[Frontiers in Bioscience S4, 722-748, January 1, 2012]

Sulphur-containing non enzymatic antioxidants: therapeutic tools against cancer

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1. ABSTRACT

The prevention of oxidation is an essential process in all cells, as decreased antioxidant protection may lead to cytotoxicity, mutagenicity and carcinogenicity. The mechanisms by which oxidative stress contributes to carcinogenesis include modulation of gene expression and induction of genetic modifications. Cellular methylation and antioxidant metabolism are linked by the transsulfuration pathway, which converts the methionine cycle intermediate, homocysteine, to cysteine, the limiting reagent in glutathione synthesis. Taurine can protect cells from oxidant-induced injury scavenging strong oxidant and cytotoxic agents, and lipoic acid can regenerate glutathione. N-acetylcysteine has anticancer properties such as counteractions against mutagens and prevention of tumor progression. The oxidizing agents react with the thiol group of these non enzymatic antioxidants determining cellular redox potential, and modulating several biological events, since different redox-sensitive molecules are involved in many cell responses such as proliferation, growth arrest, and death. The high metabolic activity characteristic of cancer cells often upregulates oxidative stress protection mechanisms. In fact, glutathione depletion is an early hallmark observed in apoptosis and it has been demonstrated as a common feature of cancer.

2. INTRODUCTION

Under normal conditions, antioxidant systems of the cell minimize the perturbations caused by reactive oxygen species (ROS). When ROS generation is increased to an extent that overcomes the cellular antioxidants, the result is oxidative stress, which can be defined as the imbalance between cellular oxidant species production and antioxidant capability (1). Antioxidants are substances that delay or prevent the oxidation of cellular oxidizable substrates. The various antioxidants exert their effect by scavenging ROS, or by activating of a battery of detoxifying/defensive proteins (2). ROS thus play different positive roles *in vivo*, being involved in energy production, phagocytosis, cell growth and intercellular signalling regulation. ROS may be also highly damaging, as they can attack biological macromolecules, namely, lipids, proteins and DNA, induce oxidation and cause membrane damage, enzyme inactivation and DNA damage (3). However, when the level of ROS exceeds the antioxidant capacity of the cell, the intracellular redox homeostasis is altered and oxidative stress ensues. Oxidative stress is considered to play a pivotal role in the pathogenesis of aging and several degenerative diseases such as cancer. In order to cope with an excess of free radicals produced upon oxidative stress, humans have developed sophisticated mechanisms to

maintain redox homeostasis (2). These protective mechanisms scavenge or detoxify ROS, block their production, or sequester transition metals that are the source of free radicals, and include enzymatic and nonenzymatic antioxidant defenses produced in the body, namely, endogenous, and others supplied with the diet, namely, exogenous (4).

Several antioxidant enzymes exist that convert ROS into less noxious compounds, for example, superoxide dismutase (SOD), catalase, thioredoxin reductase, peroxiredoxin and glutathione peroxidase (GPX). Collectively, these enzymes provide a first line of defense against superoxide anion ($O_2^{\bullet-}$) and hydrogen peroxide (H_2O_2) (2). They are of enormous importance in limiting ROS-mediated damages to biological macromolecules, but they are not able to be 100% effective because certain compounds generated by the interaction of ROS with macromolecules are highly reactive (4). It is then mandatory to detoxify these secondary products in to prevent further intracellular damage, degradation of cell components and eventual cell death (3). This second line of defense against ROS is provided by enzymes such as glutathione reductase (GR), glutathione *S*-transferase (GST), aldo-keto reductase and aldehyde dehydrogenase. The detoxified metabolites produced by these enzymes are eliminated from the cell by efflux pumps such as the glutathione *S*-conjugate transporter (4).

The third line of protection is carried out by no enzymatic antioxidants. Many biologically important compounds have been reported to have non enzymatic antioxidant functions as vitamin C (ascorbic acid), vitamin E (alpha-tocopherol), vitamin A (retinol), beta-carotene, polyamines, melatonin (5-methoxy-N-acetyltryptamine), nicotinamide adenine dinucleotide phosphate (NADPH), urate, coenzyme Q-10 (ubiquinol), polyphenols, flavonoids, phytoestrogens, terpenes, lipoic acid, glutathione (GSH), cysteine, homocysteine, taurine, methionine, adenosine, S-adenosyl-L-methionine (SAM), nitroxides and selenium (3,5). Among the huge amount of antioxidant explored in recent years, there are some of them showing important effects on cancer (2). In this review we will focus on the antioxidant function of the tripeptide glutathione, the coenzyme alpha-lipoic acid (ALA), and the amino acids cysteine, homocysteine, methionine, and taurine because all have very low toxicological profiles, and they can promote detoxification of intracellular ROS and xenobiotics (6), promising an therapeutical action on cancer through a redox-based strategy.

3. ANTIOXIDANTS AND CANCER

Antioxidants are considered as the most promising chemopreventive agents against various human cancers. However, some antioxidants play paradoxical roles, acting as "double-edged sword." A primary property of effective and acceptable chemopreventive agents should be freedom from toxic effects in population (7). In spite of identification and use of effective cancer chemopreventive agents have become an important issue in public health-related research, miscarriage of the intervention by some antioxidants makes necessary the evaluation of safety before recommending use of antioxidant supplements for chemoprevention (8). Chemopreventive agents include indoles, catechins, pharmaceutical agents, vitamins, organosulfur compounds (glutathione), sesquiterpenes (celastrol), short-chain fatty acids (lipoic acid), isoflavonoids (silymarin), and phenols (resveratrol, curcumin) (8,9). While overwhelming levels and uncontrolled/dysregulated actions of ROS lead to deleterious effects, tighter regulation of those plays an important role in cell signalling (2). As follow, decreased levels of antioxidants can lead to oxidative stress, accelerated aging and cancer (10).

Fruit and vegetable consumption has been inversely associated with the risk of cancer, with the beneficial effects attributed to a variety of protective antioxidants, carotenoids and phytonutrients (11). A consensus exists that a diet rich in fruit and vegetables is beneficial for health in preventing coronary heart disease and some forms of cancer. The nutrients responsible for the protective action are not known, but vitamins, antioxidants and flavonoids are among the likely candidates (9). Dietary supplements are generally used to increase plasma levels of these compounds. Similar results can be achieved also by increasing the proportion of vegetables and fruit in the diet (12).

Sophisticated antioxidant defenses directly and indirectly protect the host against the damaging influence of cytokines and oxidants (9). While antioxidant defenses interact when a component is compromised, the nature and extent of the defenses are influenced by dietary intake of antioxidants, including sulfur amino acids. In animal studies, *in vivo* and *in vitro* responses to inflammatory stimuli are influenced by dietary intake of NAC, cysteine, methionine, and taurine (13). Actually, every thiol-containing molecule has been tested as a sole or combinated supplement against several types of cancer (Table 1).

4. THIOL-CONTAINING COMPOUNDS AS ANTIOXIDANT AGENTS

Sulfur is the sixth most abundant macromineral in breast milk and the third most abundant mineral based on percentage of total body weight (6). The sulfur-containing amino acids are cysteine, cystine, N-acetylcysteine (NAC), homocysteine, homocystine, methionine, and taurine. From now on (together with ALA) we will refer to these sulphur-containing non enzymatic antioxidants as SCNEA. SCNEA supplementation has been indicated for athletes, children, or patients with HIV. Organic sulfur can be used to increase synthesis of SAM, GSH, taurine, and NAC (14). SCNEA may also have clinical applications in the treatment of some conditions such as depression, fibromyalgia, arthritis, interstitial cystitis, athletic injuries, congestive heart failure, diabetes, AIDS, and cancer (6). The low toxicological profiles of these sulfur compounds, combined with promising therapeutic effects, warrant continued human clinical trails (6,14,15).

Table 1. Cancer and human no-toxic dosage of sulphur-containing compounds

Sulfur nutrient	Type of cancer	Key references[1]	Dosage (mg/day)
Cysteine	Breast, colon	14,30	1500-5000 [2]
N-acetylcysteine	Lung, skin, breast, colon	30,39,40	200-1500 [3]
Homocysteine	Lung	60	400-1000 [4]
Glutathione	Melanoma, colon, neuroblastoma, lung, liver, glioma, leukemia	3,73	600-1500 [3]
Methionine	Colorectal, breast, lung, liver, prostate	17,60,104	500-1000 [3]
S-adenosylmethionine	Colorectal, liver, pancreas, colon	6,14,103,113,117,118	400-1200 [3]
Taurine	Colon, lymphoma	121,138	200-4000 [3]
Alpha-lipoic acid	Liver, colon, lung, ovary, stomach, pancreas	145,148,150,155	20-300 [3]

[1]Key references are referred to type of cancer, [2]reference 14, [3]reference 6, [4]reference 15

Thiol-containing compounds bind metals at their -SH groups and have antioxidant features. Therefore, thiol-containing antioxidants may be useful as a component of an effective treatment for metal poisoning (16). Methionine acts a precursor amino acid for glutathione which protects the cells from oxidative damage and plays vital role in detoxification (17). In addition, methionine has been shown to chelate lead and remove it from tissues (16). ALA, is a co-enzyme of pyruvate and the alpha-ketoglutarate dehydrogenase multienzyme complex of the tricarboxylic acid cycle, and has metal chelating, free radical scavenging, and antioxidant-regenerating abilities (18). NAC has antioxidant capacity to lead, including oxidative stress via stimulating GSH synthesis, thereby maintaining intracellular glutathione levels and scavenging ROS (16,19). Experimental studies have shown that SCNEA have beneficial effects against the detrimental properties of metals. For example, oxidative stress induced in rats given lead (2000ppm) with or without methionine (100mg/kg/day), NAC (800mg/kg/day), homocysteine (25mg/kg/day), ALA (50mg/kg/day) show a significant protective action by these sulphur-containing compounds (16).

It has been tested the action of several SCNEA working as free radical scavengers/antioxidants and as protectors from photodamage induced by 5-aminolaevulinic acid-mediated photodynamic therapy, employing the LM2 cell line, derived from a mammary murine adenocarcinoma (20). GSH, NAC, methionine and cysteine are the best protectors for this treatment. GSH protects organism against singlet oxygen. Intracellular thiols play a role in the mechanisms of cancer treatment modalities such as ionising radiation, chemotherapy and hyperthermia (20). Some reports have shown that resistance to chemotherapeutic agents may be due to elevated cellular GSH concentrations (21). GSH may influence DNA repair, as well as help to maintain membrane integrity via the formation of mixed disulphides (Mates 2002). SAM is a precursor of GSH which, unlike GSH itself, can readily across the cell membranes. It has been shown that supplementation of organ preservation solutions with SAM instead of GSH has improved hydroxyl radical ($^{\bullet}OH$) and singlet oxygen scavenging, as well as chelation of iron ions (20). NAC increases GSH levels by providing cysteine and it is known to prevent pathologies elicited by free radicals and ROS (22). Methionine can be converted into SAM, which through a series of transulphuration reactions yields cysteine and from cysteine comes to GSH (20). The aminothiol NAC can scavenge free radicals through binding of the reduced sulphydryl group, either by thiol mechanism or by enhancing GSH metabolism (22). NAC itself can equally act as a singlet oxygen quencher. *In vivo*, NAC forms cysteine, cystine, GSH and mixed sulphides (20).

