Biomarkers of the Antioxidant Response: A Focus on Liver Carcinogenesis

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Ricardo Sánchez-Rodríguez, Julia Esperanza Torres-Mena, Luis del Pozo Yauner, and Julio Isael Pérez-Carreón

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R. Sánchez-Rodríguez (✉) • J.E. Torres-Mena • L. del Pozo Yauner • J.I. Pérez-Carreón (✉)
Laboratorio de Bioquímica y Estructura de Proteínas, Instituto Nacional de Medicina Genómica, Mexico City, Mexico
e-mail: richikrdo@comunidad.unam.mx; jiperez@inmegen.gob.mx

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Abstract

Many studies have demonstrated the association of oxidative stress caused by excessive and sustained production of reactive species with chronic inflammatory conditions, neurodegenerative diseases, diabetes mellitus, atherosclerosis, and cancer. The main mechanism of redox control relies on the cellular antioxidant response. Nevertheless, oxidative stress and oxidative damage to biomolecules are events inherent to the process of carcinogenesis. Antioxidant response systems do not operate in isolation, as there is significant convergence among thermodynamically favored systems. Three main systems may be identified: glutathione, thioredoxins (TRX), and nicotinamide adenine dinucleotide phosphate (NADPH). Liver tumors frequently exhibit overexpression of one or more proteins belonging to the antioxidant system, for example, glutathione reductase (GSR), glutathione S-transferase P (GSTP), gamma-glutamyl transferase (GGT), glucose-6-phosphate dehydrogenase (G6PD), thioredoxin reductase (TXNR), NAD(P)H dehydrogenase [quinone] 1 (NQO1), and prostaglandin reductase 1 (PTGR1). The increased expression of these enzymes is suggested as biomarker that favors tumor development by stimulating proliferation, angiogenesis, and metastasis or by preventing cell death. The loss of expression of some antioxidant enzymes could be used as biomarkers too such as catalase (CAT) and superoxide dismutase (SOD). Therefore, the expression of these proteins shows predictive value for the prognosis and risk of liver cancer recurrence in patients. In this chapter, we discuss the most recent findings regarding the enzymatic antioxidant cellular response that occurs during liver carcinogenesis and how these systems could be used as biomarkers in the clinical practice.

Keywords

Liver cancer • Oxidative stress • Antioxidant response • Redox state • Reactive oxygen and nitrogen species

List of Abbreviation

4-HNE	4-Hydroxynonenal
8-OH-dG	8-hydroxy-20-deoxyguanosine
AKR	Aldo-keto reductase
ATF-2	Activating transcription factor

ATM	Ataxia telangiectasia mutated
CAT	Catalase
CBR1	Carbonyl reductase 1
G6PD	Glucose-6-phosphate dehydrogenase
GCL	Glutamate cysteine ligase
GGT	Gamma-glutamyl transferase
GPX	Glutathione peroxidase
GS	Glutathione synthetase
GSH and GSSG	Reduced and oxidized glutathione
GSR	Glutathione reductase
GST	Glutathione S-transferases
HCC	Hepatocellular carcinoma
HIF	Hypoxia-inducible factors
HMOX	Heme oxygenase
HNF4α	Hepatocyte nuclear factor alpha
MDA	Malondialdehyde
MDR	Multidrug resistance protein
MT	Metallothionein
NADPH	Nicotinamide adenine dinucleotide phosphate
NFE2L2	Nuclear factor (erythroid-derived 2)-like 2
NF-kB	Nuclear factor kB
NQO1	NADPH quinone oxidoreductase 1
PI3K	Phosphoinositide 3 kinase
PLC	Phospholipase C
PPAR	Peroxisome proliferator-activated receptors
PRX	Peroxiredoxin
PTEN	Phosphatase and tensin homolog
PTGR	Prostaglandin reductase
RAR	Retinoic acid receptors
RNS	Reactive nitrogen species
ROS	Reactive oxygen species
SOD	Superoxide dismutase
TRX	Thioredoxin
TXNR	Thioredoxin reductase

Key Facts of the Antioxidant Response

- Cells possess a system for the direct elimination of reactive species that is called antioxidant response.
- Antioxidant response is an important part of the cell's defense against oxidative stress.

- Antioxidant response is composed of transcription factors, enzymes, proteins, and peptides that protect against oxidative stress.
- Oxidative stress is closely associated with the development of cancer, but tumoral cells overexpress several proteins of the antioxidant response for their own protection.
- Levels of reactive species and antioxidant response determine the cellular oxide-reduction state.

Definition of Words and Terms

4-hydroxynonenal (4-HNE)	Lipid peroxidation product, which may serve as markers of oxidative stress and it can induce cell death.
Antioxidant response	Cellular system for the direct elimination of reactive species.
Glutathione	Tripeptide of glutamate, glycine, and cysteine, which include a non-proteic thiol group, glutathione plays a fundamental role in the maintenance of a reduced cellular state.
Metallothionein	Low-molecular-weight proteins that are expressed in response to stress. These proteins exhibit conserved cysteine-rich domains that are able to bind to metals to play a role in metal homeostasis.
NADPH	Nicotinamide adenine dinucleotide phosphate. It is tightly associated with the glutathione and thioredoxin systems in the maintenance of the oxidation-reduction (REDOX) balance. Additionally, NADPH is used as a cofactor by a variety of enzymes that mediate oxidation-reduction reactions as part of the phase II antioxidant response.
Oxidative stress	The loss of oxidation-reduction balance caused by either an increase in oxidant species or deficiencies in cellular antioxidant molecules.
Reactive species	Free radicals and their metabolites that are able to chemically modify various biomolecules, such as lipids, proteins, and DNA.
Redox state	Oxide and reduction status in the cells.
Thioredoxins	A group of proteins involved in oxidation-reduction recycling dependent on NADPH and these proteins exhibit in common a conserved cysteine domain.

