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Chapter IV

Glutaredoxin in Cancer Development, Progression, Chemo-Resistance and Clinical Applications

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Abstract

The Glutaredoxin (Grx) proteins, coupled with glutathione and glutathione reductase, constitute a major antioxidant system that counteracts the effects of oxidative stress in the cell. Grx proteins regulate diverse cellular functions and play an essential role in redox homeostasis. Abnormal regulation of Grx levels and/or activity has been shown in many human cancers. Recent discoveries have revealed that Grxs are important players in modulating malignant behaviors of cancer cells including uncontrolled growth, epithelial–mesenchymal transition (EMT), migration/invasion, and chemo-resistance. This chapter summarizes the current findings of Grxs in cancer biology and treatment. It describes the role and signaling pathways of Grxs in cancer with a special focus on tumorigenesis and therapy-resistance. It concludes that Grxs may serve as therapeutic targets in cancer treatment and their role in cancer warrants further investigation.

Keywords: glutaredoxin, cancer, survival, metastasis, chemotherapy, resistance

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Abbreviations

Grx: Glutaredoxin
EMT: epithelial–mesenchymal transition
ROS: reactive oxygen species
GSH: glutathione
RNRs: ribonucleoside diphosphate reductases
NTD: N-terminal domain
FasL: Fas ligand
MnSOD: manganese superoxide dismutase
TrxR1: thioredoxin reductase 1
Trx: thioredoxin
H_2O_2: hydrogen peroxide
GSS: glutathionylation
TGFβ: transforming growth factor-beta
ICAM-1: intercellular adhesion molecule-1
NF-κB: nuclear factor of kappa light polypeptide gene enhancer in B-cells
IKK: inhibitor of nuclear factor kappa-B kinase
CDDP: cis-diamminedichloroplatinum
LDH: lactate dehydrogenase

1. The Glutaredoxin System

Glutaredoxins (Grxs) are ubiquitous small heat-stable oxidoreductases that are conserved in both prokaryotes and eukaryotes [1-2]. Grxs, as part of the antioxidant system, play a central role in redox signaling, metabolism and scavenging of reactive oxygen species (ROS) that are important for both normal health and diseases as well (Figure 1). The classical dithiol Grxs contain a conserved active site sequence, namely –Cys-Pro-Tyr-Cys- [1]. In addition to this redox center, Grxs possess a binding site for glutathione (GSH) [3]. Grxs were first identified acting as electron donors to modulate the activity of ribonucleoside diphosphate reductases (RNRs) that play an important function in DNA synthesis [4-5]. When coupled with GSH and glutathione reductase in the presence of NADPH, Grxs can also catalyze the reduction of protein disulfides and of GSH-protein mixed disulfides via a dithiol or monothiol mechanism [6-7] (Figure 2), suggesting that these proteins are essential in regulating redox state of target proteins in diverse cellular processes [2, 8]. Grxs can be divided into three major groups with dithiol (Cys-X-X-Cys), monothiol (Cys-X-X-Ser), and CC-type (Cys-Cys-X-Cys or Cys-Cys-X-Ser) motifs depending on the number of active site Cys residues [9-10]. The last group has been only identified in green photosynthetic organisms, such as plants [10].

Dithiol Grxs have been extensively studied since the bacterial Grxs were discovered about forty years ago and shown to regulate diverse cellular processes [2, 4, 9] (See more details in the following sections). In contrast, monothiol Grxs were discovered quite recently and have been less characterized. Monothiol Grxs were initially identified in yeast (Grx3, 4, and 5) [11]. Yeast Grx5 encodes a mitochondrial monothiol Grx [12], whereas Grx3 and Grx4

can function in both cytoplasm and nuclei [13-15]. This group of monothiol Grxs is also conserved across organisms and has now been identified in bacteria, malarial parasites, fungi, plants, zebrafish, mice, and humans [16-21]. Monothiol Grxs can be further classified as single- and multi-domain monothiol Grxs (Figure 3).