5. TRANSSULFURATION PATHWAY

Transsulfuration pathway has a limited tissue distribution and is restricted to the liver, kidney, intestine, pancreas and adrenals (14). The transsulfuration pathway is a biochemical mechanism that links methionine metabolism to the biosynthesis of cellular redox-controlling molecules, like cysteine, glutathione, and taurine (Figure 1). Deficiencies within the transsulfuration pathway induce (i) the generation of ROS, (ii) homocysteine accumulation, and (iii) contribute to humans pathologies like tumor development (23). Methionine and cysteine can be used to increase SAM, GSH, taurine, and NAC, and to promote detoxification of xenobiotics via the sulfonation pathway (6). Uptake of cystine by the X_c^- cystine-glutamate exchanger provides the majority of cysteine, but a significant percentage may be derived from methionine, via a transsulfuration pathway (24). Re-feeding methionine to diethylmaleate-treated cells incubated in the absence of cystine or methionine resulted in a significant recovery in glutathione (25).

The transsulfuration pathway converts homocysteine to cysteine and represents the metabolic link between antioxidant and methylation metabolism (14). The first and committing step in this pathway is catalyzed by cystathionine beta-synthase (CBS), which is subject to complex regulation, including allosteric activation by the methyl donor, SAM (26). Methionine restriction can lead to a >10-fold decrease in CBS protein levels. These observations predict that under pathological conditions where SAM levels are diminished, CBS, and therefore glutathione levels, will be reduced. A mechanistic basis for the coordinate changes in redox and methylation metabolism that are a hallmark of several complex diseases is explained by these observations (26). Under pathological conditions with reduced SAM levels, namely human hepatocellular carcinoma, CBS levels are diminished (26). This decrease in CBS levels correlates with reduced glutathione (25). Absence of methionine rather than the presence of homocysteine was responsible for the observed change in CBS levels (23). The transsulfuration pathway is also blocked in some liver tumor cell lines, including HepG2 and HuH-7 cells (26). These cells are unable to form GSH from methionine but can synthesize large amounts of homocysteine (23). Regulation to metabolic changes observed in transformed liver cells in human liver cancer, suggests therapeutic strategies that may be effective

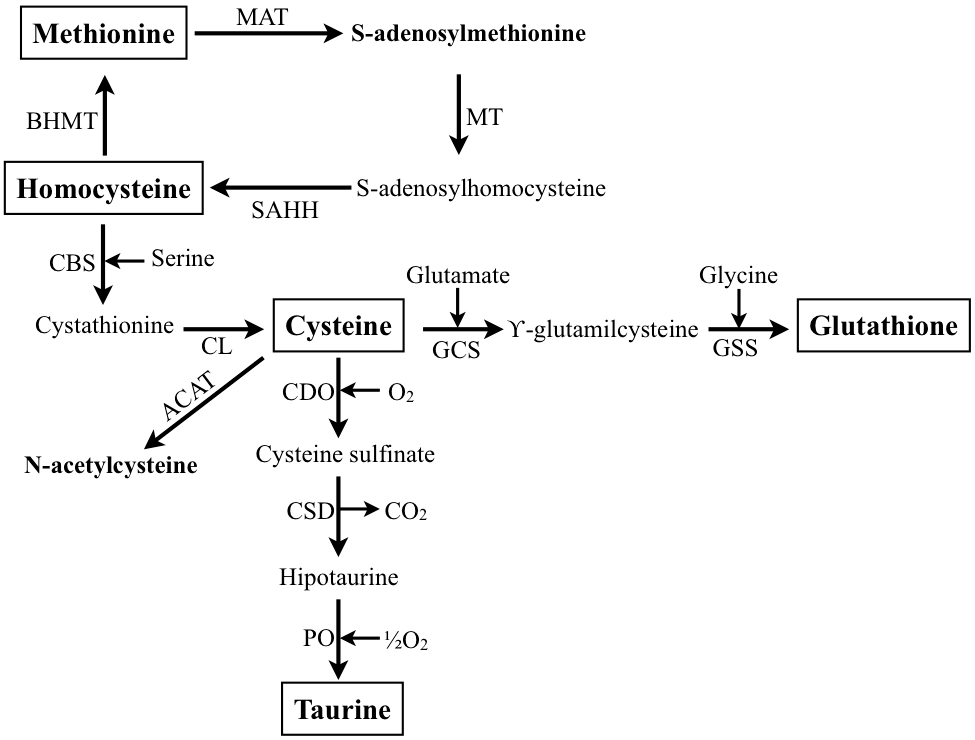

Figure 1. Transsulfuration pathway is illustrated. ACAT: acetyl-coenzyme A acetyltransferase, BHMT: betaine-homocysteine methyltransferase, CBS: cystathione beta synthase, CDO: cysteine dioxygenase, CL: cystathione gamma lyase, CSD: cysteine sulfinate decarboxylase, GCS: gamma glutamylcysteine synthase, GSS: glutathione synthetase, MAT: methionine adenosyltransferase, MT: methyltransferase, PO: peroxidase, SAHH: S-adenosylhomocysteine hydrolase.

in diminishing the enhanced oxidant burden associated with reduced transsulfuration flux (26).

The transsulfuration pathway is necessary for cellular physiology because it connects the methionine metabolic pathways to the generation of cysteine, GSH, and taurine (14). The first step of transsulfuration involves the formation of cystathionine from homocysteine and serine (23). Once formed, cystathionine is cleaved by cystathionine-gamma-lyase (CGL), releasing free cysteine (24). In the presence of high cysteine levels, cystathionine is directed into the GSH and taurine synthesis pathways (14). Thus, cysteine levels are considered the limiting step in liver GSH synthesis, where approximately 50% of the cysteine used for GSH anabolism is derived from methionine that was synthesized from the transsulfuration pathway (26). When homocysteine levels are low, cysteine flux through the transsulfuration pathway becomes down-regulated to conserve homocysteine for the methionine cycle (27).

Homocysteine can be converted into cysteine via cystathionine through the transsulfuration pathway, an irreversible process. Additionally, homocysteine can be methylated back to methionine via the remethylation pathway (14). Alternatively, homocysteine may be remethylated to methionine with either N-5-methyl-tetrahydrofolate or betaine as the methyl donor (15). Remethylation is catalised by the enzyme methylene tetrahydrofolate reductase, which uses folate and vitamin B_{12} as cosubstrate (28). Clearance of homocysteine via the transsulfuration pathway provides an endogenous route for cysteine synthesis and represents a quantitatively significant source of this amino acid needed for glutathione synthesis (29). The combination of transmethylation and remethylation pathways comprises the methionine cycle which occurs in most cells (23). Cysteine is considered a semi-indispensable amino acid whose availability is dependent upon methionine intake (27). Nonetheless, dietary cysteine can satisfy a proportion of the sulfur amino acid requirement, the so-called cysteine-sparing effect on dietary methionine requirement (30).

Cellular GSH homeostasis is maintained through *de novo* synthesis from precursor sulfur amino acids methionine and cysteine, regeneration from its oxidised form glutathione disulfide (GSSG), and uptake of extracellular intact GSH via Na^+-dependent transport systems (31). Cysteine is the rate-limiting amino acid in the intracellular synthesis of glutathione (23). Cysteine is derived from methionine via the transsulfuration pathway through homocysteine (27). Methionine and homocysteine are readily interconvertibles (25). When methionine is in excess, homocysteine is catalyzed into cysteine via cystathionine, which is facilitated by two pyridoxal 5′-phosphate-dependent enzymes, cystathionine beta-synthase

and gamma-cystathionase (14). When methionine is needed, homocysteine is remethylated to methionine using a methyl group provided by methyltetrahydrofolate; this reaction is facilitated by methionine synthase, a vitamin B_{12}-dependent enzyme, or betaine-dependent methylation (26). These interrelationships explain the positive association between plasma concentrations of total cysteine and total homocysteine (30). Maintaining normal GSH concentration is essential to most tissues, especially the intestine, which is constantly challenged by luminal toxins and oxidants derived from the diet as well as endogenous generated ROS. A marked depletion of GSH, induced by L-buthionine-(*S*,*R*)-sulfoximine (BSO), was shown to result in severe degeneration of jejunal and colonic epithelial cells in mice and this intestinal damage seemed to be prevented by concomitant GSH administration (14). Changes in the extracellular cysteine/cystine redox status *per se* have been shown to mediate proliferative signalling that is independent of the intracellular GSH/GSSG in colon cancer cells (31,32).

6. CYSTEINE

Cysteine is considered a semi-indispensable amino acid whose availability is dependent upon methionine intake (21). Cysteine is a constituent amino acid of the tripeptide glutathione, and serves also as a precursor for the synthesis of taurine, pyruvate, sulfate and hydrogen sulfide (H_2S) (15). Together with cystine (CySS), its disulphide form, they constitute an important extracellular thiol-disulphide system. Nevertheless, cysteine and cystine can be transported into cells through distinct membrane transporters (23). Within cells, CySS can participate in disulphide bond formation with intracellular thiols, such as GSH, or be reduced to cysteine (Figure 2). Conversely, intracellular cysteine can be incorporated into proteins (32). Cysteine is usually a nonessential amino acid in the diet of mammals because dietary methionine is a metabolic precursor for cysteine and, if present in an adequate amount, can completely support the cysteine requirement (30). Regardless, the conversion of methionine to cysteine does not occur in most mammalian cells so that cysteine, or its disulfide form, CySS, is needed to support individual cellular requirements (31). CySS is the predominant form in the plasma, and extensive research has characterized cysteine and CySS transport systems and utilization of cysteine and CySS for cell growth and protection against oxidative stress (23). Inhibition of cysteine incorporation into GSH by BSO sensitizes many cell types to anticancer therapy, and supply of NAC and other cysteine precursors is a common approach to enhance antioxidant defenses (31).

