluster coordination regulate the activity of human glutaredoxin 2? *Antiox. Redox Signal*. 2007, 9, 151-157.

[89] Mitra, S; Elliott, SJ. Oxidative disassembly of the [2Fe-2S] cluster of human Grx2 and redox regulation in the mitochondria. *Biochemistry*. 2009, 48, 3813-3815.

[90] Zhang, H; Du, Y; Zhang, X; Lu, J; Holmgren, A. Glutaredoxin 2 reduces both thioredoxin 2 and thioredoxin 1 and protects cells from apoptosis induced by auranofin and 4-hydroxynonenal. *Antiox. Redox Signal.* 2014, 21, 669-681.

[91] Muhlenhoff, U; Molik, S; Godoy, JR; Uzarska, MA; Richter, N; Seubert, A; Zhang, Y; Stubbe, J; Pierrel, F; Herrero, E; Lillig, CH; Lill, R. Cytosolic monothiol glutaredoxins function in intracellular iron sensing and trafficking via their bound iron-sulfur cluster. *Cell. Metab*. 2010, 12, 373-385.

[92] Linares, GR; Xing, W; Govoni, KE; Chen, ST; Mohan, S. Glutaredoxin 5 regulates osteoblast apoptosis by protecting against oxidative stress. *Bone*. 2009, 44, 795-804.

[93] Wang, Y; Amegbey, G; Wishart, DS. Solution structures of reduced and oxidized bacteriophage T4 glutaredoxin. *J. Biomol. NMR*. 2004, 29, 85-90.

[94] Bushweller, JH; Billeter, M; Holmgren, A; Wuthrich, K. The nuclear magnetic resonance solution structure of the mixed disulfide between Escherichia coli glutaredoxin(C14S) and glutathione. *J. Mol. Biol.* 1994, 235, 1585-1597.

[95] Watson, WH; Chen, Y; Jones, DP. Redox state of glutathione and thioredoxin in differentiation and apoptosis. *Biofactors*. 2003, 17, 307-314.

[96] Aslund, F; Berndt, KD; Holmgren, A. Redox potentials of glutaredoxins and other thiol-disulfide oxidoreductases of the thioredoxin superfamily determined by direct protein-protein redox equilibria. *J. Biol. Chem.* 1997, 272, 30780-30786.

[97] Starke, DW; Chock, PB; Mieyal, JJ. Glutathione-thiyl radical scavenging and transferase properties of human glutaredoxin (thioltransferase). Potential role in redox signal transduction. *J. Biol. Chem.* 2003, 278, 14607-14613.

[98] Townsend, DM; Manevich, Y; He, L; Hutchens, S; Pazoles, CJ; Tew, KD. Novel role for glutathione S-transferase pi. Regulator of protein S-Glutathionylation following oxidative and nitrosative stress. *J. Biol. Chem.* 2009, 284, 436-445.

[99] Sparaco, M; Gaeta, LM; Santorelli, FM; Passarelli, C; Tozzi, G; Bertini, E; Simonati, A; Scaravilli, F; Taroni, F; Duyckaerts, C; Feleppa, M; Piemonte, F. Friedreich's ataxia: oxidative stress and cytoskeletal abnormalities. *J. Neurol. Sci.* 2009, 287, 111-118.

[100] Johansson, M; Lundberg, M. Glutathionylation of beta-actin via a cysteinyl sulfenic acid intermediary. *BMC Biochem.* 2007, 8, 26. https://doi.org/10.1186/1471-2091-8-26 (accessed: August 28, 2018).

[101] Wang, J; Boja, E. S; Tan, W; Tekle, E; Fales, HM; English, S; Mieyal, JJ; Chock, PB. Reversible glutathionylation regulates actin polymerization in A431 cells. *J. Biol. Chem.* 2001, 276, 47763-47766.