Introduction

Reactive Oxygen and Nitrogen Species

Reactive species, or free radical molecules, are able to chemically modify various biomolecules, such as lipids, proteins, and DNA (Valko et al. 2007). A free radical is a highly reactive species with atomic orbits containing one or more unpaired electrons that are able to exist independently (Halliwell and Gutteridge 1984). Generally, free radicals and their metabolites are considered reactive species. Free radicals may be generated naturally within biological systems. The most common reactive species are those derived from oxygen (reactive oxygen species, ROS) and those derived from nitrogen (reactive nitrogen species, RNS), although reactive species derived from iron and copper also exist (Valko et al. 2007).

ROS include superoxide anions, hydrogen peroxide, hydroxyl radicals, singlet oxygen, and organic peroxides. ROS may be endogenously generated by the cell, via the activity of enzymes such as nicotinamide adenine dinucleotide phosphate (NADPH) oxidase, cytochrome P450, xanthine oxidase, or myeloperoxidase or through the activity of the mitochondrial electron transport chain. ROS may also be generated exogenously, for example, by ionizing radiation (Oyagbemi et al. 2009). The main representative species of RNS is nitric oxide, the product of the activity of nitric oxide synthase (NOS). Additionally, nitric oxide can react with superoxide to generate peroxy nitrites (Valko et al. 2007).

Reactive species may generate different metabolites upon reacting with biomolecules. For example, the reaction between reactive species and cell membrane polyunsaturated fatty acids can produce reactive metabolites with a mutagenic capacity, such as malondialdehyde (MDA) and 4-hydroxynonenal (4-HNE), which may serve as markers of oxidative stress (Wang and Liehr 1995; Bartsch et al. 2002). Oxidized nucleotides such as mutagenic 8-hydroxy-20-deoxyguanosine (8-OH-dG) are formed by the reaction of reactive species with DNA (Droge 2002; Murtas et al. 2010). Finally, 3-nitrotyrosine is formed by the reaction of RNS with proteins (Sainz et al. 2012).

Oxidative Stress

Oxidative stress is defined as the loss of oxidation-reduction balance, caused by either an increase in oxidants or deficiencies in cellular antioxidants, leading to increased levels of reactive species (Droge 2002; Valko et al. 2007; Sainz et al. 2012). Therefore, oxidative stress results from an imbalance in the redox state favoring an oxidative cellular environment.

The relationship between reduced (GSH) and oxidized (GSSG) glutathione is regarded as the best parameter for measuring the presence of cellular oxidative stress in an organism. Other such parameters include depletion of GSH (Valko et al. 2007; Franco and Cidlowski 2012) and elevated levels of MDA, 4-HNE, 8-OH-dG, and 3-nitrotyrosine.

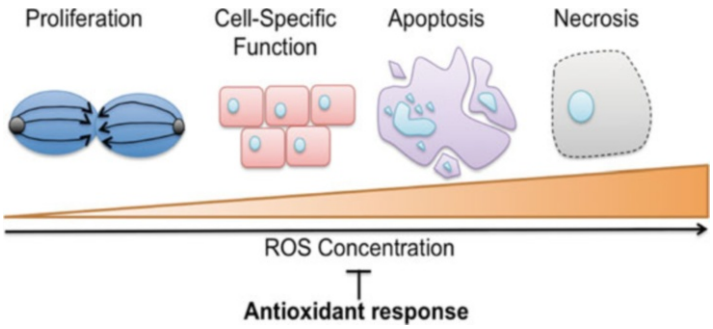

Fig. 1 ROS concentration modulates cellular functions from cell proliferation to cell death. Cell proliferation, the cell-specific function, apoptosis, and necrosis are regulated through cellular signaling, where functions of proteins and metabolites can be controlled by the oxidized and reduced state. Thus the ROS stimulus plays an important role, which can be modified by the antioxidant response

Cell Signaling and Redox Status

Several studies have demonstrated that the regulation of the redox state is important for the modulation of cellular functions, in both normal and tumor cells. Cellular signaling is altered or modified according to the concentration and duration of signals produced directly by reactive species or via the reduction of antioxidants such as GSH (Fig. 1). For example, ROS and RNS have the capacity to stimulate the release of pro-inflammatory cytokines such as IL-6 or TNF-α and anti-inflammatory cytokines such as IL-10, resulting in modulation of the immune response. Moreover, ROS may contribute to the processes of angiogenesis and cell death in a concentration-dependent fashion. For example, 4-HNE can activate pathways leading to cell rescue, or it may induce apoptosis, necrosis, senescence, or autophagy (Valko et al. 2007; Dalleau et al. 2013). Reactive species can directly oxidize protein residues, inducing changes in protein activity, as occurs in the EGF, insulin, PKC, and MAPK (Droge 2002) signaling pathways.

Proteins Regulated by the Redox State

Reactive species may induce other types of posttranslational modifications. For example, the S-nitrosylation (Sengupta and Holmgren 2012) or S-glutathionylation (Pallardo et al. 2009) of proteins (the latter of which is mediated by GSH) is dependent on the oxidized/reduced state of cysteine residues. The oxidation of cysteine residues can generate sulfenic, sulfinic, and sulfonic groups, based on the degree of oxidation, and influences the redox potential of the protein. As described below, these modifications play important roles in protein signaling and inactivation. Additionally, glutathionylation of DNA and histones is thought to be relevant to the epigenetic regulation of gene expression (Pallardo et al. 2009; Zhang and Forman 2012). The proteins ATM, PLC, PI3K, PTEN, and HIF are sensitive to the

concentration of GSH, which is generally associated with hypoxia due to the failure of respiratory chain complex III (Bae et al. 2011). The concentration of GSH is closely correlated with the level of reactive species and contributes to various cellular processes. For example, low levels of GSH favor the activation of apoptosis or cell cycle arrest (Reddy et al. 2008; Franco and Cidlowski 2012). In contrast, increased GSH levels favor resistance against apoptosis. The nucleus/cytoplasm ratio of GSH levels is essential for cell cycle regulation (Pallardo et al. 2009); as this ratio is influenced by fluctuations in the redox environment, it affects functional role of cell cycle proteins such as cdks, p53, and p21 (Valko et al. 2007).