Previous studies have shown that cysteine stimulated cell proliferation in various cells (32). However, as cysteine is a precursor of GSH synthesis and the administration of cysteine increases intracellular GSH under physiological conditions, it is still unclear as to whether the stimulatory effects of exogenous cysteine are entirely mediated by intracellular GSH synthesis or mediated, at least in part, by a glutathione-independent mechanism (14). Moreover, because cystine can be readily reduced to cysteine, the mechanism by which exogenous CySS mediates cell proliferation is similarly unresolved. It has been demonstrated that extracellular thiol-disulphide redox status (cysteine-cystine balance in the medium) regulated proliferation in the human colon cancer cell line, CaCo-2 cells. In other study, the administered cysteine was minimally converted to CySS in the medium, showing that exogenous cysteine or CySS alone was as effective in stimulating CaCo-2 cell proliferation as the combination of the two amino acids (32). Hence, it is unlikely that extracellular cysteine-cystine balance was a critical determinant in modulating the stimulatory effect (23). Accordingly, it was not possible to rule out the possibility of a direct effect of cysteine or CySS on extracellular membrane receptors, stating that exogenous cysteine administration stimulated proliferative activity in GSH-deficient CaCo-2 cells in the absence of GSH synthesis. This mitogenic effect of exogenous cysteine could be associated with enhanced expression of cyclin D1, and facilitation of cell transition from G_1-to-S phase (32).

A deficiency of cysteine has been shown down-regulate O^6-methylguanine DNA methyltransferase (MGMT) expression and lead to increased toxicities by alkylating agents (33). L-2-oxothiazolidine-4-carboxylic acid (OTC), is metabolized to cysteine by 5-oxoprolinase, an essential enzyme in the gamma-glutamyl cycle, and NAC, degraded by cellular acylases to cysteine; both these compounds have been demonstrated to be effective and non-toxic cysteine delivery agents. The apparently similar influence of methionine and cysteine can be explained by their precursor-product relationship through the transsulfuration pathway (34). Catabolism of GSH through the reactions in gamma-glutamyl cycle provides a significant pool of cellular cysteine, whose availability is critical for protein synthesis, as well as the resynthesis of glutathione (14). Gamma-glutamyl transpeptidase (GGT), which catalyzes the hydrolysis of glutathione, and glutathione S-conjugates, is the first enzymatic step in GSH catabolism (23). To investigate whether the degradation of GSH and the consequent cysteine production plays a role in MGMT expression, it has been used acivicin, an irreversible inhibitor of GGT (34). Intracellular cysteine content significantly affects the level of *MGMT* gene expression, and supports the notion of using GSH-enhancing drugs for MGMT-targeted chemoprevention. Glutathione depletion down-regulates MGMT activity in HT29 cells and cysteine may up-regulate MGMT (34). Development of dietary or synthetic compounds as potential cancer chemopreventative agents against alkylating chemicals is highly attractive because of their safety, low toxicity and ease of oral intake for prolonged periods (33). Cysteine prodrugs (OTC and NAC) increased MGMT activity in human lymphocytes and cancer cell lines. Administration of these chemicals to animals has been shown to result in significant increases of cysteine and GSH levels in various tissues, including the liver, kidney, lung and brain (34). This thiol enhancement has been shown to prevent hepatic and other toxicities due to acetaminophen, carcinogens, and anticancer drugs (33). Consequently, cysteine may act as a metabolic signal for cellular synthesis of MGMT and this raises the possible

Table 2. Suggested antitumour activity of NAC in combined treatments with other anticancer drugs

Drug	Cancer/tumour cells	Main NAC effect	Key reference
Gingko biloba	Oral cancer HSC-2 cells	Increase apoptosis	41
Withaferin A	Leukemia	Decrease caspase-3	42
Mitoxantrone	Lung, leukemia	Increase G1-phase	43
Etoposide	Lymphoblastoid TK6 cells	Increase G1-phase	44
Paclitaxel	Ovarian HO8910 cells	Increase colony formation	45
(-)-Gossypol	Squamous HNSCC cells	Increase apoptosis	46
Cisplatin	Kidney	Decrease p53	47
Vitamin D3 analogs	Leukemia HL60 cells	Increase differentiation	49
Cytosine arabinoside	Leukemia P388 cells	Increase apoptosis	51
Antimycin A	Lung Calu-6 cells	Increase GSH	98
Thymoquinone	Colon cancer cells	Decrease phosphorilation	157

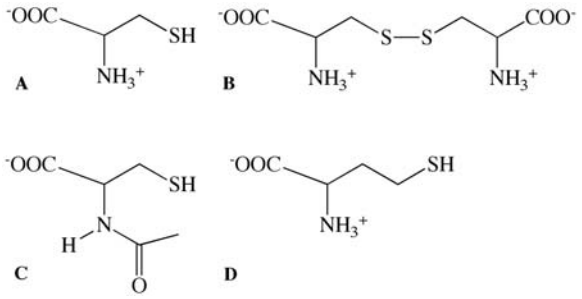

Figure 2. Cysteine (A), cistine (B), N-acetylcysteine (C), homocysteine (D).

dietary regulation of MGMT expression in human tissues. In contrast to GSH augmenting agents which increased cellular MGMT, depletion of GSH reduced the MGMT activity. While GSH may influence protein functions in several ways including the maintenance of protein sulfhydryls in a reduced state, other data suggested that cysteine derived from GSH catabolism may regulate the extent of MGMT synthesis (34).

Higher plasma total cysteine concentrations were significantly associated with a lower risk of breast cancer because cysteine is the precursor of glutathione, a powerful detoxifying agent of carcinogens, which facilitates the elimination of heavy metals, drug metabolites, and DNA-damaging chemicals (35). As a result, cysteine or its precursors might have the potential to be chemopreventive against breast cancer (30). Moreover, cysteine, glutathione, and NAC, can modulate immune responses (30). Cysteine and cysteine derivatives inhibit both the transcription factor nuclear factor-kappaB (NF-kappaB) that is induced under physiological conditions by ROS and the expression of several nuclear factor-κB-dependent genes including interleukin-2 receptor α chain, tumor necrosis factor alpha (TNF-alpha), the major histocompatibility complex, and c-*fos*. Cysteine and cysteine derivatives also stimulate DNA synthesis of several cycling T-cell clones and activate cytotoxic T cells and thus play a regulatory role in T-cell-mediated immune responses (30). Higher plasma total cysteine concentrations were associated with lower risk of breast cancer. An inhibitory effect of cysteine on breast tumor initiation could be in part related to its role as the rate-limiting amino acid in the intracellular synthesis of glutathione (21). Plasma total cysteine levels were found to be inversely associated with risk of cervical dysplasia (30).

6.1. N-acetylcysteine

NAC, a synthetic precursor of cysteine and glutathione, is commonly used as a mucolytic agent and as an antidote against acetaminophen-induced hepatotoxicity (Figure 2). NAC has effectively blocked DNA binding by a variety of carcinogens including benzo(*a*)pyrene, acetylaminoflurorence, and cigarette smoke (21). Exposure of NIH3T3 cells to NAC, increases the intracellular level of GSH and thereby the elimination of ROS via the GPX system (36). NAC inhibits cell proliferation and tumor metastasis, increases DNA repair by protecting related enzymes, and modulates immune responses as well as gene expressions of a variety of tumor suppressor genes and oncogenes. Hence, NAC has been considered to be one of the most promising drugs for cancer chemoprevention. In the dimethylbenzanthracene-induced rat mammary carcinogenesis model, a low dose of NAC modestly decreased the occurrence of mammary cancers (21). In many experimental models, NAC has antigenotoxic and anticarcinogenic properties mediated at different stages of the carcinogenesis process (35). NAC can equally enhance the anchorage-independent growth of cells transformed by activated ABL tyrosine kinases or Ras. This effect was not dependent on loss of focal adhesion kinase activation (37). NAC rescued cell growth that was suppressed by heat shock protein (HSP) 90 inhibitors possibly by chemical modification of their quinone moiety. NAC rendered Rat1/BCR-ABL cells resistance to a Ras inhibitor manumycin. In the absence of HSP90 inhibitors, NAC stimulated the activation of MAP kinase in BCR-ABL-transformed but not in the parental Rat1 cells (37). NAC decreased IKK and IkB-a phosphorylation, and Rel-A/p65 and NF-kappaB binding, though the last two were affected with less intensity compared to the NF-kappaB inhibitor (38).

Dietary supplementation with a nutrient mixture containing NAC has inhibitory potential on the development of mouse lung tumors induced by urethane (39). A similar nutrient mixture exhibited strong potential as a useful anticancer therapeutic tool for skin cancer by significantly inhibiting the incidence and tumor multiplicity of 7,12-dimethylbenz-alpha-anthracene (DMBA)-induced skin tumors both *in vitro* and *in vivo* (40). Additionally, NAC has been used in combined treatments with other anticancer drugs (Table 2). The antiproliferative effects of a Gingko biloba leaf extract to cells from tissues of the human oral cavity were interestingly studied. Toxicity to the HSC-2 cells was lessened by NAC, through induction of apoptosis (41). Induction of apoptosis in cancer cells has

become the major focus of anti-cancer therapeutics development. NAC has shown antitumour activity in combined treatments with other anticancer drugs, including withaferin A (42), mitoxantrone (43) and etoposide (44). Increasing evidence indicates that ROS are also involved in paclitaxel cytotoxicity. The cytoprotective roles of HSP27 include chaperoning cellular proteins, regulating apoptotic signalling and modulating oxidative stress. Reduction of HSP27 expression increased the *in vitro* chemosensitivity of HO8910 ovarian cancer cells to paclitaxel and increased paclitaxel-induced apoptosis and ROS production, although NAC partly offset the effects of HSP27 siRNA (45). Tumor cells with wild-type p53 and high levels of Bcl-xL are cisplatin resistant but are induced to undergo apoptosis by (-)-gossypol, making this a promising agent for overcoming cisplatin resistance. As (-)-gossypol is known to undergo oxidative metabolism *in vivo*, ROS generation may be responsible for both off-target cytotoxicity and inactivation of the drug. In agreement with this hypothesis, oxidation of (-)-gossypol by pre-treatment with H_2O_2 eliminated its activity. Combined treatment with NAC to block ROS increased (-)-gossypol-induced cytotoxicity to tumor but not normal cells. Furthermore, NAC increased the induction of apoptosis, in both cisplatin sensitive and resistant cells. Treatment with antioxidant to block ROS prevents oxidative inactivation of (-)-gossypol and limits off-target toxicity allowing more potent (-)-gossypol-induced anti-tumor activity (46).