[102] Anathy, V; Aesif, SW; Guala, AS; Havermans, M; Reynaert, NL; Ho, YS; Budd, RC; Janssen-Heininger, YM. Redox amplification of apoptosis by caspase-dependent cleavage of glutaredoxin 1 and S-glutathionylation of Fas. *J. Cell. Biol*. 2009, 184, 241-252.

[103] Huang, Z; Pinto, JT; Deng, H; Richie, JP. Jr. Inhibition of caspase-3 activity and activation by protein glutathionylation. *Biochem. Pharmacol*. 2008, 75, 2234-2244.

[104] Allen, EM; Mieyal, JJ. Protein-thiol oxidation and cell death: regulatory role of glutaredoxins. *Antiox. Redox Signal*., 2012, 17, 1748-1763.

[105] Shi, Q; Xu, H; Kleinman, WA; Gibson, GE. Novel functions of the alpha-ketoglutarate dehydrogenase complex may mediate diverse oxidant-induced changes in mitochondrial enzymes associated with Alzheimer's disease. *Biochim. Biophys. Acta*. 2008, 1782, 229-238.

[106] Di Domenico, F; Cenini, G; Sultana, R; Perluigi, M; Uberti, D; Memo, M; Butterfield, DA. Glutathionylation of the pro-apoptotic protein p53 in Alzheimer's disease brain: implications for AD pathogenesis. *Neurochem. Res*. 2009, 34, 727-733.

[107] Sampathkumar, R; Balasubramanyam, M; Sudarslal, S; Rema, M; Mohan, V; Balaram, P. Increased glutathionylated hemoglobin (HbSSG) in type 2 diabetes subjects with microangiopathy. *Clin. Biochem*. 2005, 38, 892-899.

[108] Shelton, MD; Kern, TS; Mieyal, JJ. Glutaredoxin regulates nuclear factor kappa-B and intercellular adhesion molecule in Muller cells: model of diabetic retinopathy. *J. Biol. Chem.* 2007, 282, 12467-12474.

[109] Craghill, J; Cronshaw, AD; Harding, JJ. The identification of a reaction site of glutathione mixed-disulphide formation on gammaS-crystallin in human lens. *Biochem. J.* 2004, 379, 595-600.

[110] Zhang, S; Chai, FY; Yan, H; Guo, Y; Harding, JJ. Effects of N-acetylcysteine and glutathione ethyl ester drops on streptozotocin-induced diabetic cataract in rats. *Mol. Vis.* 2008, 14, 862-870.

[111] Velu, CS; Niture, SK; Doneanu, CE; Pattabiraman, N; Srivenugopal, KS. Human p53 is inhibited by glutathionylation of cysteines present in the proximal DNA-binding domain during oxidative stress. *Biochemistry*. 2007, 46, 7765-7780.

[112] Ichijo, H; Nishida, E; Irie, K; ten Dijke, P; Saitoh, M; Moriguchi, T; Takagi, M; Matsumoto, K; Miyazono, K; Gotoh, Y. Induction of apoptosis by ASK1, a mammalian MAPKKK that activates SAPK/JNK and p38 signaling pathways. *Science*. 1997, 275, 90-94.

[113] Song, JJ; Rhee, JG; Suntharalingam, M; Walsh, SA; Spitz, DR; Lee, YJ. Role of glutaredoxin in metabolic oxidative stress. Glutaredoxin as a sensor of oxidative stress mediated by H_2O_2. *J. Biol. Chem.* 2002, 277, 46566-46575.

[114] Wu, B; Dong, D. Human cytosolic glutathione transferases: structure, function, and drug discovery. *Trends Pharmacol. Sci.* 2012, 33, 656-668.

[115] Hayes, JD; Flanagan, JU; Jowsey, IR. Glutathione transferases. *Ann. Rev. Pharmacol. Toxicol.* 2005, 45, 51-88.

[116] Tew, KD; Townsend, DM. Glutathione-s-transferases as determinants of cell survival and death. *Antiox. Redox Signal*. 2012, 17, 1728-1737.