Antioxidant Response

Cells possess a system for the direct elimination of reactive species. This system includes various antioxidant enzymes, such as superoxide dismutase, catalase, and peroxidases, as well as other proteins with oxidoreductase activity or with thioredoxin domains. Moreover, nonenzymatic systems such as the GSH system allow the redox homeostasis within cells to be maintained. The expression of these proteins is regulated by the activation of transcription factors such as NFE2L2, NF-kB, and AP-1, which modulate the presence of reactive species. A global view of the antioxidant response system is illustrated in Figs. 2 and 3. Antioxidant response systems do not operate in isolation, as there is significant convergence among thermodynamically favored systems. Three main systems may be identified: glutathione, thioredoxins, and NADPH (Penney and Roy 2013).

Stress and Oxidative Damage in Carcinogenesis

Studies on carcinogenesis, particularly those involving chemical compounds, have demonstrated a correlation between persistent oxidative stress and damage to DNA, proliferation, adhesion, and cell survival. Moreover, persistent oxidative stress is implicated in the inactivation of tumor suppressor genes, overexpression of proto-oncogenes, and genetic instability. Sustained DNA damage alters signal transduction and transcription, increases the frequency of errors during replication, and induces genetic instability, ultimately promoting carcinogenesis. High levels of ROS are essential during the initiation and promotion stages of chemical carcinogenesis. Nevertheless, recent findings support the role of antioxidants in the carcinogenesis process (Saeidnia and Abdollahi 2013; Sayin et al. 2014), possibly via perturbation of the redox equilibrium or the production of stable radical species. Therefore, maintenance of the redox equilibrium is essential for cellular adaptation and survival. Perturbation of the redox equilibrium may lead to the activation of specific signaling pathways, depending on the signal type, duration, and intensity affecting the redox balance. Thus, numerous components of oxidative stress such as 4-HNE and antioxidant pathways such as the glutathione metabolism can be used as biomarkers of tissue damage or disease state (Ooi et al. 2011).

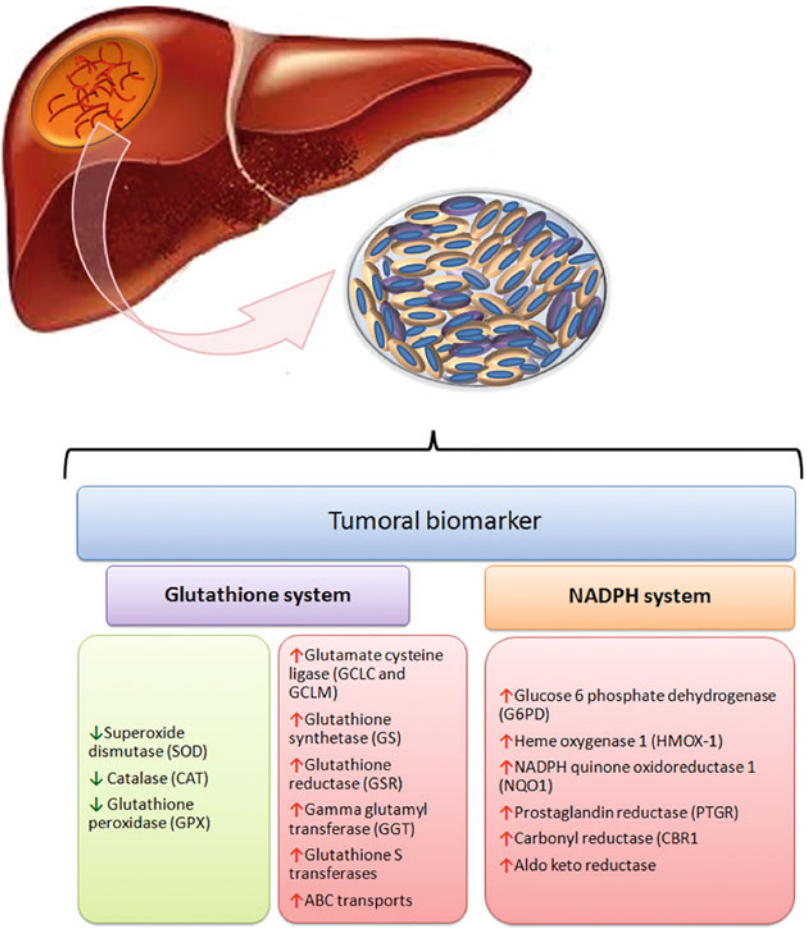

Fig. 2 Proteins of the antioxidant response could be tumoral biomarkers. The antioxidant proteins can modulate the oxidation-reduction (REDOX) cellular equilibrium. The loss or reduced expression of these enzymes is generally associated with an increased risk of cancer development; however their overexpression in tumors has been associated with poor prognosis, resistance to chemotherapy, and recurrence. Thus, they could be good biomarkers of cellular perturbations

Oxidative Stress and Liver Cancer

Hepatocellular carcinomas (HCC) and cholangiocarcinomas are the most frequent types of liver carcinomas, and their classification is dependent on the type of cells that give rise to these tumors (Llovet et al. 2003). Risk factors for liver cancer include hepatitis B (HBV) and hepatitis C (HCV) viral infections, alcoholic cirrhosis, metabolic syndrome, biliary cirrhosis, chronic hepatic lesions, hemochromatosis, and consumption of foods contaminated with aflatoxin B1 (El-Serag and