NAC also attenuated $^{\bullet}OH$ accumulation, and importantly, diminished p53 activation during cisplatin treatment. This was accompanied by the suppression of PUMA-alpha, a p53-regulated apoptotic gene. Concomitantly, mitochondrial cytochrome c release and apoptosis were ameliorated. Notably, NAC when added as part of a post-cisplatin treatment, was inhibitory to p53 activation and apoptosis (47). Apoptosis was induced with the chemotherapy drugs VP-16 and cisplatin, in B lymphoma cells. NAC inhibited cisplatin-induced cell death and this is attributed to its known ability to react directly with and inactivate cisplatin before it enters the cell (48).

NAC significantly increased the viability of lead-induced apoptotic HepG2 cells in a dose-dependent manner. Interestingly, the addition of NAC to lead nitrate-treated HepG2 cells significantly decreased cellular content of ROS (19). 1alpha,25-dihydroxyvitamin D3 (VD3) and the EB1089 analog are well known for their roles in the modulation of proliferation and the differentiation of several malignant cells. NAC associations with antioxidants induced a slight increase of differentiated HL60 cells. The highest differentiation effect occurred in the case of the EB1089-NAC association. Thus, antioxidant agents demonstrated a protective effect against VD3 and EB1089 oxidative cytotoxicity and an enhancement of the monocyte differentiation (49). Treatment for 13 weeks with NAC of human prostatic cancer cell lines DU145 and PC3 caused inhibition of cellular proliferation, reducing ROS levels (50). In addition, cytosine arabinoside (1-beta-d-arabinofuranosylcytosine; Ara-C) is the most important antimetabolite chemotherapeutic drug used for acute leukemia. Interestingly, NAC, significantly blocked Ara-C-induced cytotoxicity (51).

7. HOMOCYSTEINE

Homocysteine is a sulfur-containing amino acid present in the blood and tissues but not incorporated into protein (Figure 2). Numerous clinical studies have shown that elevated plasma homocysteine levels are strongly associated with increased risk for pathological status (10,11,14). Homocysteine is a very prominent uremic toxin. Homocysteine, formed by demethylation of methionine, displays an antioxidant effect on cellular systems at micromolar levels (18). As stated before, homocysteine can be converted into cysteine via cystathionine through the transsulfuration pathway, an irreversible process. Homocysteine can also be methylated back to methionine via the remethylation pathway. The combination of transmethylation and remethylation pathways comprises the methionine cycle which occurs in most cells (14). Experimental evidence has indicated that homocysteine promote vascular damage through oxidative stress. High homocysteine levels are linked to poor nutrition, in particular, deficient levels of folate, and vitamins B_6 and B_{12} (16,28). Homocysteine elevation may result of inherited disorders of enzyme activity or from enzyme cofactor deficiencies of cobalamin, folate, or pyridoxine with subsequent interruption of homocysteine metabolic pathways (15). Both *in vitro* and *in vivo* work shows that homocysteine can activate cells to proliferate being a potent stimulant of DNA synthesis (52). Oral administration of homocysteine-producing hyperhomocysteinemia leads to increased cellular proliferation rates (53). Oxidative stress, altered glutathione levels, and hyperhomocysteinemia play critical roles in Alzheimer's disease. Hyperhomocysteinemia was correlated with increased reduced/oxidized brain glutathione ratio, with decreased GST activity and increased lipid peroxidation in mice (54).

The level of plasma homocysteine, long known to be B vitamin dependent, has recently been shown to be inversely associated with plasma selenium concentration in human subjects. Although the effect of folate, PLP and vitamin B_{12} concentrations on plasma homocysteine correlated with selenium concentration, supplementation with selenium does not affect total homocysteine concentration. Glutamate-cysteine ligase (GCL) converts cysteine to gamma-glutamyl cysteine (on the pathway to GSH) and may thereby aid removal of homocysteine (25). Men have higher plasma levels of total homocysteine than do women, but the mechanism of this sex-dependent difference is not known. It has been investigated regulation by testosterone of CBS. Testosterone downregulates CBS expression via a posttranscriptional mechanism in the androgen-responsive prostate cancer cell line, LNCaP (29). This diminution in CBS levels is accompanied by a decrease in flux through the transsulfuration pathway and by a lower intracellular GSH concentration (14).

Low millimolar concentrations of homocysteine-induced micronuclei in L5178Y and HL60 cells but did not

enhance the DNA damage. Homocysteine protected cells challenged with H_2O_2 from oxidative stress. This was accompanied by an increased cellular GSH level (27). Homocysteine is a normal product in the metabolism of the essential amino acid methionine (25). While healthy adults have homocysteine levels lower than 12 μM, the levels of end-stage renal disease patients frequently exceed 100 μM. Homocysteine has often been considered a strong predictor of both cardiovascular and non-cardiovascular mortality in the general population and of cardiovascular mortality in end-stage renal disease patients (27).

Additionally, hyperhomocystenemia has been discussed as a new risk factor for cancer and as a potential tumour marker. This is in line with studies revealing a positive correlation between plasma homocysteine and genomic damage (micronuclei) in healthy individuals (55). The DNA of dialysis patients suffering from hyperhomocysteinemia is often hypomethylated, which may be due to the inhibition of methyltransferases by the accumulation of homocysteine or the homocysteine precursor SAM (27). If we consider that methionine-dependent tumors produce large amounts of homocysteine from methionine, it is plausible that homocysteine is one of the signals required to recruit macrophages to the site of tumor. Further studies will be needed to analyze the effect of local hyperhomocysteinuria that has resulted from transsulfuration deficiencies on the development of optimal microenvironments for tumor development (23). It has been also described homocysteine-induced genomic damage as revealed by the micronucleus frequency in L5178Y and HL60 cells (55). Indeed, it has been proposed that some of the adverse effects associated with hyperhomocysteinemia may be due to the generation of ROS (56). Homocysteine treatment did not increase the amount of ROS in HL60 cells. This is in line with observations in HUVEC and LLC-PK1 cells, in which homocysteine failed to elicit an oxidative stress response. Thus, the generation of oxidative stress by homocysteine may be cell type-specific (27). This idea is supported by a publication stating that homocysteine can both inhibit and promote low-density lipoprotein oxidation depending on the experimental conditions (57). However, it was observed a reduction of oxidative stress in cells challenged with H_2O_2 (27). This reduction may be due to the conversion of homocysteine to GSH by the transsulfuration pathway, which would be a fast reaction (23). It can be concluded that homocysteine can exert dual effects *in vitro*: genotoxic on the one hand but antioxidant on the other. Whether the detected antioxidant capacity of homocysteine is relevant for any situation in patients remains to be elucidated (Fink 2007).

Recent findings emphasize a relationship between elevated homocysteine levels and human hepatoma (30), colorectal cancer (58), and prostate cancer (59). Increasing supplemental folic acid can reduce homocysteine concentrations, which may have implications for pathological status (10,12), including lowering cancer risk (60). Serum homocysteine decreased significantly throughout the course of treatment of patients with metastatic colorectal carcinoma treated with the combination of bevacizumab, oxaliplatin, 5-fluorouracil and leucovorin (61). A combination of adenosine and homocysteine potentiates TNF-alpha-mediated cytotoxicity, but does not modulate activation of NF-kappa B transcription factor controlling the expression of various TNF-alpha-inducible genes (62). Adenosine and homocysteine at concentrations that enhance TNF-alpha-induced cytotoxicity accumulate S-adenosyl-L-homocysteine (SAH), a potent inhibitor of SAM-dependent methylation reactions (63). In addition, preloading L929 cells with SAH resulted in enhanced responses to TNF-alpha, suggesting that SAH potentiates TNF-alpha-induced cytotoxicity (62). Moreover, the combination of adenosine and homocysteine changed the dependency of the TNF-alpha-mediated cytolysis on ROS (63). In the absence of adenosine and homocysteine TNF-alpha-mediated cytotoxicity was inhibited by antioxidants. In the presence of adenosine and homocysteine, TNF-alpha-mediated cytotoxicity is not inhibited by these antioxidants (62). A combination of adenosine and homocysteine selectively modulates TNF-alpha-mediated cytotoxicity without changing the TNF-alpha-induced activation of NF-kappa B (63). It has also been shown that adenosine and homocysteine enhance cAMP responses. Adenosine and homocysteine-induced modulation of TNF-alpha cytotoxicity seems to be a general phenomenon and can be observed in other cancer cell lines including HeLa and WEHI cells (62). Besides oxidative stress and vascular damage, homocysteine has a great importance in regulating DNA methylation through SAM, the main methyl donor in eukaryotes (23). Alterations of SAM and methylation were evidenced in Alzheimer disease and in elderly. Homocysteine accumulation induced through vitamin B deprivation could impair the methylation capacity with consequent amyloid-beta upregulation. Moreover, it was found that homocysteine alterations had an effect on neuroblastoma but not on glioblastoma cells in Alzheimer disease (64).

Dehydroascorbic acid (DHA) has the remarkable ability to eliminate the aggressive mouse tumours, L1210, P388, Krebs sarcoma, and Ehrlich carcinoma (65). Cancer cells (but not normal cells) contain measurable quantities of homocysteine thiolactone (HTL). Dehydroascorbic acid reacts with homocysteine thiolactone converting it to the toxic compound, 3-mercaptopropionaldehyde (66). Rapidly-dividing tumour cells make unusually large amounts of HTL and that administered dehydroascorbic acid enters the cells and converts the thiolactone to mercaptopropionaldehyde which kills the cancer cells. HTL synthesis is mammalian cells was first demonstrated in three malignant cell lines, human cervical carcinoma (HeLa), mouse renal adenocarcinoma (RAG), and Chinese hamster ovary carcinoma (CHO) (65). Homocysteine itself does not occur in proteins and there is no enzyme for activating it. Nonetheless, homocysteine is erroneously activated by methionyl-tRNA synthetase in mammals. This is recognized as an error and the homocysteinyl-AMP is edited-out whereupon it converts to homocysteine thiolactone (66). The presence of HTL in cancer cells can be a finding of major interest which has not yet been fully developed. It may be related to two conditions in rapidly-dividing cells: (i) the high demand for methyl groups which

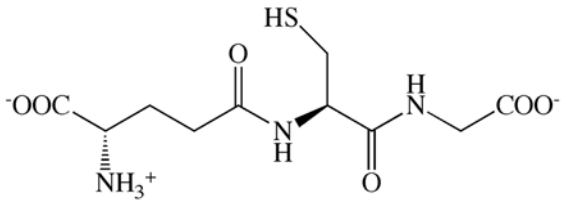

Figure 3. Glutathione.

leads to homocysteine formation, (ii) the high rate of protein synthesis which increases the risk of homocysteine misactivation. Injected DHA would enter the tumour cells where it would convert the endogenous HTL to 3-mercaptopropionaldehyde (65). The latter compound combines the toxicities of aldehydes and mercaptans and would be expected to kill the tumour cells. The indicated mechanism has obvious advantages for cancer therapy; the toxic agent is generated inside the cancer cells and appears to be selective for cancer cells (66). The therapeutic potential of this mechanism might be further increased by giving methionine or a folic acid antagonist (methotrexate) along with DHA to increase HTL formation in cancer cells (65).