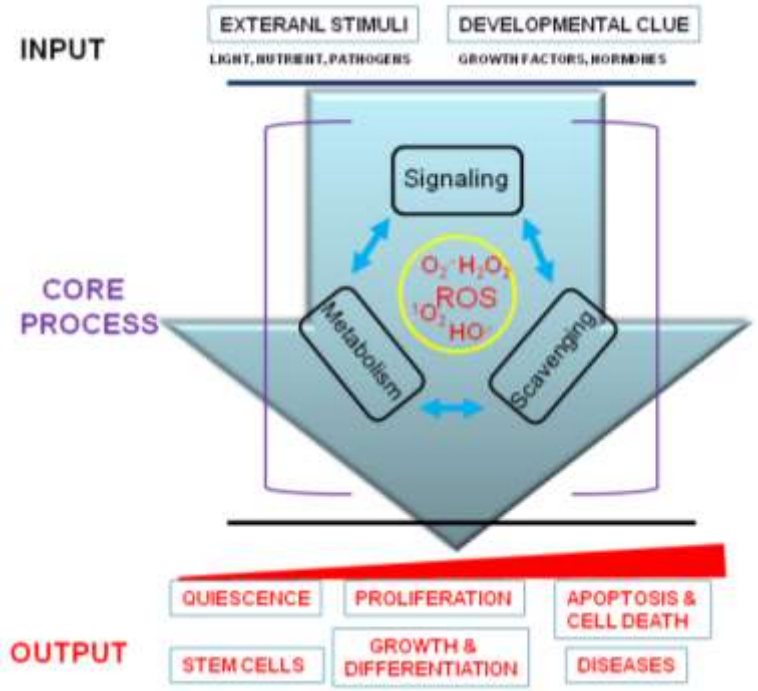

Figure 1. Reactive oxygen species (ROS)-mediated signaling network. The heart of the system is a core process, comprising ROS production and metabolism, scavenging, redox signaling that are tightly coupled to each other. Grxs are thought to play a central role in this core process that interfaces with two sets of components: two input modules that come from either external stimuli, such as light, temperature, nutrient, toxins, and pathogens or developmental cues including growth factors and hormones. The output of the core process is translated to ROS levels that regulate various cellular processes and determine cell fates, normal growth and differentiation, and pathogenesis.

Unlike dithiol Grxs that can act as thioreductases in reversible glutathionylation [22], whether monothiol Grxs catalyze protein deglutathionylation remains to be determined. Several recent studies suggest that monothiol Grxs may be able to deglutathionylate disulfides and/or GSH-protein mixed disulfides in different species [11, 23-25]. Specifically, Zaffagnini *et al.* recently reported that a CGFS-type monothiol Grx from *Chlamydomonas reinhardtii* could be reduced by photoreduced ferredoxin and ferredoxin-thioredoxin reductase in the chloroplast under light and was able to efficiently catalyze A(4)-glyceraldehyde-3-phosphate dehydrogenase deglutathionylation, whereas cytosolic and chloroplastic thioredoxins were inefficient [26]. This finding provides experimental evidence that glutathionylated A(4)-glyceraldehyde-3-phosphate dehydrogenase is the first physiological substrate identified for a CGFS-type Grx [26]. Interestingly, bacterial Grx4, unlike other previously characterized Grxs, can serve as a substrate for thioredoxin reductase instead of NADPH/glutathione reductase [27]. Together, those studies suggest that monothiol Grxs have distinct functions compared to dithiol Grxs.

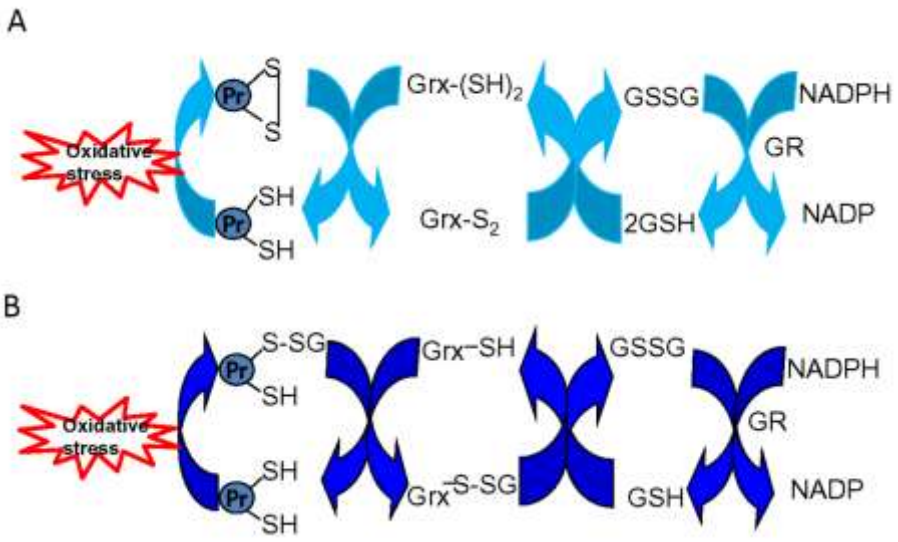

Figure 2. The catalytic mechanism of the glutaredoxin systems. Dithiol (A) and monothiol (B) catalytic mechanisms have been proposed for the glutaredoxin system. In the glutaredoxin action, electrons are received from reduced nicotinamide adenine dinucleotide phosphate (NADPH) and transferred to glutathione reductase (GR), then glutathione (GSH), and relayed to glutaredoxins (Grxs). Grxs then reduce disulfide bonds in target proteins (Pr) caused by oxidative stress.