[117] Allocati, N; Masulli, M; Di Ilio, C; Federici, L. Glutathione transferases: substrates, inihibitors and pro-drugs in cancer and neurodegenerative diseases. *Oncogenesis*. 2018, 7, 8. https://www.nature.com/articles/s41389-017-0025-3 (accessed: August 28, 2018).

[118] Krajewski, MP; Kanawati, B; Fekete, A; Kowalski, N; Schmitt-Kopplin, P; Grill, E. Analysis of Arabidopsis glutathione-transferases in yeast. *Phytochemistry*. 2013, 91, 198-207.

[119] Gallagher, EP; Gardner, JL; Barber, DS. Several glutathione S-transferase isozymes that protect against oxidative injury are expressed in human liver mitochondria. *Biochem. Pharmacol.* 2006, 71, 1619-1628.

[120] Morel, F; Aninat, C. The glutathione transferase kappa family. *Drug Metab. Rev.* 2011, 43, 281-291.

[121] Morgenstern, R; Zhang, J; Johansson, K. Microsomal glutathione transferase 1: mechanism and functional roles. *Drug Metab. Rev*. 2011, 43, 300-306.

[122] Brautigam, L; Zhang, J; Dreij, K; Spahiu, L; Holmgren, A; Abe, H; Tew, KD; Townsend, DM; Kelner, MJ; Morgenstern, R; Johansson, K. MGST1, a GSH transferase/peroxidase essential for development and hematopoietic stem cell differentiation. *Redox Biol*. 2018, 17, 171-179.

[123] Mandal, AK; Skoch, J; Bacskai, BJ; Hyman, BT; Christmas, P; Miller, D; Yamin, TT; Xu, S; Wisniewski, D; Evans, JF; Soberman, RJ. The membrane organization of leukotriene synthesis. *Proc. Natl. Acad. Sci. USA.* 2004, 101, 6587-6592.

[124] Mohana, K; Achary, A. Human cytosolic glutathione-S-transferases: quantitative analysis of expression, comparative analysis of structures and inhibition strategies of isozymes involved in drug resistance. *Drug Metab. Rev*. 2017, 49, 318-337.

[125] Oakley, A. Glutathione transferases: a structural perspective. *Drug Metab. Rev.* 2011, 43, 138-151.

[126] Prabhu, KS; Reddy, PV; Jones, EC; Liken, AD; Reddy, CC. Characterization of a class alpha glutathione-S-transferase with glutathione peroxidase activity in human liver microsomes. *Arch. Biochem. Biophys.* 2004, 424, 72-80.

[127] Ronis, MJ; Mercer, KE; Gannon, B; Engi, B; Zimniak, P; Shearn, CT; Orlicky, DJ; Albano, E; Badger, TM; Petersen, DR. Increased 4-hydroxynonenal protein adducts

in male GSTA4-4/PPAR-alpha double knockout mice enhance injury during early stages of alcoholic liver disease. *Am. J. Physiol. Gastrointest. Liver Physiol.* 2015, 308, G403-415.

[128] Manevich, Y; Feinstein, SI; Fisher, AB. Activation of the antioxidant enzyme 1-CYS peroxiredoxin requires glutathionylation mediated by heterodimerization with pi GST. *Proc. Natl. Acad. Sci. USA.* 2004, 101, 3780-3785.

[129] Lindahl, M; Mata-Cabana, A; Kieselbach, T. The disulfide proteome and other reactive cysteine proteomes: analysis and functional significance. *Antiox. Redox Signal.* 2011, 14, 2581-2642.

[130] Xiong, Y; Uys, JD; Tew, KD; Townsend, DM. S-glutathionylation: from molecular mechanisms to health outcomes. *Antiox. Redox Signal.* 2011, 15, 233-270.

[131] Adler, V; Yin, Z; Fuchs, SY; Benezra, M; Rosario, L; Tew, KD; Pincus, MR; Sardana, M; Henderson, CJ; Wolf, CR; Davis, RJ; Ronai, Z. Regulation of JNK signaling by GSTp. *EMBO J.* 1999, 18, 1321-1334.