Modulation of gene expression by oxidative damage affects carcinogenesis by altering the epigenetic effects and chromosomal rearrangements (23). Epigenetic effects on gene expression stimulate growth signals and proliferation, while chromosomal rearrangements contribute to neoplasic progression (67). In addition, a redox imbalance is shown to stimulate protein kinase and poly(ADP ribosylation) pathways, affecting signal transduction and promoting tumor development (23). Interestingly, studies examining the effects of high homocysteine levels show that poly(ADP-ribose) polymerase (PARP) enzymes repair single strand DNA breaks induced by genotoxic agents or oxidative stress (67). In all related cases, the initiation potential of oxidants seems to contribute to carcinogenesis by inducing DNA base changes in certain oncogenes and tumor suppressor genes (23). It is shown that the $^{\bullet}OH$ is able to activate certain oncogenes, such as K-ras and C-Raf-1 (68). Point mutations in CpG dinucleotides are frequently found in certain tumor suppressor genes, such as p53 and ras, leading to inactivation (23). Furthermore, $^{\bullet}OH$ attacks cells containing mutant or absent p53, resulting in failure to arrest in G1, and reducing cell capacity to repair damaged DNA (67). This increase in replication errors can initiate additional oncogene activation and tumor suppressor gene inactivation, ultimately contributing to malignancy (23).

8. GLUTATHIONE

Glutathione, (2*S*)-2-amino-5-[[(2*R*)-1-(carboxymethylamino)-1-oxo-3-sulfanylpropan-2-yl]amino]-5-oxopentanoic acid, is a very important tripeptide present in significant concentrations in all tissues (69). Glutathione is not an essential nutrient since it can be synthesized from the amino acids L-cysteine, L-glutamic acid and glycine, forming L-gamma-glutamyl-L-cysteinylglycine (Figure 3). Its reduced form (GSH) and oxidized form (GSSG), which can be inter-converted by the enzymes GPX and GR, constitute the major intracellular thiol-disulphide redox system in mammalian cells (14). Glutathione serves several vital functions, including scavenging peroxides, modulating critical cellular processes (cell proliferation, apoptosis, microtubular-related processes, and immunological function), maintaining the essential thiol status of proteins by preventing oxidation of -SH groups or by reducing disulphide bonds induced by oxidative stress, detoxifying electrophiles, and providing a reservoir for cysteine (32).

Glutathione is synthesized in two sequential ATP-dependent reactions catalyzed by gamma-glutamylcysteine synthetase (GCS), the rate-limiting enzyme, and glutathione synthetase (GSS) (23). Other factors in the regulation of the *de novo* GSH synthesis are the availability of cysteine and the concentration of GSH itself that inhibits, by a feedback mechanism, GCS activity (26). In the presence of oxidative stress, GSH concentration rapidly decreases while GSSG, potentially highly cytotoxic, increases because of the reduction of peroxides or as a result of free radical scavenging (4). This has two important consequences: First, the thiol redox status of the cell will shift and activate certain oxidant response transcriptional elements, and second, GSSG may be preferentially secreted from the cell and degraded extracellularly, increasing the cellular requirement for *de novo* GSH synthesis (4). Glutathione disulfide can also be reduced back to GSH by the action of GR utilizing NADPH as a reductant (3). The high intracellular concentration of glutathione (usually 5-10 mM) together with GPX and GR makes the molecule an effective redox buffer to absorb excessive free radicals (70). In mammalian cells, the cycling between GSH and GSSG serves to remove ROS such as H_2O_2 produced due to either cellular respiration or metabolism of toxic substances, and protect cells from oxidative injuries (31). Besides the two-step enzymatic pathway catalyzed by GCS and GSS, cells can synthesize glutathione from a reduction of GSSG to GSH by NADPH. Elevated tissue GSH has been shown to prevent cancer formation (1). A method of assessing cellular redox state was proposed by Jones (71) basing on the Nerst equation: $Eh = -252 + 30 \log ([GSS]/[GSH]^2)$. The increase in GSH level in response to xenobiotics has been reported to be associated with an elevation in the activity of GCS (70). It was suggested that GSH can feedback inhibit GCS activity and a transient decrease in GSH can release the inhibition on its activity. While a high GSH content may be important for carrying out detoxification, the elevated GSH level could cause a disturbance in the balance in cellular functions (72). One of the problems associated with a high GSH/GSSG is the suppression of cell growth and proliferation (70).

The central role of reduced GSH appears clear in intracellular endogenous antioxidant defenses as it is involved in all the lines of protection against ROS (72). Under normal cellular redox conditions, the major portion of this regulator is in its reduced form and is distributed in nucleus, endoplasmic reticulum and mitochondria (73). Additionally, GSH may be covalently bound to proteins through a process called glutathionylation and acts as a

coenzyme of numerous enzymes involved in cell defense (2). Glutathione can directly scavenge free radicals or act as a substrate for GPX and GST during the detoxification of H_2O_2, lipid hydroperoxides and electrophilic compounds (3). Glutathione peroxidases constitute a family of enzymes, which are capable of reducing a variety of organic and inorganic hydroperoxides to the corresponding hydroxy compounds, utilizing GSH and/or other reducing equivalents (4). There are several tissue-specific GPX's that exhibit tissue-specific functions. All of them are selenoproteins and their primary function is to counteract oxidative attack (3). During the catalytic cycle, selenium is oxidized by the hydroperoxides to a selenic acid derivative. This intermediate is subsequently reduced by the electron donor (69). When GSH is used, a seleno-disulfide is formed, which is cleaved by a second GSH molecule to yield the reduced GPX. During catalysis the oxidation state of the enzyme depends on the relative concentration of the reducing (GSH) and oxidized (hydroperoxides) substrates. Glutathione peroxidases are ubiquitously distributed (4). GSTs are three enzyme families (cytosolic, mitochondrial and microsomal) that detoxify noxious electrophilic xenobiotics, such as chemical carcinogens, environmental pollutants and antitumor agents (69). GSTs exert those protective effects because they are able to catalyze the conjugation of GSH with oxidation end products and represent a second line of defense against the highly toxic spectrum of substances produced by ROS-mediated reaction (4). Both GPX and GST activities can eventually lower the level of total intracellular GSH (2). GR is a flavoenzyme and is represented by a single-copy gene in humans. It has been observed that exposure to agents that lead to increased oxidative stress also leads to an increase in its mRNA content (4). The recycling pathway for GSH formation is fundamental in the metabolism of GSH-dependent defense reactions (2). In conclusion, the presence of GSH is essential, but not in itself sufficient, to prevent the cytotoxicity of ROS, being of fundamental importance the functionality of the glutathione-dependent enzymes, which participate in the first and second lines of defense (4).

Many foreign chemicals get attached to GSH, which is really acting as a detoxifying agent (72). In fact, alteration of GSH metabolism is one of the most essential antioxidative defense mechanisms against oxidative damage and cancer (69). In the reduced state, the thiol group of cysteine is able to donate a reducing equivalent ($H^+ + e^-$) to other unstable molecules, such as ROS (2). In donating an electron, glutathione itself becomes reactive, but readily reacts with another reactive glutathione to form GSSG. Such a reaction is possible due to the relatively high concentration of glutathione in cells (up to 5 mM in the liver). In healthy cells and tissue, more than 90% of the total glutathione pool is in the reduced form and less than 10% exists in the disulfide form. An increased GSSG/GSH ratio is considered indicative of oxidative stress. For example, breast tumor and hepatoma cell lines show altered GSH production (73). GSH redox status is reshaped in blood of Ehrlich ascites-bearing mice (1). This effect is mainly due to an increase in oxidized GSH levels. Two reasons may explain the increase in blood oxidized GSH: (i) the increase in peroxide production, in addition to changes affecting the GSH-related antioxidant enzyme activities, leading to GSH oxidation within the red blood cells; and (ii) an increase of oxidized GSH release from different tissues into the blood. Nevertheless, GSH and peroxide levels are higher in the cells when they proliferate actively, like cancer cells (3). Aromatic amines, such as benzidine, initiate bladder cancer in humans. Glutathione inhibited transformation of benzidine by reactive nitrogen oxygen species (RNOS) like NO^+ and $ONOO^-$. Inhibition is at least in part due to conjugate formation because GSH is known to react with RNOS forming *S*-nitrosoglutathione (74).

Cancer cells have a high demand for cysteine to provide GSH and drive cell growth and proliferation. C6 glioma cell line is a model system for astrocytoma/glioblastoma cells in which to study synthesis of glutathione. Incubation of cells with a sub-toxic concentration of glutamate analogues (gliotoxin) leads to an increase in the contribution of the transsulfuration pathway to GSH synthesis because glutathione depletion causes a JNK- and p38 mitogen activated protein kinase (MAPK)-mediated increase in expression of CGL (24). Notably, in the presence of both methionine and cystine, the concentration of GSH exceeded the resting level (14). Transsulfuration provides a compensatory mechanism that is upregulated in response to depletion of GSH or oxidative stress in C6 cells. These results validate the use of gliotoxins as a tool for studying the contribution of the transsulfuration pathway to GSH synthesis in response to depletion of intracellular cysteine (24). JNK phosphorylation takes place in the final step of the stress activated protein kinase (SAPK) pathway that occurs in many cells as a prelude to apoptosis (72). It has been proposed that a depletion in GSH triggers activation of this pathway, therefore stimulating the transsulfuration pathway and channelling methionine towards production of cysteine (24). It is significant that GSH depletion caused by mild oxidative stress in hepatocytes led to a sustained increase in JNK activity, hence sensitizing the cells to TNF-induced apoptosis (75). Consequently, blockade of the exchanger is viewed as a potential target for cancer therapy (24). However, other recent evidence indicates that methionine uptake in gliomas is high and correlates positively with tumour viability thus questioning the potential effectiveness of blockade of the X_c^- exchanger as a means of limiting GSH synthesis and tumour cell survival (23). Alternatively, transsulfuration pathway functions as a pathway to ensure continued supply of cysteine for GSH synthesis during oxidative stress or when cystine import may be required to drive a cystine/cysteine redox cycle in C6 glioma cells (24).