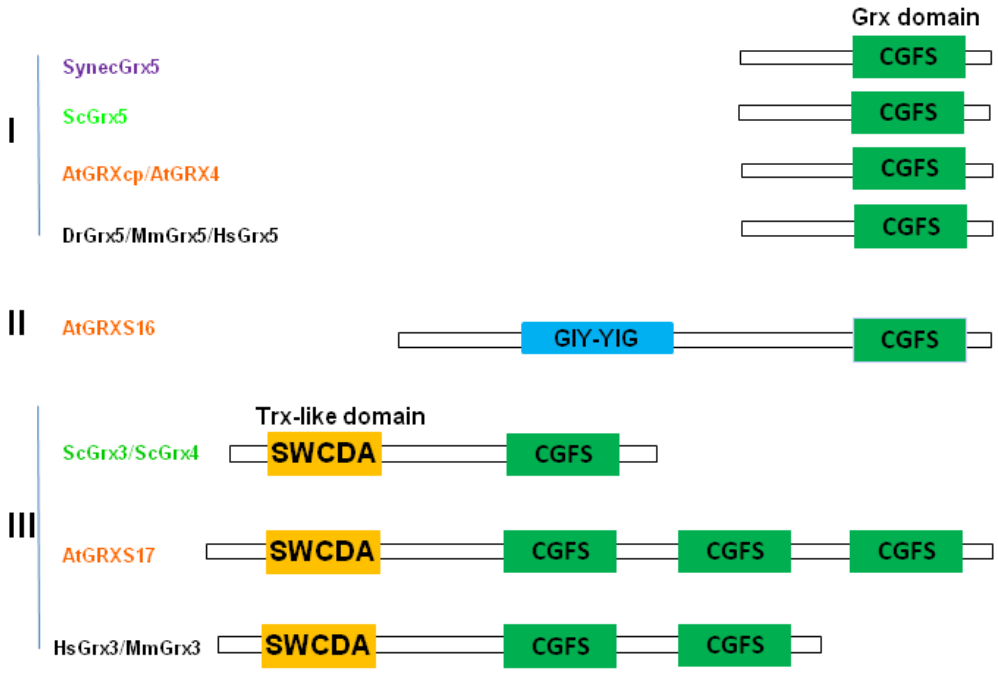

Figure 3. Structure of CGFS-type monothiol glutaredoxins from different organisms. The green boxes indicate Grx domains with "CGFS" motifs; the blue boxes indicate a unique GIY-YIG endonuclease domain in AtGRXS16; the orange boxes indicate the N-terminal Trx-like domains. At represents *Arabidopsis thaliana*, Dr is *Danio rerio*, Hs is *Homo sapiens*, Mm is *Mus musculus*, Sc is *Saccharomyces cerevisiae*, Synec is *Synechocystis*.

Recently, the human mitochondrial dithiol Grx2 has been identified as an iron-sulfur cluster protein, in which Grx2 binds a [2Fe-2S] cluster that bridges two Grx2 molecules through two structural Cys residues to form a Grx2 dimer [28]. Structure-function analyses also indicate that poplar dithiol Grx C1, like human Grx2, contains a [2Fe–2S] cluster in the holo form [29-30]. This [2Fe-2S] cluster is proposed to act as a redox sensor for the activation of Grx under stress conditions [31]. Compared to dithiol Grxs, biochemical analysis indicates that the vast majority of monothiol Grxs are able to bind a Fe-S cluster in the dimer form [32]. Only a few structures of monothiol Grxs have been determined [33-37]. The structures of two monothiol Grxs, *Escherichia coli* Grx4 and the Trx-like domain of yeast Grx3, have been reported. However, the active-site motif regions are not visible or are partially disordered in two of these monothiol Grx structures [33-34]. The structure of poplar GrxS12 has recently been determined [38]. This enzyme possesses an unusual monothiol CSYS active-site sequence and is similar to yeast ScGrx6 which contains the CSYS motif [38-39]. In contrast to some other monothiol Grxs, GrxS12 does not incorporate an iron–sulfur cluster in its original form, whereas bacterial Grx4, Arabidopsis AtGRXcp (AtGRXS14), and human Grx5 have been shown to bind an iron–sulfur cluster in their homodimeric forms [35-38]. It has been proposed that monothiol Grx dimers function in Fe-S cluster biogenesis in both prokaryotes and eukaryotes and serve as a carrier to deliver the intact Fe–S cluster to the apoproteins [35. 40-42] or form a [2Fe–2S] cluster–ligand complex to mediate signaling events in the cell [43].

Grxs are known to be involved in DNA synthesis through modulating RNR activity [9]. A recent study implicates that activation of phage T4 intron endonuclease, I-TevI, is redox-dependent and regulated by Grx1, suggesting that there may be a link between endonuclease activity and redox homeostasis [44]. Most significantly, Liu *et al.* reported that a chloroplastic monothiol glutaredoxin, AtGRXS16, from *Arabidopsis thaliana*, comprises two distinct functional domains, an N-terminal domain (NTD) with GlyIleTyr-TyrIleGly (GIY-YIG) endonuclease motif and a C-terminal Grx module, to coordinate Fe-S cluster biosynthesis and DNA cleavage in chloroplasts [45]. This study further demonstrated that these two functional domains were negatively regulated through the formation of an intramolecular disulfide bond [45]. Given the emerging evidence that Fe–S clusters may be essential components of diverse nucleic acid processing machinery [46-47] and may also be critical for DNA damage recognition [48-50], these findings unravel a novel manner of regulation for Grxs and provide insight into a mechanistic link between redox regulation and Fe-S cluster-mediated DNA metabolism in the cell.