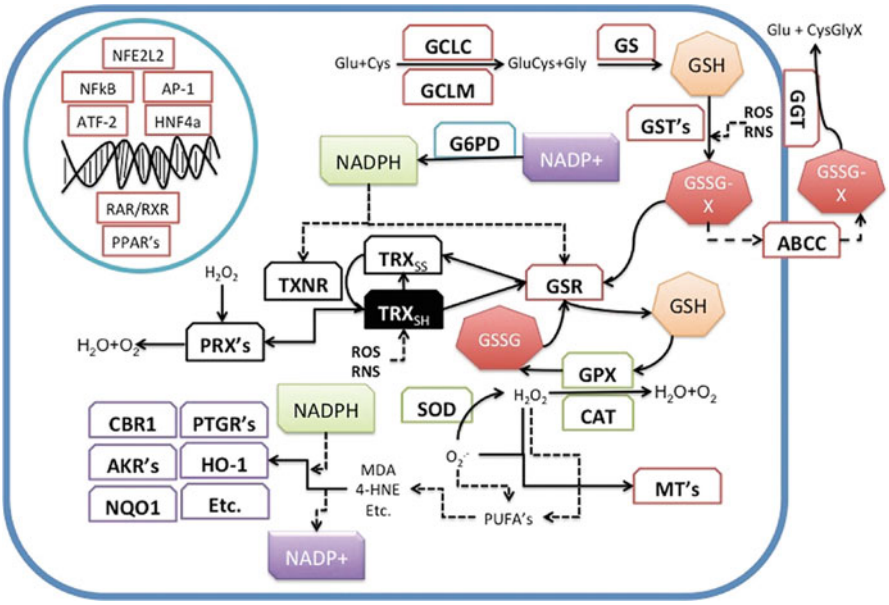

Fig. 3 Principal pathways of the antioxidant response. The transcription factors NFE2L2, NF-kB, AP-1, ATF-2, HNF4a, RAR/RXR, and PPARs are the main factors that regulate the expression of the proteins constituting the cellular antioxidant system. Various pathways for the detoxification of reactive species are interconnected with the GSH, NADPH, and thioredoxin (TRX) systems. The concentrations of ROS, O_2^{-}, and H_2O_2 can be directly modulated by enzymes such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPX), peroxiredoxin (PRX), and metallothioneins (MTs). The concentration of the remaining ROS and RNS may be regulated via conjugation with GSH (GS-X) mediated by GSTs or reduced by TRX, or these species may be metabolized by enzymes with oxidoreductase activity. A sustained antioxidant response may trigger an increase in the synthesis of GSH or TRX or may lead to activation of recycling pathways mediated by thioredoxin reductase (TXNR) and glutathione reductase (GSR) enzymes, which depend on the presence of NADPH. Alternatively, increased GSH synthesis may result from the recycling of amino acids such as glutamate via the activity of gamma-glutamyl transferase (GGT)

Rudolph 2007). These risk factors are diverse, but they share a similar tumorigenic mechanism that is dependent on the generation of oxidative stress. This has been demonstrated in experimental models of chemical hepatocarcinogenesis, in which ROS (Sanchez-Perez et al. 2005) are formed through the metabolic activation of chemical carcinogens, leading to the oxidation of lipids and proteins.

Antioxidant Response Enzymes as Biomarkers

Antioxidant Enzymes

The main enzymes involved in the antioxidant response are superoxide dismutase, catalase, and glutathione peroxidase (GPX). These enzymes are able to directly metabolize ROS, modulating the oxidation-reduction balance in cells, and loss or

Table 1 Antioxidant response enzymes used as a biomarker

Antioxidant enzyme	Gain or loss	Type of biomarker	References
SOD	Loss	Increase oxidative stress	(Marra et al. 2011)
SOD	Gain	Reduces tumor aggressiveness, good response to treatment, and prolonging survival time in patients	(Fu et al. 2011)
CAT	Loss	Increase oxidative stress	(Matos et al. 2009)
CAT	Gain	Positive response to treatment	(Matos et al. 2009)
GPX	Loss	Tumor progression	(Czeczot et al. 2006)

reduced expression of these enzymes is generally associated with an increased risk of cancer development. They are thus good biological indicators of cellular perturbations, in other words biomarkers (Fig. 2 and Table 1).

Superoxide Dismutase

SOD represents the main mechanism for the elimination of superoxide radicals in the mitochondria, through the activity of the xanthine oxidase and NADPH oxidase system, whereby superoxide radicals are transformed into hydrogen peroxide. Cells contain two main superoxide dismutase enzymes: SOD Cu/Mg or SOD1, localized to the cytoplasm, and SOD Mn or SOD2, localized to the mitochondria (Droge 2002). In general, an absence of SOD or reduced SOD activity is a marker that is associated with increased oxidative stress. In contrast, SOD expression reduces tumor aggressiveness, increasing treatment effectiveness and prolonging survival time in patients.

Catalase

The CAT protein participates in the metabolism of hydrogen peroxide, generated by peroxisomes via the activity of SOD or the cytochrome P450 system. Metabolism of hydrogen peroxide by CAT results in the production of water and oxygen. In hepatocellular carcinoma, reduced levels of CAT have been found within tumors or marker of increased levels of reactive species in neoplastic cells. CAT re-expression has been indicated as a possible biomarker for a positive response to treatment (Matos et al. 2009).