Protein kinases C (PKCs) are a family of isoenzymes sensitive to oxidative modifications and involved in the transduction signal pathways that regulate cell growth (72). PKC isoforms are specifically influenced by the amount of intracellular GSH (76). The greatest GSH depletion is associated with a maximal ROS reduction and accompanied by an increase in the activity of the delta isoform (77). ROS generation induced early morphological changes in GSH-depleted neuroblastoma cells

characterized, at the intracellular level, by the modulation of PKC-delta activity that was involved in the pathway leading to apoptosis (76). These results define a novel role of PKC-delta in the cell signaling pathway triggered by GSH loss normally employed in the treatment of neuroblastoma (77). GSH/GSSG ratio can be regulated by glutathione-S-thiolation with protein cysteine residues that may be irreversibly oxidized, and it may constitute both a first line of defense against oxidative stress and a system through which a redox change can be transformed into a cellular signaling event (72). Depletion of intracellular GSH has been reported to occur with the onset of apoptosis in numerous studies (73) and is quite often accompanied by an increase in ROS (3). GSH depletion modulates the activity of specific PKC isoforms. GSH depletion induces translocation to the mitochondria of PKC-delta (76). The GSSG/GSH ratio is important in balancing the effects of alkylating agents and ROS frequently produced at membrane surfaces in response to growth factors and cytokine stimulation (77). Once generated, ROS can alter signaling pathways governing cell growth by specifically altering the oxidation of protein cysteine residues that may be reduced by GSH present at millimolar concentrations inside cells (76). Direct oxidative injury and GSH depletion are separate but related processes influencing the protein oxidation state (73). Indeed, a decrease in cellular glutathione levels consequent to the attack of free radicals and alkylating agents enhances oxidant-induced effects such as changes in gene expression (34). Understanding the role of PKC-delta in the induction of early morphological changes induced by diminished GSH levels accompanied by the formation of oxyradicals might be useful in the commitment of neuroblastoma cells to apoptosis and in the clinical approach to brain cancers and their complications (76).

There is considerable evidence both in animal models and in human studies that alcohol-induces hepatic injury involves GSH depletion and consequent oxidative stress (72). Ethanol ingestion decreased glutathione levels in the plasma, lung tissue, and lung lavage fluid, and increased oxidized glutathione levels in the lung lavage fluid (78). Furthermore, ethanol ingestion decreased cell glutathione content by 95%, and decreased cell viability, *in vitro*. Finally, treatment with the glutathione precursors SAM and NAC during ethanol ingestion significantly reduced lung damage (79). Polycyclic aromatic hydrocarbons increases GSH content as a defense mechanism against oxidative stress as well as to promote its detoxification in the human hepatoma cells HepG2. The increase in intracellular GSH level was due to activation of GCS activities (70).

Many cancer therapeutic drugs are apoptosis inducers as they can disrupt the redox balance by depleting the intracellular thiol buffer system through extrusion or redistribution of GSH (73). Redox regulation is an important component of malignant cell survival. Tipping the cellular redox balance through pharmacologic regulation in favor of increasing intracellular ROS and/or depleting protective reducing metabolites (such as glutathione) may lead to oxidative stress and resultant induction of apoptosis for the treatment of cancer (80). Deplete intracellular GSH leads to a depletion of protein thiols as well as enhancement of ROS which eventually leads to apoptotic cell death (81). Lower blood levels of glutathione were also observed among patients with cervical dysplasia or invasive cancer (78).

8.1. Glutathione in the glutamine/glutamate network

Eventually, glutamine via glutamate, is one of the precursors for the synthesis of GSH, the major endogenous antioxidant in mammalian cells, which protects them from oxidative injury and cell death (3). Cancer cells have higher GSH levels than the surrounding normal cells, which attributes to a higher rate of cell proliferation and resistance to chemotherapy (1). Therefore, selective tumour depletion of GSH presents a promising strategy in cancer treatment. Experimental studies have associated decreased GSH levels with inhibition of proliferation and stimulation of apoptosis (82). Glutamine metabolism is another target for alteration in cancer development. Both glutamine uptake and the rate of glutaminolysis (i.e., catabolism of glutamine to generate ATP and lactate in the mitochondria) are known to increase in tumors (83). In glutamine metabolism, mitochondrial glutaminase (GLS) is central in the conversion of glutamine to glutamate (3). Glutamate participates in regulation of mitochondrial bioenergetics in many normal and cancer cells via the tricarboxylic acid cycle for ATP production as well as antioxidant defense through GSH synthesis (83). The human glutaminase 2 gene (GLS2) encodes a mitochondrial glutaminase that catalyzes the hydrolysis of glutamine to glutamate (83). P53-inducible GLS2 regulates intracellular glutamine metabolism and ROS levels and promotes antioxidant defense through controlling the GSH/GSSG ratio, although it exists the additional possibility that regeneration of GSH from GSSG is increased by GLS2 expression (84). Other results show how modified Ehrlich ascites tumour cells, expressing antisense mRNA for GLS2, contain lower levels of GSH than normal ascites cells. In this context, these cells contain a higher number of apoptotic cells and are more sensitive to both methotrexate and H_2O_2 toxicity than normal cells. Taken together, these results provide insights into the role of GSH in cancer development by demonstrating that GSH levels can alter apoptosis and tumorigenic capacity (1).

GLS2 is a unique p53 target gene to mediate the role of p53 in both cellular energy metabolism and antioxidant defense mechanisms (85). P53 increases GLS2 expression under both nonstressed and stressed conditions, which results in enhanced mitochondrial respiration and ATP generation and, furthermore, increased glutathione levels and decreased ROS levels in cells, which in turn protects cells from oxidative stress-induced apoptosis (85). Furthermore, GLS2 expression is lost or greatly decreased in hepatocellular carcinomas and the overexpression of GLS2 greatly reduced tumor cell colony formation (84). These results demonstrated that as a unique p53 target gene, GLS2 is a mediator of p53's role in energy metabolism and antioxidant defense, which can contribute to its role in tumor suppression (85). Glutamine must be converted to glutamate by glutaminase before entering the TCA cycle: cancer cells that express the Myc oncogene overexpress glutaminase and are killed by glutamine

withdrawal (83). However first-generation glutamine antagonists such as 6-diazo-5-oxo-L-norleucine and acivicin are neurotoxic, safe glutaminase inhibitors are under development (86).

Malignant glioma cells kill surrounding neurons in the brain specifically by secreting the amino acid glutamate, an obligatory waste product of glutathione synthesis (87). Glutamate release is a plausible mechanism that may account for the pathologic changes in bone metastasis, since bone, like brain, is also highly sensitive to glutamatergic disruption (88). Glutamate is released from cancer cells as a side effect of cellular oxidative stress protection, and that this process may be common to a variety of cancers (83). Many cancer cells must sustain significantly upregulated antioxidant defence mechanisms to survive, progress, and potentially metastasize (72). To generate sufficient GSH, an abundant source of intracellular cysteine is required (14). Since cysteine is rapidly oxidized to cystine in the extracellular environment, an amino acid transport system is necessary to move the oxidized form into the cell for a readily available cellular waste metabolite, glutamate (88). Although the released glutamate can act as an autocrine/paracrine signal to encourage cell invasion, the functional consequences to the host organ are for far greater significance for glioma patients (88). As neurons do not have the enzymatic capacity to effectively terminate the glutamate signal, this excess glutamate will cause hyperexcitation, eventually leading to neuronal death (88). While glutamate release has been suggested as providing an adaptive advantage for glioma cells, the host pathology may simply be a side effect of the interaction between the cancer´s requirements for high levels of GSH biosynthesis and the brain´s inherent sensitivity to glutamate (88).

8.2. Glutathione and drug resistance of cancer cells

GSH content of cancer cells is particularly relevant in regulating mutagenic mechanisms, DNA synthesis, growth, and multidrug and radiation resistance (73). In malignant tumors, as compared with normal tissues, that resistance associates in most cases with higher GSH levels within these cancer cells (21). Hence, approaches to cancer treatment based on modulation of GSH should control possible growth-associated changes in GSH content and synthesis in these cells. Recent works show that a high percentage of metastatic cells with high GSH levels survive the combined nitrosative and oxidative stresses. GGT overexpression and an inter-organ flow of GSH, by increasing cysteine availability for tumor GSH synthesis, function in combination as a metastatic-growth promoting mechanism. Experimental evidence shows that acceleration of GSH efflux facilitates selective GSH depletion in metastatic cells, and sensitization of metastatic cells to therapy (73). Efflux of GSH from melanoma cells may facilitate metastatic cell resistance against endothelium-induced oxidative/nitrosative stress. GSH efflux was abolished in melanoma cells incubated with methionine (likely related to a methionine-sensitive GSH carrier previously detected in hepatocytes). GSH depletion in metastatic cells can be achieved by using Bcl-2 antisense oligodeoxynucleotide, in combination with inhibition of GGT, which limits GSH synthesis by preventing cysteine generation from extracellular GSH (21).

Drug-resistant cells have the inherent ability to maintain physiological GSH, and cellular redox state and maintain increased cellular resistance to DNA damage (72). GSH loss was followed by a rapid increase in ROS levels (89). The resistant, but not drug-sensitive cells normalized the intracellular GSH concentration along with ROS levels and survived drug treatment (1). GSH levels are elevated in many types of tumor cells that show increased resistance to chemotherapeutic agents (89). GSH levels vary over a range of 0.5-10 mM, depending on the cell type, and play a role in the detoxification of a variety of electrophilic compounds and peroxides via catalysis by GST and GPX. GSH levels are rapidly depleted during apoptosis, and this is considered a biochemical hallmark of oxidant-induced, programmed cell death (31). Maintaining intracellular GSH levels by either GSH precursors (such as NAC) or inhibitors of GSH efflux can repress or delay apoptosis. Drug-resistant cancer cells, whether derived directly from patients or maintained in culture, have high GSH levels and show increased GCS activity (89). GCS, along with GSS is involved in the *de novo* synthesis of GSH (14). Low molecular weight, non-enzymatic antioxidants (e.g., SCNEA), protect cells from ROS-mediated damage or pathological signaling (9).