The mammalian glutaredoxins: There are four members of Grxs that have been discovered in mammals: Grx1, Grx2, Grx3 (also known as PICOT and TXNL2), and Grx5. The 12 kDa dithiol Grx1 possesses Cys-Pro-Tyr-Cys active site and is mainly localized in the cytosol, but also can be located in the nucleus [51], mitochondria [52], and secreted from the cell [53]. Unlike Grx1, the 14 kDa dithiol Grx2 possesses Cys-Ser-Tyr-Cys active site and is located in mitochondria, but its cancer-specific isoforms show different subcellular localizations [54]. The 38 kDa monothiol Grx3 is a multi-domain protein that contains an N-terminal Trx-like domain with the active site motif Ala-Pro-Gln-Cys and two repeated C-terminal monothiol Grx domains with the active site Cys-Gly-Phe-Ser (Figure 3). It is localized in the cytosol and the nucleus [55]. The 17kDa monothiol Grx5 is localized in mitochondria and harbors the same active site motif of Grx3. Among the four human Grxs, Grx1 and Grx2 have been studied more comprehensively than the other two members, Grx3

and Grx5. Generally, Grxs can function as electron donors, catalyze reversible glutathionylation, and participate in iron metabolism [9]. Grx2 [56], Grx3 [57], and Grx5 [37] are able to bind [2Fe-2S] clusters. An earlier finding shows that Grx expression and activity can be induced by tumor promoters such as phorbol ester 12-O-tetradecanoylphorbol-13-acetate (TPA) in mouse models, suggesting a role of Grx in tumorigenesis [58]. Studies on Grxs have been directed more towards human diseases, including cancer (Figure 4). However, the role of this family in the process of carcinogenesis and the potential applications in therapeutic approaches by targeting Grxs await further exploration. This chapter will update the current understanding of Grxs in human cancers and offer insights for future directions.

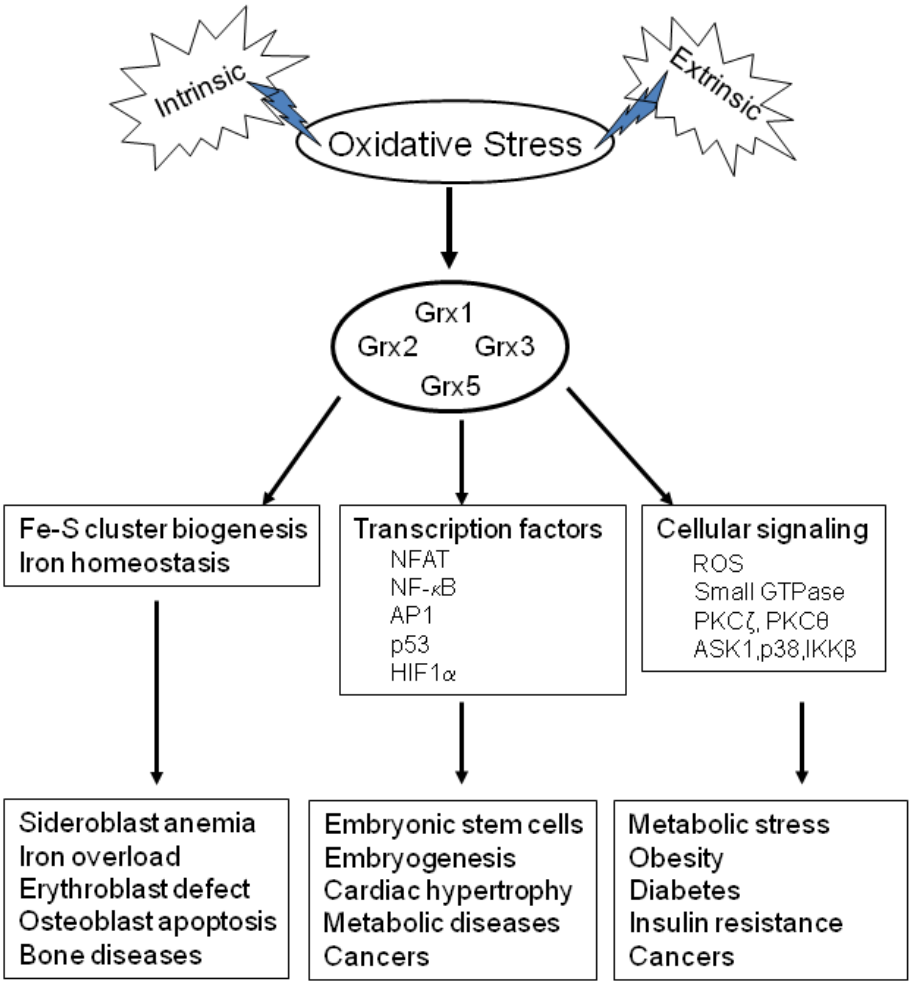

Figure 4. Mammalian Grx systems play a central role in normal human health and disruption of their systems is closely linked to many human diseases. Grxs have crucial functions in Fe-S cluster assembly and iron regulation in the cell and critical in the pathogenesis of anemia and bone diseases [73, 119-120]. Grxs are able to modulate the activity of many transcription factors that control both physiological and pathological processes [63, 75, 77, 121-123]. Furthermore, the Grx systems regulate many cellular signaling pathways that are essential for the development of metabolic disorders and cancer [16, 53, 124-125].