Glutathione Peroxidase

The enzyme GPX belongs to a family of enzymes containing eight isoforms that specialize in the catabolism of hydrogen peroxide using the tripeptide glutathione (GSH) as an electron donor (Valko et al. 2007; Reszka 2012). The expression of GPX is heterogeneous in solid tumors. Although the activity of GPX increases

Table 2 Phase II detoxification enzymes used as biomarker

Enzyme	Gain or loss	Type of biomarker	References
GCLC-GCLM	Gain	Biomarker of HCC	(Cheng et al. 2015)
GSR	Gain	Biomarker of tumor tissue	(Kekec et al. 2009)
GGT	Gain	Biomarker of tumorigenesis	(Corti et al. 2010; Zhang and Forman 2012)
ABCC3	Gain	Liver damage, resistance to drug treatment in HCC	(Colak et al. 2010; Zuniga-Garcia et al. 2015)
PRDX1	Gain	Angiogenesis in cancer	(Sun et al. 2014)
TRX1	Gain	Poor prognosis in secondary tumors	(Noike et al. 2008)
HMOX-1	Gain	Resistance to treatment and increased invasiveness in HCC	(Cheng et al. 2015)
NQO1	Gain	Resistance to treatment and poor prognosis in liver tumors	(Petrelli et al. 2014) (Wakai et al. 2011; Buranrat et al. 2012)
PTGR1	Gain	Biomarker of HCC	(Sanchez-Rodriguez et al. 2014)
CBR1	Gain	Progression of HCC	(Tak et al. 2011)
AKR1B10	Gain	Biomarker and progression of HCC	(Heringlake et al. 2010)

globally, specific isoforms may show reduced expression mediated by mechanisms of DNA hypermethylation.

In liver cancer, it has been found that global GPX activity as a biomarker is reduced compared with healthy and cirrhotic tissue (Czeczot et al. 2006). Nevertheless, the expression of GPX2 increases in liver tumors from the initial stages of hepatocarcinogenesis up to the final stages of metastases (Suzuki et al. 2013).

Phase II Detoxification Enzymes in Antioxidant Systems

The phase II detoxification enzymes are considered antioxidant enzymes due to their capacity to metabolize and eliminate reactive species, maintaining the cellular redox equilibrium. The majority of these enzymes participate in complexes converging on thioredoxins and nonprotein molecules such as GSH and NADPH, and they could be used as biomarker (Fig. 2 and Table 2).

Biomarkers of the Intracellular Glutathione System

Glutathione in its reduced form (GSH), with a non-proteic thiol group, is the tripeptide that is most abundant in cells (Franco and Cidlowski 2012). GSH is the main reducing agent and antioxidant involved in the fine control of the cellular redox status and plays a fundamental role in the maintenance of a reduced cellular state.

Changes in the intracellular balance of GSH/GSSG are the best biomarkers that determine the cellular redox status (Droge 2002; Pallardo et al. 2009; Lin et al. 2014); in fact, cytosolic depletion of GSH is the main characteristic of cell death by apoptosis (Circu and Aw 2008).

GSH depletion caused by oxidative stress or via active flow through the cell membrane generates an imbalance between reduced/oxidized glutathione (GSH/GSSG), resulting in the formation of reactive nitrogen species. GSH is able to induce posttranslational modifications in proteins via glutathionylation, a process that is dependent on the reduced/oxidized status of cysteine residues and the redox potential of the protein (Reddy et al. 2008; Franco and Cidlowski 2012). Therefore, the pathways for the synthesis and recycling of GSH play a central role in the antioxidant response. In solid tumors, GSH and dependent pathways are active in the regulation of the redox status, and abundant GSH confers greater resistance to antitumor treatment and reactive species; moreover, it results in greater tumor aggressiveness. Therefore, we next focus on the antioxidant enzymes that participate in the GSH system, reported to be associated with liver cancer (Fig. 4).

Glutamate Cysteine Ligase

The glutamate cysteine ligase (GCL) complex catalyzes the first and rate-limiting step in the synthesis of GSH: the union of the glutamate and cysteine at the gamma position. Glutamate cysteine ligase consists of a heterodimer with a catalytic subunit (GCLC) and a modifier subunit (GCLM) (Mougiakakos et al. 2012).

During the development of HCC, the expression of the GCLC and GCLM subunits increases from the early stages of preneoplastic lesions, and high expression levels are maintained during carcinogenesis in experimental models (Perez-Carreon et al. 2006; Albrethsen et al. 2011). In clinical samples of HCC, the GCLC and GCLM subunits were found to be under- and overexpressed in tumors, respectively, compared with cirrhotic tissue (Cheng et al. 2015) suggesting their use as biomarker for HCC. In contrast, in glioblastomas, inhibition of GCLC-GCLM complexes has been observed and is thought to be induced by posttranslational modifications induced by aldehydes (Backos et al. 2013). Therefore, another aspect that is important for understanding the functionality of enzymes involved in the antioxidant response in liver cancer is their transcriptional and transductional regulation.

Glutathione Synthetase

Glutathione synthetase (GS) catalyzes the second step in the synthesis of GSH, forming the peptide bond between γ-glutamyl-cysteine and glycine, resulting in the tripeptide (Franco and Cidlowski 2012). In experimental models of hepatocellular carcinoma, an increase in GS is observed from preneoplastic stages to the cancer