[132] Bartolini, D; Galli, F. The functional interactome of GSTP: A regulatory biomolecular network at the interface with the Nrf2 adaption response to oxidative stress. *J. Chromatogr. B. Anal. Technol. Biomed. Life Sci*. 2016, 1019, 29-44.

[133] Wu, Y; Fan, Y; Xue, B; Luo, L; Shen, J; Zhang, S; Jiang, Y; Yin, Z. Human glutathione S-transferase P1-1 interacts with TRAF2 and regulates TRAF2-ASK1 signals. *Oncogene*. 2006, 25, 5787-5800.

[134] Sharma, A; Patrick, B; Li, J; Sharma, R; Jeyabal, PV; Reddy, PM; Awasthi, S; Awasthi, YC. Glutathione S-transferases as antioxidant enzymes: small cell lung cancer (H69) cells transfected with hGSTA1 resist doxorubicin-induced apoptosis. *Arch. Biochem. Biophys.* 2006, 452, 165-173.

[135] Dorion, S; Lambert, H; Landry, J. Activation of the p38 signaling pathway by heat shock involves the dissociation of glutathione S-transferase Mu from Ask1. *J. Biol. Chem*. 2002, 277, 30792-30797.

[136] Matsuzawa, A; Nishitoh, H; Tobiume, K; Takeda, K; Ichijo, H. Physiological roles of ASK1-mediated signal transduction in oxidative stress- and endoplasmic reticulum stress-induced apoptosis: advanced findings from ASK1 knockout mice. *Antiox. Redox Signal.* 2002, 4, 415-425.

[137] Ralat, LA; Misquitta, SA; Manevich, Y; Fisher, AB; Colman, RF. Characterization of the complex of glutathione S-transferase pi and 1-cysteine peroxiredoxin. *Arch. Biochem. Biophys*. 2008, 474, 109-118.

[138] Tew, KD; Manevich, Y; Grek, C; Xiong, Y; Uys, J; Townsend, DM. The role of glutathione S-transferase P in signaling pathways and S-glutathionylation in cancer. *Free Rad. Biol. Med*. 2011, 51, 299-313.

[139] Ralat, LA; Manevich, Y; Fisher, AB; Colman, RF. Direct evidence for the formation of a complex between 1-cysteine peroxiredoxin and glutathione S-transferase pi with activity changes in both enzymes. *Biochemistry*. 2006, 45, 360-372.

[140] Hardie, DG; Ross, FA; Hawley, SA. AMPK: a nutrient and energy sensor that maintains energy homeostasis. *Nat. Rev. Mol. Cell. Biol*. 2012, 13, 251-262.

[141] Zhao, Y; Hu, X; Liu, Y; Dong, S; Wen, Z; He, W; Zhang, S; Huang, Q; Shi, M. ROS signaling under metabolic stress: cross-talk between AMPK and AKT pathway. *Mol. Cancer*. 2017, 16, 79.

[142] Klaus, A; Zorman, S; Berthier, A; Polge, C; Ramirez, S; Michelland, S; Seve, M; Vertommen, D; Rider, M; Lentze, N; Auerbach, D; Schlattner, U. Glutathione S-transferases interact with AMP-activated protein kinase: Evidence for S-glutathionylation and activation *in vitro*. *PLoS One*. 2013, 8, e62497. https://doi.org/10.1371/journal.pone.0062497 (accessed: August 28, 2018).

[143] Zmijewski, JW; Banerjee, S; Bae, H; Friggeri, A; Lazarowski, ER; Abraham, E. Exposure to hydrogen peroxide induces oxidation and activation of AMP-activated protein kinase. *J. Biol. Chem.* 2010, 285, 33154-33164.