2. Grxs in Cancer Development

2.1. Expression of Grxs in Human Cancers

The expression of Grxs in cancers has been widely investigated. An immune-histochemical analysis performed by Nakamura *et al.* [59] reveals an increased expression of Grx1 in 90% of pancreatic cancer tissues. In a screening of 588 genes by differential hybridization of a human cDNA microarray, upregulation of Grx1 has been found in basal cell carcinoma tissues [60]. Grx1 and Grx2 expression have been found to be correlated with the degree of differentiation in non-small lung adenocarcinoma [61]. Grx2 mRNA levels are elevated in breast, colon and lung cancer tissues and Grx5 mRNA levels are elevated in lung cancers [62]. Grx3 protein is found to be dramatically increased in lung and colon cancers, while the levels of other Grxs are relatively unaltered [62]. Similarly, Ohayon *et al.* reveal that Grx3 expression is increased in Hodgkin's lymphoma/Reed Sternberg cells [63]. Another study reported by Qu *et al.* [64] shows that the levels of Grx3 are elevated in breast cancers and are correlated with patient survival and metastasis to the lung and brain. These findings suggest that Grx3 has a positive role in supporting/promoting tumor cell growth and/or survival. There is also contradictory finding which shows downregulation of Grx1 in malignant gallbladder tissues [65]. Nevertheless, increased Grxs levels were found to be associated with cancers in the majority of studies.

2.2. Cancer Specific Isoforms and Subcellular Localizations

Cancer specific isoforms of Grx members have been recently discovered. Grx2 has long been established as a mitochondrial oxidoreductase and is suggested to play a critical role in protection against apoptotic stimuli. Recent discoveries by Lönn *et al.* show that Grx2b and Grx2c, which are derived from alternative transcription initiation and splicing [54], are enzymatically active isoforms of Grx2 whose expression is restricted to the testes in normal tissues, but broadly expressed in various cancer cell lines [66]. Unlike Grx2a, Grx2b and Grx2c show both cytosolic and nuclear localization. Their novel findings provide evidence for the presence and functions of Grx isoforms. Although the role of these isoforms in tumorgenesis and/or progression needs further investigation, targeting cancer-cell-specific isoforms is a prospective strategy for an effective and secure therapy against cancer.

Besides its well-known cytosolic localization, Grx1 is also localized in the inter-membrane space of mitochondria [52]. This differs from Grx2a, which is localized exclusively in the mitochondrial matrix. The role of Grx1 in mitochondria is correlated with SOD activity [67]. Grx2 is pivotal in reversible interactions of protein thiols with the mitochondrial glutathione pool and protects cells from apoptosis and oxidant damage [68-70]. The localization and presence of cancer-specific Grx isoforms may also provide novel mechanisms involving tumorigenesis and progression. More detailed investigations on the mechanisms of Grxs subcellular distribution and/or translocation may provide new insights into better understanding cancer and new clues for therapeutic strategies in cancer therapy.

2.3 The Role of Grxs in Cancer Cell Proliferation and Survival

ROS can act in dual roles as inhibitors or accelerators of malignant transformation. Elevated oxidative status has been observed in many types of cancer cells due in part to their high metabolic rate. On the other hand, many tumor cells possess stronger antioxidative defense mechanisms to counterbalance excessive ROS, maintain their redox status, and thus suppress apoptosis [71]. As one of the major antioxidant systems, the Grx system protects cancer cells from oxidative stress-induced apoptosis and enables them to better adapt/utilize stress. Anathy *et al.* show that stimulation with the Fas ligand (FasL) induces S-glutathionylation of Fas, and thereby induces and amplifies apoptosis through caspase-dependent degradation of Grx1 [72]. This apoptosis can be attenuated by overexpression of Grx1 [72]. Grx5 also protects cells from apoptosis induced by oxidative stress [74]. Grx5 deficiency reduces manganese superoxide dismutase (MnSOD) activity and increases caspase activity [73]. Human Grx2 has been shown to protect HeLa cells from oxidative stress-induced apoptosis [69] by preventing the release of cytochrome c [70]. In particular, Grx2 prevented loss of cardiolipin, the phospholipid anchoring cytochrome c to the inner mitochondrial membrane. In Hela cells, the Grx system plays a backup role to keep Trx1 reduced in conditions that lack of thioredoxin reductase 1 (TrxR1) and thus prevents ROS accumulation as well as subsequent cell death [74].