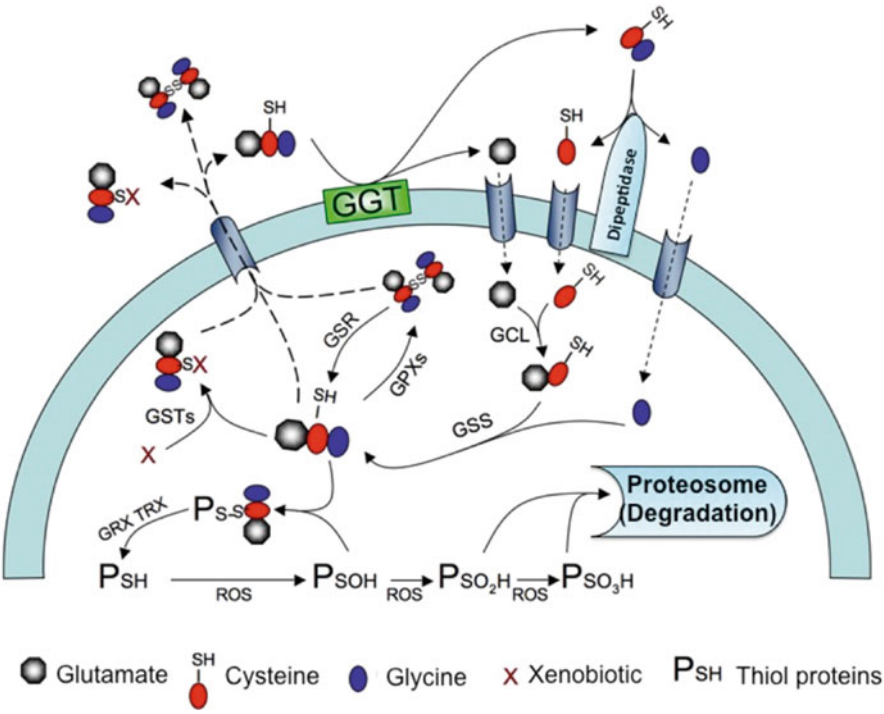

Fig. 4 Glutathione metabolism. GGT catabolizes extracellular glutathione via transfer of the gamma-glutamic group, and dipeptidases hydrolyze the cysteinylglycine. The synthesis of glutathione occurs intracellularly in two steps and catalyzed by the enzymes γ-glutamate cysteine ligase (GCL) and glutathione synthetase (GS). Under conditions of oxidative stress, GSH may be oxidized into GSSG via the activity of glutathione peroxidase (GPX) and reduced via the activity of GSR. One function of GSH is the detoxification of xenobiotics (X) through conjugation reactions, forming GS-X, which is performed by glutathione S-transferase enzymes (GSTs). Glutathione conjugates (GSH and GSSG) may be exported from the cell via glutathione transporters. Reactive oxygen species (ROS) may oxidize protein thiol groups (P-SH) to form proteins with sulfenic (P-SOH), sulfinic (P-SO_2H), and sulfonic (P-SO_3H) acid groups

stage. GS overexpression has also been observed during hepatic regeneration, which is differentiated only by a specific splice variant (Uchida et al. 2010). Therefore, future studies should investigate the types of GS isoforms present in liver tumors to be used as markers.

Glutathione Reductase

Glutathione reductase (GSR) belongs to the GSH recycling system and is involved in catalyzing the reduction of oxidized glutathione (GSSG) to reduced glutathione (GSH) using NADPH as the electron donor. In gastric cancer, hepatocellular

carcinoma, and lung cancer, an increase in the activity of GSR is a biomarker in tumor tissue (Kekec et al. 2009; Sorokina et al. 2010; Pastor et al. 2013).

Gamma-Glutamyl Transferase

GGT is a membrane protein that catalyzes the degradation of extracellular glutathione and conjugates with glutathione between glutamate and cysteine residues. The activity of GGT favors the recovery of free amino acids, which may be used in subsequent intracellular GSH resynthesis (Corti et al. 2010). Work conducted by Hanigan and collaborators several decades ago demonstrated the presence of GGT in various types of solid tumors, such as renal carcinoma, papillary thyroid carcinoma, lung adenocarcinoma, hepatocellular carcinoma, and pancreatic, colon, prostate, ovary, breast, skin, and brain cancer (Hanigan et al. 1994; Kim et al. 2012). In scientific research, GGT is considered a biomarker of tumorigenesis (Corti et al. 2010; Zhang and Forman 2012).

Glutathione S-Transferases

Glutathione S-transferases (GST) are a family of enzymes that catalyze the conjugation of GSH to a variety of endogenous and exogenous molecules. The activity of these enzymes favors the elimination of xenobiotics (X). Glutathione S-transferases may be divided into five classes (α, μ, π, σ, and τ) (Valko et al. 2007). In both clinical and experimental HCC, reduced global activity of GST has been observed within tumors (Czeczot et al. 2006), and the specific isoforms GSTπ1 and GSTα1 (Colak et al. 2010; Albrethsen et al. 2011; Suzuki et al. 2013) have been shown to be overexpressed and have been used as biomarkers in experimental models of HCC.

ABC Transporters

ABC transporters are the best-studied proteins in regard to the development of resistance to antitumor pharmacological treatments and may also be referred to as multidrug resistance proteins (MDRs). This group is encoded by 48 genes, divided among seven families, and is involved in the transport of GSH and GSH conjugates (Chen and Tiwari 2011; Ooi et al. 2011); therefore, these proteins contribute to the regulation of the antioxidant response by eliminating toxic products and controlling the GSH/GSSG balance. As indicated by their name (MDRs), the expressions of these proteins in solid tumors are the biomarkers for resistance to drug treatment and greater rates of recurrence. In liver cancer, overexpression of ABCC9 has been observed. Overexpression of ABCC3 has been detected in both experimental and clinical HCC. ABCC3 has also been found to be overexpressed in

cholangiocarcinoma as well as in cirrhotic tissue and tissue infected with HVB (Colak et al. 2010; Yu et al. 2012; Zuniga-Garcia et al. 2015) suggesting as biomarker for damage to liver tissue.

Thioredoxin Domain Proteins as Biomarkers

This antioxidant response system consists of a group of proteins such as TRX and peroxiredoxins (PRXs) as well as proteins involved in oxidation-reduction recycling dependent on NADPH, as is the case for TXNR. These proteins exhibit in common a conserved cysteine domain that is capable of modulating reactive species and regulating the cellular redox status, and this domain allows these proteins to participate in various cellular processes, such as apoptosis and DNA synthesis. The lethality of TRX knockout in mice (Sengupta and Holmgren 2012; Penney and Roy 2013) demonstrated the importance of this protein for biological systems. Overexpression of one or more TRX proteins in cancer is common, suggesting that this system may play an important role in metabolism within tumor tissue. An important challenge in research is functional analysis of the activities of the various TRX isoforms and elucidating the specific functional differences between these isoforms.

In hepatocellular carcinoma, PRDX1 is overexpressed in tumors and is a biomarker associated with angiogenesis and cancer progression (Sun et al. 2014). In contrast, in secondary tumors resulting from colon cancer metastases, overexpression of TRX1 is common and is a biomarker associated with a poor prognosis (Noike et al. 2008). Overexpression of TRX has been observed in preneoplastic lesions and in both experimental and clinical cholangiocarcinomas (Yoon et al. 2010).