H_2O_2 and other peroxides are mostly eliminated by GPX, which utilizes GSH as an electron donor in the reduction reaction, and produces GSSG as an end product (3). GSH was extruded equally from the sensitive and resistant cells via methionine- or cysteine-inhibitable GSH transporters after drug treatment (89). This GSH loss caused a subsequent increase in ROS levels in many cell types (1). Restoration of GSH levels in drug-resistant cells consistent with the high GSH levels in tumor cells possessing increased resistance to platinum-containing compounds, alkylating agents, anthracyclines, and arsenic trioxide (89). GSH hinders the arsenic trioxide apoptosis induction through ROS-dependent survivin downregulation in human lung cancer cells. Survivin is a member of the inhibitor of apoptosis protein family. It is modulated by ROS in several types of neoplasms including leukemias (90). Drug-resistant cells possess high GCS activity. GSH extrusion was significantly inhibited by methionine or cysteine, suggesting that GSH loss occurs via a specific transporter (89). The mitochondrial GSH level is dependent on the cytosolic GSH concentration, and so it is possible that a decrease in the GSH concentration impairs the GSH-dependent ROS detoxification system (primarily GPX and GR) in mitochondria, leading to an excess of ROS that is released into the cytoplasm (3). Resistance to commonly used alkylating or platinum chemotherapeutic agents is multifactorial, involving altered drug transport along with enhanced drug biodegradation, detoxification, and DNA repair (89). Increased GSH levels due to an enhanced gamma-GCS activity were reported in several drug-resistant tumor cell lines, and in tumor cell cultures isolated from patients whose tumors were clinically resistant to drug therapy (89).

Assuming that cysteine levels are maintained by the breakdown of GSH, there is a shift in the redox balance following the induction of oxidative stress, such that the ratio of GSH to GSSG is altered in favor of GSSG (14). GSH acts as a reducing agent, is involved in the metabolism of xenobiotics, is a free-radical scavenger, aids in cell-cycle regulation and microtubular-related mechanisms, regulates Ca^{2+} homeostasis, regulates protein function and gene expression via thiol-disulfide exchange reactions, and participates in the mitochondrial mechanisms that link the opening of the permeability transition pore complex to the activation of cell death (23). Different types of tumor, including melanoma, bladder carcinoma, lung cancer, colon cancer, and breast tumors that are multidrug and radiation resistant, are also found to have a high GSH content (89). Considering the homeostatic redox buffer function of GSH and its role in inactivating some carcinogens and protecting cells against DNA-damaging free radicals and lipid peroxidation, it is plausible that tumor cells may need more GSH for their survival than other cell types (23). GST is shown to be associated with multidrug resistance of tumor cells (31). High GSH concentrations in cancer cells, induction of GSH in murine melanocytes following c-H-ras oncogene-induced transformation, or impairment of metastatic spread by GSH depletion have been described, further highlighting the importance of GSH pathways in tumor development and growth (23). Under metastatic conditions, high levels of GSH can support a rapid cell cycle, an elevated rate of DNA synthesis, and a block in apoptosis (3). Therefore, GSH can be characterized as a double edged sword, protecting non-tumor cells against oxidative stress induced by metabolism or exogenous compounds and at the same time, protecting tumor cells from apoptosis and chemotherapeutic treatments, thus furthering tumor development and metastasis (23).

Free radical-induced cytotoxicity may initiate carcinogenesis by depleting the normal cell population and promoting the clonal expansion of more resistant cells (23). Glutathione depletion is widely used to sensitize cells to anticancer treatment inducing the progression of programmed cell death and overcoming chemoresistance (1). It has been reported that neuroblastoma cells with MYCN amplification are unable to start TRAIL-dependent death and MYCN, in concert with cytotoxic drugs, efficiently induces the mitochondrial pathway of apoptosis through oxidative mechanisms (77). These results describe a novel pathway of apoptosis dependent on ROS formation and PKC-delta activation and independent of p53, Bcl-2, and Bax levels (76). GSH loss induced by BSO leads to overproduction of ROS and triggers apoptosis of MYCN-amplified neuroblastoma cells (77). BSO is a GSH-depleting agent that inhibits GCS, which is often up-regulated in chemotherapy-resistant tumors (81). GSH depletion is responsible for DNA damage and apoptosis in MYCN-amplified NB cells in consequence of ROS overproduction and activation of PKC-delta (77).

Retinoid N-(4-hydroxyphenyl) retinamide (4-HPR) induces the overproduction of ROS in human leukemia cells (91). 4-HPR generated large quantities of ROS in cell lines which expressed low glutathione levels, these cells being the most sensitive to the retinoid (92). The sensitivity of leukemia cells to 4-HPR could be modulated, either by increasing intracellular glutathione contents using all-trans retinoic acid, or by decreasing it using DL-BSO (91). All-trans-retinoic acid increased the level of expression of tissue transglutaminase, whereas inhibition of this enzyme led to enhanced apoptosis (93). Our findings indicate that the glutathione content contributes to determining the sensitivity of cells to 4-HPR and points to the potential application of glutathione-inhibiting agents as enhancers in 4-HPR-based therapies (91). The cytotoxicity of 4-HPR was analyzed in four other leukemia/lymphoma lines (CCRF-HSB2, Molt-4, KG-1, HL-60). Cytotoxicity of 4-HPR correlated with the amount of ROS produced in many cell lines (92). The intracellular glutathione level varied among all the assayed cell lines, the highest levels occurring in Molt-4 and KG-1, which were less sensitive to 4-HPR. Suppression of glutathione by BSO enhanced the level of 4-HPR-induced ROS production and apoptosis in Molt-4 (91).

Glutathione pathway was also altered in DND-1A, HeLa (94) and HSC-2 cancer cell lines (41). Recovery of GSH depletion and the reduction of $O_2^{\bullet-}$ levels in Calu-6 lung cancer cells were accompanied by the inhibition of apoptosis (95). Data provide strong support for the hypothesis that variation in GSH is associated with prognosis of some types of cancer (96). Of note, GSH contents and the activities of antioxidant enzymes such as GR, and GST were adversely reduced by cytotoxic and genotoxic agents on human lung carcinoma pulmonary type II-like epithelium cell (A-549 cells) (97). It has been reported that the intracellular GSH content has a decisive effect on anticancer drug-induced apoptosis, which indicates that apoptotic effects are inversely comparative to GSH content (98). Hence, effects of tamoxifen were prevented when mitochondrial nitric oxide synthase was inhibited and GSH was supplemented (99). Depletion of GSH sensitizes follicles and granulose cells to toxicant-induced apoptosis and that supplementation of GSH is protective. Suppression of GSH synthesis reversed resistance to radiation as GSH synthesis ameliorates various sequelae associated with exposure to oxidative stress and irradiation (100).

9. METHIONINE

Methionine is an essential amino acid that influences cellular metabolism (Figure 4). Methionine metabolism can be divided into five major but interdependent biochemical pathways: the methionine salvage pathway, the methionine cycle, the transsulfuration pathway, the GSH synthesis pathway, and the taurine synthesis pathway (23). Methionine metabolites are essential for phosphatidyl choline regeneration in the cytoplasmic membrane, and methionine is required for the metabolic pathways that regulate gene expression, chromatin structure, transcription, post-transcriptional processing, and protein synthesis (101). Deficiencies are responsible for many kinds of tumors, including non-small cell lung cancer, leukemia, glioma, rectal adenocarcinoma,

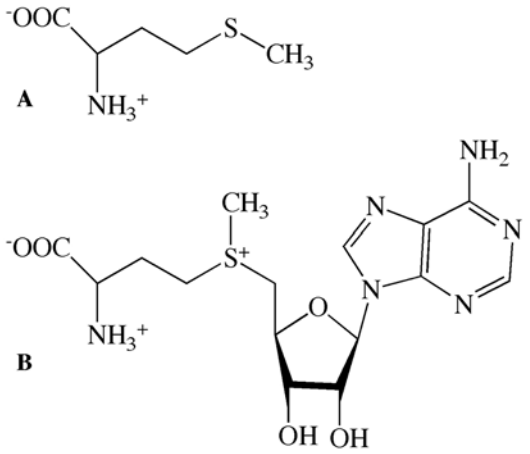

Figure 4. Methionine (A), S-adenosylmethionine (B).

and melanoma (102). The methionine cycle is equally responsible for (i) converting betaine into dimethyl-glicine, which regenerates methionine from cysteine in a reaction catalyzed by betaine homocysteine methyltransferase, and (ii) converting 5-methylenetetrahydrofolate (5-THF) into tetrahydrofolate (THF) by methionine synthase, which produces methionine from homocysteine (102). It is demonstrated that approximately 50% of all tumors are incapable of proliferating when methionine is replaced by homocysteine, resulting in cell cycle arrest, and eventual death (103).

Methionine is transmethylated intracellularly to homocysteine via SAM, the principal biological methyl donor in mammalian cells and a precursor for polyamine synthesis (102). Dietary factors on cancer risk include methionine supply (Table 2), that may interact with some genes associated with breast, lung and colorectal cancer (17). Mitochondrial generation of ROS is increased in mice with fatty livers induced by methionine-deficient diets (104). Both nuclear and mitochondrial DNA are targets for ROS-induced damage and accumulate hydroxylated bases, that introduce mutations that promote cancer as well as cell death (73). Fatty livers are unusually vulnerable to damage from oxidative stress and also exhibit an increased risk for hepatocellular carcinoma (105). Restriction of methionine modulates Raf and Akt survival pathways and affects the function of mitochondria in DU145 and PC3, two human androgen-independent prostate cancer cell lines (104). This restriction inhibits energy production (ATP synthesis) and induces generation of ROS. Restriction of methionine in DU145 and PC3 reduces mitochondrial membrane potential and induces release of cytochrome c from mitochondria and caspase-independent apoptosis (106). In DU145, methionine restriction reduces activity of Akt, activates caspase-9, decreases mitochondrial distribution of phosphorylated Raf and apoptosis inducing factor, and increases mitochondrial distribution of Bak (104). The inhibition of Akt activity alone does not explain the ability of methionine restriction to induce apoptosis of PC3 (106). In fact, mitochondrial Bcl-xL is also decreased in methionine-restricted cells (107). Under methionine restriction, reduced mitochondrial Raf does not inactivate the pro-apoptotic function of Bak. Conversely, apoptosis induced by methionine restriction was not associated with alterations in intracellular distribution of Raf, Bcl-2 family proteins, or apoptosis inducing factor (104).

Methionine works as a micronutrient protective agent involved in DNA methylation, synthesis, and repair. Methionine shows significant protection for rectal cancer in second and third quintiles of consumption but not for colon cancer (108). Some risk factors for breast cancer are dependent upon CYP1A1 genotype. Low intakes of methionine appear to increase the risk of breast cancer in individuals with specific genotypes (109). It is likely that the elevated production of homocysteine by methionine-dependent cancer cells is an adaptation that has allowed tumor cells to survive and colonize a constantly changing, biological environment (23). Cisplatin is an anti-neoplastic drug extensively used in cases of head and neck cancer that induces ototoxicity. Antioxidant drug D-methionine (300 mg/kg) can partially protect from cisplatin ototoxicity (110). Sulphurs containing antioxidants have the potential to compensate the side-effects of cisplatin, and it has been observed that both isomers of methionine block the cisplatin toxic effects in the ear and in the kidney (111). On the other hand, intraperitoneal administration of rats within a select dose range of seleno-L-methionine reduces cyclophosphamide-induced toxicity (myelosuppression) on blood cells and bone marrow (112).