Knockdown of Grxs show severe phenotypes; however, only Grx3-knockout mice are embryonically lethal [75]. Grx3 inhibits apoptosis through its role in cell activation-associated signaling pathways or in the cellular response to stress signals [55]. Consistent with its role in anti-apoptosis, the expression of Grx3 showed significant positive correlation with Survivin [62], which is a known anti-apoptotic factor and is positively correlated with tumor stage in lung cancer [76]. Qu *et al.* also showed an anti-apoptotic effect of Grx3 in breast cancer cells. Grx3 knockdown increased intracellular ROS levels and decreased the GSH/GSSG ratio, thereby inhibiting cell growth and survival [64]. Impaired cell proliferation caused by Grx3 depletion was observed in another study, which further showed that Grx3 depletion rendered cell cycle arrest at G2/M phase and cytokinesis failure [77]. In summary, Grxs can regulate cancer cell growth through inhibiting apoptosis and controlling cell cycle progression.

2.4. Reversible Glutathionylation of Cancer-Associated Proteins by Grxs

Glutaredoxin acts either as a dithiol protein disulfide reductase, similar to thioredoxin (Trx), or as a catalyst for the reduction of mixed disulfides of glutathionylated proteins [22] (Figure 2). This process is also called deglutathionylation, which is exclusively catalyzed by Grxs. Glutathionylation is often considered to be a process that protects sensitive cysteinyl residues from irreversible oxidation [22, 78]. In addition to this protective effect, glutathionylation also results in protein-specific functional changes during the regulation of signaling mediators.

Grxs are known to regulate the activity of many proteins through reversible glutathionylation, such as Ras, Fas, ASK1, NF-κB, and procaspase-3, all of which play important roles in the process of tumorigenesis [79]. Glutathionylation is one of the common types of post-translational regulation of protein functions which can be both activating and suppressing. The glutathionylation of Ras (GSS-Ras) activates this oncoprotein, leading to the

downstream phosphorylation of AKT, p38, as well as increased cell proliferation [80]. Grx1 overexpression decreases GSS-Ras and inhibits its activity [80]. On the other hand, glutathionylation can inhibit protein functions as well. AKT undergoes disulfide bond formation between Cys-297 and Cys-311 under stress, which subsequently causes dephosphorylation at Thr-308 and Ser-473 and loss of function [81]. Grx1 can directly regulate AKT activity through reducing oxidized AKT by deglutathionylation accordance with GSH. Overexpression of Grx protects cells from hydrogen peroxide (H_2O_2)-induced apoptosis by regulating the redox state of AKT [81]. Grx1 participates in the deglutathionylation of Sirt1, a redox-sensitive deacetylase which regulates p53 activity and plays critical role in tumorigenesis [82]. Grx5 appears to function in the reversal of glutathionylation as well. Kim *et al.* showed that Grx5 could reduce oxidized tumor suppressor PTEN caused by oxidative stress such as H_2O_2 *in vitro* [83].

NF-κB is another redox-sensitive transcriptional factor that is critical in tumor development and progression. Qanungo's group first reported that Grx1-dependent S-glutathionylation of p65-NF-κB in hypoxic pancreatic cancer cells suppressed NF-κB activity and induced apoptosis [84]. Qu *et al.* also discovered that S-glutathionylation of NF-κB under Grx3-depletion-induced severe oxidative stress suppresses its activity [64]. Therefore, the reversible glutathionylation catalyzed by Grxs plays both promoting and inhibiting roles in cancer which also reflects the delicacy of the Grxs and ROS in modulating the activity of key signaling pathways.

2.5. The Role of Grxs in the Cancer Microenvironment

Tumor microenvironment is critical not only in supporting tumor cell growth, but also in reprogramming tumor cells to develop malignant features [85-86]. In 1995, a study by Rubartelli *et al.* showed that reduced extracellular conditions caused by extracellular Trx inhibit HepG2 cell growth [87]. Their finding suggests a critical role of secreted antioxidant proteins in regulating tumor cell biology and reveals a novel mechanism linking intracellular redox status with extracellular redox status and the modulation of cell functions [88-89]. Grx1 and Grx2 can also be secreted and detected in human plasma by ELISA [90]. The secretion of Grx1 and its presence in plasma suggests extracellular functions. Björnstedt *et al.* discovered that extracellular Grx can function as a reductant for the selenium-dependent peroxidase [91]. Grx1 plays an important role in both autocrine and paracrine pro-inflammatory responses [53]. Extracellular Grx1 is a potential redox modulatory protein that regulates extracellular homeostasis of glutathionylated proteins and GSH [92]. Lundberg *et al.* analyzed intra- and extracellular Grx levels in different cell lines, and showed that Grx was found in all cell lines tested and in the extracellular media of several cell lines [90]. It is possible that secreted Grxs play a role in regulating the redox status of the tumor microenvironment. Currently, the role of secreted Grxs in cancer is largely unknown.