Biomarkers of NADPH System

NADPH is the principal cofactor used as an electron donor in various reactions. NADPH is primarily synthesized via the pentose phosphate pathway, which is the main pathway for the synthesis of ribose required for the production of DNA and RNA. NADPH contributes to cell survival via modulation of the cellular pH and redox status (Furuta et al. 2010). In fact, NADPH is tightly associated with the glutathione and thioredoxin systems (Penney and Roy 2013) in the maintenance of the oxidation-reduction balance. Additionally, NADPH is used as a cofactor by a variety of enzymes that mediate oxidation-reduction reactions as part of the phase II antioxidant response.

Glucose-6-Phosphate Dehydrogenase

The enzyme glucose-6-phosphate dehydrogenase (G6PD) plays a fundamental role in the synthesis of ribose-5-phosphate and NADPH and is essential for the antioxidant response (Furuta et al. 2010). The presence of G6PD in solid

tumors is a biomarker due to its critical role for the synthesis of nucleic acids and maintenance of the cellular redox status. In fact, the complete pentose phosphate pathway is overexpressed in hepatocellular carcinomas compared with non-tumor tissues (Perez-Carreon et al. 2006; Shimizu et al. 2014). Future studies should focus on understanding how G6PD participates with other enzymes such as NADP transhydrogenase, NADP-dependent malate, and NADP-dependent isocitric dehydrogenase in the production of NADPH within hepatic tumors.

Heme Oxygenase-1

Heme oxygenase-1 (HMOX-1) is a microsomal enzyme that is highly inducible by various stimuli, such as UV light, reactive species, and hypoxia (Yin et al. 2012). HMOX-1 enzymes catalyze the rate-limiting reaction in the metabolism of heme groups, giving rise to biliverdin, iron, and carbon monoxide. The enzyme and its resulting metabolites present antioxidant, anti-inflammatory, anti-apoptotic, and immunomodulating functions (Noh et al. 2013). Therefore, HMOX-1 enzymes participate in the process of redox control by maintaining cellular homeostasis. The expression of HMOX-1 in liver cancer is a biomarker associated with increased resistance to treatment and increased invasiveness. Expression of the HMOX-1 enzyme is not limited to tumor tissue, as it is also expressed in adjacent and cirrhotic tissue (Cheng et al. 2015).

NADPH Quinone Oxidoreductase 1

NAD(P)H dehydrogenase [quinone] 1 (NQO1) is a cytosolic enzyme that uses NADPH to directly reduce quinones and hydroquinones (Lin et al. 2014). Additionally, NQO1 participates in the metabolism of superoxide and the maintenance of endogenous vitamins (Yang et al. 2014). Some of the functions of NQO1 enzymes include protection against cytotoxic and carcinogenic quinone insults and protection from oxidative stress. The NQO1 enzyme interacts directly with the NF-kB, NFE2L2, and p53 pathways (Wakai et al. 2011; Jamshidi et al. 2012). Expression of NQO1 in tumors favors resistance to treatment and is considered a biomarker of a poor prognosis in patients.

In patients with cholangiocarcinoma, overexpression of NQO1 has been reported in late and differentiated stages; however, NQO1 expression is weak in tumors in intermediate and early differentiated stages. How the expression of this enzyme affects the prognosis of patients with cholangiocarcinomas remains under study (Wakai et al. 2011; Buranrat et al. 2012). Overexpression of NQO1 has also been described in cases of hepatocellular carcinoma in both experimental models and patients (Petrelli et al. 2014).

Prostaglandin Reductase

The prostaglandin reductase (PTGR) enzymes catalyze NADPH-dependent oxidoreductive reactions. PTGRs are primarily involved in the catabolism of leukotrienes, prostaglandins, aldehydes, and ketones. Additionally, PTGRs catabolize products generated during oxidative stress, as is the case of alpha,beta-unsaturated 4-HNE, produced via lipid peroxidation in cells (Dick et al. 2001). In solid tumors, the expression of PTGR is associated with the modulation of 4-HNE and prevention of 4-HNE-induced cell death.

In experimental models and clinical samples of hepatocellular carcinomas, PTGR1 overexpression is present as an early biomarker (Sanchez-Rodriguez et al. 2014). Moreover, expression of PTGR1 is included in the list of genes that has been associated with progenitor cells for hepatocellular carcinoma (Ho et al. 2012).

Carbonyl Reductase 1

Carbonyl reductase 1 (CBR1) is a monomeric NADPH-dependent cytosolic enzyme that catalyzes the metabolism of compounds carrying a carbonyl group, such as antibiotics, antitumor drugs, and prostaglandins (Murakami et al. 2012). CBR1 is also involved in the metabolism of products of lipid peroxidation reactions, such as 4-oxonon-2-enal, a reactive species that modifies proteins and DNA. The role of CBR1 in carcinogenesis is not currently clear; however, CBR1 is a biomarker overexpressed in hepatocellular carcinomas, and its overexpression appears to correlate with the stage of progression. CBR1 is thought to provide protection against reactive species in cells (Tak et al. 2011).

Aldo-Keto Reductase

The superfamily of aldo-keto reductase enzymes (AKRs) includes 15 genetically related families. These enzymes participate in the reduction of aldehydes and ketones, which are reactions that depend on NADPH as a cofactor (Wang et al. 2010). Additionally, these enzymes contribute to the metabolism of steroids, carbohydrates, and prostaglandins (Endo et al. 2010). AKRs are also thought to play a role in the cellular processes of proliferation and angiogenesis (Chellappa et al. 2012). The AKR1 family is more consistently expressed in neoplastic cells, and these proteins are biomarkers associated with a poor prognosis and greater invasiveness in cases involving solid tumors. In liver cancer, cholangiocarcinomas, and hepatocellular carcinoma, overexpression of AKR1B10 has been confirmed in clinical samples at early and intermediate

stages relative to healthy tissue, while AKR1B10 expression is lost in advanced tumor stages (Heringlake et al. 2010). In experimental models of hepatocellular carcinoma, both AKR7A3 and AKR1B1 have been found to be overexpressed at early stages in preneoplastic tissue in comparison with healthy tissue (Albrethsen et al. 2011).