[144] Zou, MH; Kirkpatrick, SS; Davis, BJ; Nelson, JS; Wiles, WG; IV. Schlattner, U; Neumann, D; Brownlee, M; Freeman, MB; Goldman, MH. Activation of the AMP-activated protein kinase by the anti-diabetic drug metformin *in vivo*. Role of mitochondrial reactive nitrogen species. *J. Biol. Chem.* 2004, 279, 43940-43951.

[145] Xie, Z; Dong, Y; Zhang, M; Cui, MZ; Cohen, RA; Riek, U; Neumann, D; Schlattner, U; Zou, MH. Activation of protein kinase C zeta by peroxynitrite regulates LKB1-dependent AMP-activated protein kinase in cultured endothelial cells. *J. Biol. Chem.* 2006, 281, 6366-6375.

[146] Trewin, AJ; Berry, BJ; Wojtovich, AP. Exercise and mitochondrial dynamics: Keeping in shape with ROS and AMPK. *Antioxidants (Basel).* 2018, 7, 7. https://doi.org/10.3390/antiox7010007 (accessed: August 28, 2018).

[147] Tikhanovich, I; Cox, J; Weinman, SA. Forkhead box class O transcription factors in liver function and disease. *J. Gastroenterol. Hepatol*. 2013, 28 Suppl. 1, 125-131.

[148] Colombo, SL; Moncada, S. AMPKalpha1 regulates the antioxidant status of vascular endothelial cells. *Biochem. J.* 2009, 421, 163-169.

[149] Wang, S; Dale, GL; Song, P; Viollet, B; Zou, MH. AMPKalpha1 deletion shortens erythrocyte life span in mice: role of oxidative stress. *J. Biol. Chem*. 2010, 285, 19976-19985.

[150] Greer, EL; Oskoui, PR; Banko, MR; Maniar, JM; Gygi, MP; Gygi, SP; Brunet, A. The energy sensor AMP-activated protein kinase directly regulates the mammalian FOXO3 transcription factor. *J. Biol. Chem.* 2007, 282, 30107-30119.

[151] Xie, Z; Zhang, J; Wu, J; Viollet, B; Zou, MH. Upregulation of mitochondrial uncoupling protein-2 by the AMP-activated protein kinase in endothelial cells attenuates oxidative stress in diabetes. *Diabetes*. 2008, 57, 3222-3230.

[152] Jang, EJ; Kim, DH; Lee, B; Lee, EK; Chung, KW; Moon, KM; Kim, MJ; An, HJ; Jeong, JW; Kim, YR; Yu, BP; Chung, HY. Activation of proinflammatory signaling

by 4-hydroxynonenal-Src adducts in aged kidneys. *Oncotarget*. 2016, 7, 50864-50874.

[153] Zarkovic, N; Ilic, Z; Jurin, M; Schaur, RJ; Puhl, H; Esterbauer, H. Stimulation of HeLa cell growth by physiological concentrations of 4-hydroxynonenal. *Cell. Biochem. Funct*. 1993, 11, 279-286.

[154] Pizzimenti, S; Barrera, G; Dianzani, MU; Brusselbach, S. Inhibition of D1, D2, and A-cyclin expression in HL-60 cells by the lipid peroxydation product 4-hydroxynonenal. *Free Rad. Biol. Med.* 1999, 26, 1578-1586.

[155] Barrera, G; Pizzimenti, S; Laurora, S; Moroni, E; Giglioni, B; Dianzani, MU. 4-Hydroxynonenal affects pRb/E2F pathway in HL-60 human leukemic cells. *Biochem. Biophys. Res. Commun.* 2002, 295, 267-275.

[156] McElhanon, KE; Bose, C; Sharma, R; Wu, L; Awasthi, YC; Singh, SP. Gsta4 null mouse embryonic fibroblasts exhibit enhanced sensitivity to oxidants: Role of 4-hydroxynonenal in oxidant toxicity. *Open J. Apoptosis.* 2013, 2, 1-11. http://dx.doi.org/10.4236/ojapo.2013.21001 (accessed: August 28, 2018).