3. Grxs in Cancer Metastasis and Therapy Resistance

3.1. Grxs in Cancer Metastasis

ROS is considered to be a double-edged sword in regulating cell motility in human cancer cells. EMT, which promotes tumor progression, is regulated by ROS [93]. Grxs are scavengers of ROS, and can play positive and negative roles in regulating EMT and metastasis. A moderate increase of ROS promotes tumorigenesis by activating tumor-promoting signaling pathways, but a more drastic increase of ROS inhibits tumor formation and progression [94-95]. Elevation of ROS is concomitant with some EMT signaling pathways. Transforming growth factor-beta (TGFβ) is a cytokine important in inducing EMT in cancer cells. A study reported by Lee *et al.* showed that the loss of Grx1 expression is required for TGFβ induced EMT in H-Ras transformed mammary epithelial cells [62]. On the other hand, other studies have shown that up-regulation of Grx1 is concomitant with NF-κB activation, induction of intercellular adhesion molecule-1 (ICAM-1) [78] and interleukin-6 [53], and activation of IKK activity [53]. Grx3 knockdown increases intracellular ROS levels, reverses EMT, and suppresses metastasis in breast cancer cells through oxidative inhibition of NF-κB activity [64], a well-known transcription factor critical for tumor development and progression. This observation is consistent with previous findings that Grx1 modulates the inhibitory effects of H_2O_2 on IKK-β and NF-κB through deglutathionylation [96]. The contradictions seem to imply that the role of Grx as a metastasis promoter or inhibitor is cellular-context dependent and needs further detailed investigation.

3.2. The Role of Grxs in Therapy Resistance

In general, ROS generated from mitochondria is higher in cancer cells than in normal cells [97]. It is agreed that increased ROS production is a common feature associated with cancer cells [97]. The vast majority of the agents or modalities used to kill cancer cells, such as radiotherapy, chemotherapy and targeted therapies, act either directly or indirectly by generating ROS to block key steps in the cell cycle and apoptosis induction. The classic antitumor drugs such as Cisplatin and Adriamycin appear to produce ROS at excessive levels, causing oxidative stress, which in turn may cause cellular damage. It was also found that the effectiveness of ROS-generating agents, including Paclitaxel and other chemotherapy drugs, in various cancer cell lines varies widely because the antioxidant capacities of those cell lines are different [98]. Thus, as the major cellular defense system that protects cells against oxidative stress, high levels of Grxs in cancer cells may play a critical role in drug resistance, thus making them potential targets for cancer treatment.

3.3. Grx Levels and Drug Sensitivity

Previous findings have provided links between Grx levels and drug resistance in cancer cells. High Grx levels are concomitant with resistance to different chemotherapeutic drugs.

Grx is overexpressed in Adriamycin-resistant MCF-7 [99], a human breast cancer cell line, and inhibits apoptosis caused by adriamycin cytotoxicity [100]. CDDP (cis-diamminedichloroplatinum) is a first-line chemotherapeutic drug for a large number of malignant tumors; it is genotoxic and works by cross-linking DNA, resulting in cell-cycle arrest and ultimately triggering apoptosis. Nakamura *et al.* showed that CDDP-resistant subclones of Hela cells exhibit higher levels of Grx compared to parental cells [59]. Overexpression of Grx1 increases the resistance of MCF7 breast cancer cells to Doxorubicin, a widely used anti-cancer agent [101]. siRNA-mediated silencing of Grx2 dramatically sensitizes the cells to doxorubicin- and phenylarsine oxide-induced cell death [69]. Overexpression of both mitochondrial and cytosolic Grx2 decreases the susceptibility of HeLa cells to doxorubicin-induced apoptosis as well as the antimetabolite 2-deoxy-D-glucose by attenuation of cytochrome c release and caspase activation induced by both agents [70]. Their findings demonstrate a crucial role of Grx2 in regulating the mitochondrial redox status and cell death at the mitochondrial checkpoint. Notably, there is one report showing the pro-apoptotic function of Grx3 [102]. In this study, it was found that Grx3 depletion and overexpression of caspase-cleaved Grx3, normally induced by chemotherapy drugs, inhibited the activation of caspase-3 by chemotherapy drugs and accordingly apoptosis [102]. The seemingly paradoxical conclusions drawn by different studies may reflect the complexity of Grx functions, which require further investigation.