Metallothionein as Biomarkers

Metallothioneins (MTs) belong to a family of low-molecular-weight proteins that are expressed in response to stress. These proteins exhibit conserved cysteine-rich domains that are able to bind to metals such as zinc, copper, cadmium, mercury, and platinum, thus playing a role in metal homeostasis (Werynska et al. 2013). In addition, MT proteins efficiently bind to reactive species. Various agents, such as hormones, cytotoxic agents, and inflammatory cytokines, induce MT expression. These proteins participate in cellular protection against UV radiation, modulation of reactive species, and protection against chemotherapeutic agents (Gumulec et al. 2014). The expression of MTs is elevated in solid tumors, though the exact levels are isoform specific. Moreover, their expression is a biomarker that tends to unfavorably affect the response to chemotherapy, as these proteins critically influence cell proliferation.

In papillary thyroid carcinoma and breast, oral, colon, kidney, and lung cancers, the MT1 and MT2 isoforms are overexpressed. Reports concerning the expression levels in HCC are inconsistent, but it is known that the MT1H and MT1G subtypes may participate as suppressors of proliferation (Liu et al. 2009; Gumulec et al. 2014). The activation and silencing mechanisms of these proteins in liver cancer are of particular interest; for example, in colorectal cancer, the expression of MT1G, MT1F, MT1H, MT1M, MT1X, and MT2A is lost during the transition from a normal mucosa to a tumor. The main mechanism thought to play a role in reducing expression of these isoforms is silencing via DNA hypermethylation (Arriaga et al. 2012).

Potential Applications to Prognosis, Other Diseases, or Conditions

The various enzymes of the antioxidant response are overexpressed from early stages of liver carcinogenesis and often are indicative of response to chemotherapy, cancer diagnosis, prognosis, and recurrence. Considering the high heterogeneity of tumors, the use of molecular diagnosis such as expression profile of antioxidant enzymes could contribute to better classification of tumors and precise diagnosis. This application could be extended for other cancers such as kidney, lung, ovarian, brain, and colon (Hanigan et al. 1994; Bartsch et al. 2002; Liu et al. 2009; Pastor et al. 2013; Gumulec et al. 2014), where high protein expression of antioxidant response enzymes has been reported also; this profile has been associated with resistance to cell death, increased proliferation, resistance to chemotherapy treatment, and metastasis. The antioxidant proteins as

biomarkers of tumors may allow chemical design for pharmacological treatments, for example, antitumor agents such as mitomycin C and the acylfulvenes, which are activated by enzymatic bioreduction. Thus, molecular diagnosis using antioxidant biomarkers will contribute to a better personalized therapy for cancer patients.

The antioxidant enzymes such as GGT, SOD, and PRX1 were measured with high levels in diseases such as coronary astery disease (Paolicchi et al. 2004; Rivollier et al. 2006; Franzini et al. 2009). Additionally, neurodegenerative diseases such as Huntington, Parkinson, Alzheimer, and amyotrophic lateral sclerosis are closely related with oxidative stress, so the roles of the antioxidant response and the use of related proteins to the redox metabolism have been considered as a therapeutic target, and they are subject of current research (Gan and Johnson 2014).

Conclusion and Future Biomarker Research

The antioxidant enzymes and antioxidant pathways are known to be present and active in tumor cells, and we could use them as biomarkers for diagnosis, recurrence, and prognosis associated to liver carcinogenesis. Furthermore the enzymatic redox capabilities of these kinds of biomarkers could be used as target for chemotherapy of HCC. Most studies show that even though tumors overexpress a variety of antioxidant enzymes, high levels of reactive species are still present; this is more complicated considering the difficulty to measure the exact oxidation-reduction balance in tumor cells, due to their heterogeneity and asynchrony. The high level of GSH and the reduced state of cancer cells, which are conditions that would favor cell proliferation and the cell death evasion, may contribute to tumor burden (Bobko et al. 2012). The biomarker research on antioxidant response may help to understand whether oxidative stress is present or absent in tumor cells or whether tumor cells exhibit a completely new redox equilibrium status (Droge 2002) that may allow a cellular reduced state or tolerance to high levels of reactive species. Considering the tumor microenvironment complexity of the biomarker research, it is also important to explore the differences in the redox potential between neoplastic cells and normal surrounding cells. Moreover, the mechanisms responsible for redox adaptation in tumor cells merit clarification; also several studies are necessary to determine a gene expression profile of antioxidant response genes in liver tumors. This profile of biomarkers of the antioxidant response may contribute to better diagnosis and classification of tumors in liver carcinogenesis.

Summary Points

- This chapter focuses on biomarkers of antioxidant response in the liver in particular a focus on cancer.
- Antioxidant response is the cellular mechanism that counteracts oxidative stress.

- Oxidative stress is a measurable index. But very often the stress response is measurable after the initiation of cellular events.
- Biomarkers, particularly those related to genes or proteins, have the potential to be measurable before cellular events.
- Biomarkers of the antioxidant response include three main protein-metabolite systems: glutathione, thioredoxins, and nicotinamide adenine dinucleotide phosphate.
- Several proteins involved in antioxidant response are overexpressed in liver tumors, and they are associated with diagnosis, prognosis, and response to therapy.
- The antioxidant response may contribute to tumoral development as an adaptive response to maintain a reduced redox balance.
- These include glutathione reductase, glutathione S-transferase P, gamma-glutamyl transferase, glucose-6-phosphate dehydrogenase, thioredoxin reductase, NAD(P)H dehydrogenase [quinone] 1, and prostaglandin reductase 1.

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