[157] Raza, H. Dual localization of glutathione S-transferase in the cytosol and mitochondria: implications in oxidative stress, toxicity and disease. *FEBS J.* 2011, 278, 4243-4251.

[158] Desmots, F; Rissel, M; Gilot, D; Lagadic-Gossmann, D; Morel, F; Guguen-Guillouzo, C; Guillouzo, A; Loyer, P. Pro-inflammatory cytokines tumor necrosis factor alpha and interleukin-6 and survival factor epidermal growth factor positively regulate the murine GSTA4 enzyme in hepatocytes. *J. Biol. Chem*. 2002, 277, 17892-17900.

[159] Curtis, JM; Grimsrud, PA; Wright, WS; Xu, X; Foncea, RE; Graham, DW; Brestoff, JR; Wiczer, BM; Ilkayeva, O; Cianflone, K; Muoio, DE; Arriaga, EA; Bernlohr, DA. Downregulation of adipose glutathione S-transferase A4 leads to increased protein carbonylation, oxidative stress, and mitochondrial dysfunction. *Diabetes*. 2010, 59, 1132-1142.

[160] Uys, JD; Knackstedt, L; Hurt, P; Tew, KD; Manevich, Y; Hutchens, S; Townsend, DM; Kalivas, PW. Cocaine-induced adaptations in cellular redox balance contributes to enduring behavioral plasticity. *Neuropsychopharmacol*. 2011, 36, 2551-2560.

[161] Sun, KH; Chang, KH; Clawson, S; Ghosh, S; Mirzaei, H; Regnier, F; Shah, K. Glutathione-S-transferase P1 is a critical regulator of Cdk5 kinase activity. *J. Neurochem.* 2011, 118, 902-914.

[162] Davis, W; Jr. Ronai, Z; Tew, KD. Cellular thiols and reactive oxygen species in drug-induced apoptosis. *J. Pharmacol. Exp. Ther*. 2001, 296, 1-6.

[163] Board, PG; Menon, D. Glutathione transferases, regulators of cellular metabolism and physiology. *Biochim. Biophys. Acta*. 2013, 1830, 3267-3288.

[164] Pathania, S; Bhatia, R; Baldi, A; Singh, R; Rawal, RK. Drug metabolizing enzymes and their inhibitors' role in cancer resistance. *Biomed. Pharmacother*. 2018, 105, 53-65.

[165] Tew, KD; Townsend, DM. Regulatory functions of glutathione S-transferase P1-1 unrelated to detoxification. *Drug Metab. Rev*. 2011, 43, 179-193.

[166] Castro-Caldas, M; Carvalho, AN; Rodrigues, E; Henderson, C; Wolf, CR; Gama, MJ. Glutathione S-transferase pi mediates MPTP-induced c-Jun N-terminal kinase activation in the nigrostriatal pathway. *Mol. Neurobiol*. 2012, 45, 466-477.

[167] Hayes, JD; Pulford, DJ. The glutathione S-transferase supergene family: regulation of GST and the contribution of the isoenzymes to cancer chemoprotection and drug resistance. *Crit. Rev. Biochem. Mol. Biol*. 1995, 30, 445-600.

[168] Higgins, LG; Hayes, JD. Mechanisms of induction of cytosolic and microsomal glutathione transferase (GST) genes by xenobiotics and pro-inflammatory agents. *Drug Metab. Rev.* 2011, 43, 92-137.

[169] Bryan, HK; Olayanju, A; Goldring, CE; Park, BK. The Nrf2 cell defence pathway: Keap1-dependent and -independent mechanisms of regulation. *Biochem. Pharmacol*. 2013, 85, 705-717.

[170] McMahon, M; Thomas, N; Itoh, K; Yamamoto, M; Hayes, JD. Dimerization of substrate adaptors can facilitate cullin-mediated ubiquitylation of proteins by a "tethering" mechanism: a two-site interaction model for the Nrf2-Keap1 complex. *J. Biol. Chem.* 2006, 281, 24756-24768.

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