3.4. The Involment of Grxs in Drug Metabolism

Human Grxs are involved in the metabolism of chemotherapeutic drugs because they are the main contributors to the formation of the pharmacologically active metabolite of clopidogrel from its GSH conjugate in the human liver [103]. Selenium compounds are known to decrease cell proliferation, cause DNA fragmentation and induce apoptosis in tumor cells at high concentrations [104-105]. For this reason, selenium is a potential therapeutic agent in cancer treatment and exhibits cytotoxicity through ROS generation [104]. Wallengerg's group discovered that selenium compounds, including selenite, GS-Se-SG, Se-DL-cystine and DL-cystine, are substrates of Grx1; suggesting Grx1 is involved in selenium metabolism [106]. They also showed that suppression of Grx1 increased cell viability after selenium compounds treatment. This effect may be explained by diminished redox-cycling of Grx with these compounds, generating less mixed disulfides and ROS. The finding reported by Olm *et al.* also indicates that Grxs contribute to selenium cytotoxicity by the reduction of cystine to cysteine intracellularly, leading to a more efficient uptake and consequently higher cytotoxicity [107]. In contrast to TrxR, which protects cells from selenium cytotoxicity [108], Grx1 contributes to selenium toxicity [106]. It is noteworthy that the Grx system is inhibited by bis-(glutathionato) platinum (II) (GS-Platinum complex, GS-Pt), a major route for cellular elimination of CDDP [109]. This observation also explains why CDDP-resistant cancer cells show high levels of Grxs. The role of Grx in drug metabolism and activity is a novel theme in studying the mechanisms of redox-based cancer treatment.

4. Grxs as Potential Targets in Cancer Treatment

4.1. Killing Cancer Cells by Targeting Grxs

As mentioned earlier in this chapter, Grxs protect cancer cells from oxidative stress generated by cancer cells themselves (due to high metabolism) or therapeutic chemicals to support their uncontrolled proliferation and malignant invading behaviors. The idea of selectively killing cancer cells by exploiting their metabolic and oxidative traits makes Grxs valuable targets in anticancer treatment. Inhibition of Grxs in cancer cells may shift the balance between high oxidative stress and antagonizing antioxidant capacity, and further magnify their vulnerabilities to oxidative stress. The combination of chemotherapeutic drugs with Grxs inhibition (e.g., knockdown or inhibitors) may be more effective in killing cancer cells than chemotherapy alone and thus reduce the occurrence of drug resistance and the side-effects of chemotherapy. Alternatively, Grx inhibition in combination with ROS-inducing drugs such as elesclomol [110], a chemical that selectively transporting copper to mitochondria to induce oxidative stress [111], may overwhelm cancer cells with ROS and thus induce apoptosis. Recently, a phase III clinical trial of elesclomol in combination with paclitaxel in melanoma shows that patients with low and normal baseline levels of lactate dehydrogenase (LDH), a well-known indicator for hypoxia and poor prognosis in cancer, had an improvement in progression-free survival [112]. This suggests that ROS-generating agents may hold promise as novel anticancer therapies. Therefore, it is reasonable to speculate that Grxs blockade may also render cancer cells more sensitive to cytotoxic therapies.

4.2. Detection of Grxs in Serum as Biomarkers

Grx levels are found to be correlated with disease status. A study by Godoy *et al.* shows that Hela cells overexpressing Grx1-3 and 5 displayed higher survival/ proliferation rates and lower oxidative damage compared to control cells when subjected to hypoxia and reoxygenation [101]. As mentioned earlier, Trx and Grx can be detected in the serum from patients. More importantly, extracellular Trx and Grx levels are both capable of modulating the uptake of selenium by cancer cells, which is crucial to the specific selenite (a type of selenium compounds) cytotoxicity [107]. Detection of serum Trx1 showed diagnostic value in malignant diseases [113-114]; therefore, whether Grx levels in serum can serve as a diagnostic or recurrent marker for cancer patients is an issue worth investigating.

4.3. Detection of Mutations and Post-Translational Modifications

The mutations in the Grx family, which are related to functional changes, have been discovered in other species [115-117]. Whether these similar mutations involving the function of Grx can also be found in human cancers is waiting to be solved. Despite the fact that Grxs are major regulators in maintaining redox homeostasis, they are also substrates of redox-related modifications. Grx1 can be regulated by oxidative stress through modification of its cysteinyl residues and this modification is related to activity loss [118]. In contrast,

modification of Grx2 actually leads to the activation of its function [118]. Tyrosine phosphorylation of Grx3 renders its translocation to the nucleus under oxidative stress [55]. The mutations or post-translational modifications of Grxs may not only reflect their physical function, but their disease-specific roles as well.

Conclusion

The importance of the Grx system in cancer is increasingly recognized. Emerging evidence has strongly suggested a critical role of Grxs in the process of tumorigenesis and treatment resistance, though there are several studies that show opposite results. Whether redox stress is a friend or foe is the debate of the century. Likewise, the role of Grxs in cancer cells is also intricate and complex. For example, high levels of Grxs can either promote or suppress EMT. To make matters worse, different Grx members may exert distinct effects on cell functions. The molecular mechanisms of Grx members involved in signaling pathways in cancer are worthy of attention. The comprehensive investigations on Grxs will provide better understanding of cancer biology and contribute to the development of clinical applications. It is hoped that this review generates substantial interests in the role of Grxs in cancer, and that further investigations will establish Grx members as biomarkers and therapeutic targets in cancer. The increasingly obvious complexity in the biology of ROS in cancer development creates a compelling reason to unravel the mechanisms underlying the function of Grx.

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