9.1. S-adenosylmethionine

SAM is the major biological methyl donor for other molecules such as DNA and plays an important allosteric role in the regulation of sulfur metabolism (23). Reduced SAM concentrations, as a consequence of low methionine intake or folate deficiency, mainly lead to a deregulation in DNA methylation and are implicated in various cancers, including colorectal cancer (Figure 4). Decreased methylation of DNA may contribute to loss of the normal controls on proto-oncogene expression (14). In humans, hypomethylation of DNA has been observed in colorectal cancers (113). Accumulation of DNA methylation abnormalities, observed during progression of human neoplasias, may be influenced by certain dietary factors (114). Low dietary folate and methionine may reduce levels of SAM (14). High dietary folate was inversely associated with risk of colorectal adenoma, and methionine intake was inversely associated with risk of adenomas (113).

Under normal circumstances, most cellular oxygen is consumed in the cytochrome *c* oxidase complex of the respiratory chain, a system that does not generate ROS (2). However, within the respiratory chain, the ubiquinone pool of complex III of respiration generates $O_2^{\bullet-}$ as a result of single electron transfer to molecular oxygen (115). Ethanol metabolism leads to the generation of acetaldehyde and free radicals that bind rapidly to numerous cellular targets, including components of cell signaling pathways and DNA (116). In addition to direct DNA damage, acetaldehyde depletes GSH (115). Chronic ethanol abuse leads to induction of hepatocyte microsomal cytochrome P450 2E1, an enzyme that metabolizes ethanol

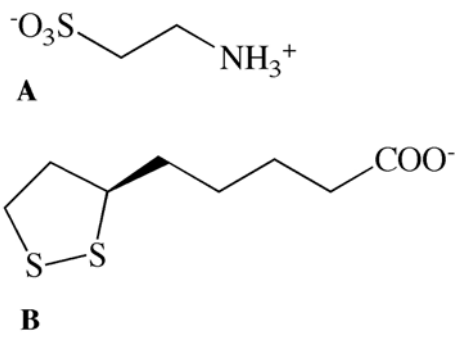

Figure 5. Taurine (A), alpha-lipoic acid (B).

to acetaldehyde and causes further free radical production and aberrant cell function leading to cancer development (116). SAM is employed as dietary antioxidants for therapeutic purposes. SAM has generated considerable interest as an antioxidant for use in protecting against the deleterious effects of ethanol on the liver (115). The role of SAM in transsulfuration is central as a precursor in GSH synthesis, GSH representing a main hepatocellular antioxidant (25). Despite this, the role of SAM as an exogenous antioxidant therapy remains controversial (115).

SAM exhibits a chemoprotective effect (54). Likely this effect is mediated by counteracting, oxidative stress and NF-kappaB activation. To test this hypothesis F344 rats were subjected to hepatocarcinogenesis with or without SAM (38). Lipid-peroxidation was decreased by antioxidants, but only SAM increased glutathione. SAM, in its regulation from IKK downwards, abolished the NF-kappaB activation. Although all antioxidants inhibited oxidative stress as shown by reduction of lipid peroxidation, not all exerted the same effect on NF-kappaB signalling pathway and only SAM increased GSH. The mechanisms exerted by SAM makes this compound a potential liver cancer therapeutic agent (38). SAM potentiated SOD and GST activity and restored altered brain glutathione and erythrocytes lipid peroxidation. These results underline the importance of SAM as neuroprotective compound, acting both on methylation and oxidation metabolism (54).

Accumulation of genetic changes characterizes the progression of cells, initiated by carcinogens, to full malignancy (102). A decrease in SAM content in the liver, associated with DNA hypomethylation in rat liver, during the development of preneoplastic foci, and in neoplastic nodules and hepatocellular carcinomas (103). Reconstitution of the methyl donor level in the liver by SAM administration inhibits growth and induces phenotypic reversion and apoptosis of preneoplastic cells (102). A 6-month SAM treatment results in a sharp and persistent decrease in development of neoplastic nodules, suggesting a long duration of SAM chemopreventive effect. SAM treatment results in overall DNA methylation and partial methylation of c-Ha-ras, c-Ki-ras, and c-myc (103).

SAM is a labile molecule that, at physiologic temperature and pH, is spontaneously cleaved into 5′-methylthioadenosine (MTA) and homoserine lactone (117). Treatment of rats with exogenous SAM results in recovery of SAM level in preneoplastic liver cells, as well as in a partial reconstitution of the MTA pool, which is greatly reduced in preneoplastic cells. Administration of MTA to rats induces a dose-dependent inhibition of the development of preneoplastic liver lesions, associated with inhibition of DNA synthesis and increase in remodeling of preneoplastic lesions. It was considered that the SAM chemopreventive effect might depend, at least partially, on its transformation into MTA (103). The MTA chemopreventive effect is associated with a marked dose-dependent inhibition of ornithine decarboxylase activity, a key enzyme in polyamine synthesis (118). Results of recent research on the protective effect of SAM and MTA on oxidative liver damage by carbon tetrachloride have shown an antioxidative effect of both molecules. There is evidence that the two molecules exert their effect by different mechanisms: maintenance of a high GSH pool occurs in carbon tetrachloride-intoxicated rats, as a consequence of SAM treatment, whereas MTA, being a thioether compound, may exert a direct antioxidant effect, even without affecting GSH pool. The question arises whether the therapeutic use of SAM is preferable to that of the more stable MTA molecule. In human beings, dysplastic and regenerative liver nodules and neoplastic nodules are preneoplastic lesions. At present, it is easier to envision a therapeutic use of SAM, as a chemopreventive agent, because of its current use in human beings for treatment of liver injury (103). SAM and MTA exert a proapoptotic effect in colon cancer HT-29 and RKO cells. HT-29 and RKO differ in p53 status: HT-29 cells express inactive p53, whereas RKO cells express wild-type p53. Hence, the proapoptotic effects of SAM and MTA are independent of p53. Although SAM and MTA are proapoptotic in colon cancer cells, they have no toxic effects in NCM460 cells, a normal colon epithelial cell line (117). In contrast to liver cancer cells, SAM and MTA had no effect on Bcl-xS expression in colon cancer cells (119). In conclusion, SAM and MTA are proapoptotic in colon cancer cells but not normal colon epithelial cells. The evidence that SAM is non-toxic to normal colon epithelial cells is consistent with its excellent safety profile and lack of significant side effects. Thus, SAM and MTA may be attractive agents in the chemoprevention and in the treatment of colonic neoplasia (117).

10. TAURINE

Taurine (2-aminoethanesulphonic acid) is the most abundant intracellular amino acid in humans, being a non-essential sulfur-containing β-amino acid with membrane-stabilizing and cytoprotective properties (99). Taurine is a free amino acid in inflammatory cells, where it is thought to be cytoprotective (Figure 5). Therapeutically, taurine possesses antidiabetic, antihypertensive, antioxidant and hepatoprotective activities (120). One of the possible mechanisms of its pharmacological action involves the free radical scavenger activity. Taurine treatment showed reduced lipid peroxidation and protein oxidation (measured as protein carbonyl content) in the liver mitochondria (99). Taurine is a conditionally essential non-proteinogenic amino acid that is required for many aspects of mammalian

metabolism. Taurine is required for the development and survival of cells, being the most abundant single amino acid in leukocytes (20–50 mM) (121). In addition, taurine can protect cells from oxidant-induced injury by forming taurine chloramine (TauNHCl) (122). TauNHCl synthesis occurs through a reaction between taurine and plasma hypochlorous acid (HOCl), resulting in the inactivation of this strong oxidant and cytotoxic agent (23). HOCl is the final product of the reaction between H_2O_2 and chloride (Cl^-) ion that is catalyzed by myeloperoxidase (MPO) (23). $O_2^{\bullet-}$ dismutates to H_2O_2, which is converted to HOCl by the peroxidase (123). HOCl is also found in equilibrium with molecular chlorine (Cl_2), and responsible for damaging lipids, proteins, and DNA. High levels of HOCl production by polymorphic variants of MPO are associated with risk of leukemia, lung cancer, and laryngeal cancer. The inflammation-mediated chlorination of cytosine residues in DNA may account for several DNA alterations observed in human tumors (23). Under normal conditions, taurine controls cell and tissue levels of Cl^- and HOCl by reacting with these molecules to generate TauNHCl (124). Additionally, TauNHCl downregulates the immunologic response by reducing the production of proinflammatory mediators like nitric oxide, TNF-K, prostaglandin E2, and monocyte chemotactic protein-1, which blocks the cascade effect of chronic inflammation that leads to tumor progression (23).

Taurine equally exert its cytoprotective effect by scavenging and inactivating H_2O_2 and $^{\bullet}OH$ or by binding Fe^{2+} like a chelator. Taurine significantly restored the decreased activities of mitochondrial Mn-SOD and GPX. Taurine has no adverse effects, considered to be safe even at very high dietary intake and has shown protection against some oxidative stress conditions induced by ammonia, acetaminophen and gentamicin. Therefore, pharmacologically taurine appears to be promising agent, especially in reducing the toxicity of antineoplastic drugs (99). Taurine appears to be located in the cytosol rather than the cytoplasmic granules, suggesting that taurine would not be secreted into the phagolysosome and would have a protective effect in the neutrophil cytosol and the extracellular medium. Due to the low pKa of the sulfonic acid moiety, taurine is a charged compound with low membrane permeability and would not concentrate in an acidified compartment (125), having membrane stabilizing and antioxidant properties (121). Taurine reacts slowly with neutrophil components so that these oxidants accumulate in the extracellular medium and do not inhibit neutrophil functions. Taurine represents up to 50 % of the released amines and the amount of taurine increased 2- to -3 fold upon stimulation with phorbol myristate acetate (122). Taurine and other neutrophil amines also protect neutrophils and other cells by competing with endogenous NH_4^+ for reaction with HOCl (99).

TauNHCl increased cellular glutathione and gamma-GCS mRNA expression in T84 cells, whereas as little as 62.5 µM TauNHCl decreased CFTR mRNA expression (126). TauNHCl is one of the most abundant compounds generated by activated neutrophils (122). In contrast to HOCl, which causes necrosis, TauNHCl is a potent inducer of apoptosis in tumor cells. Apoptosis induced by TauNHCl in human B lymphoma cells is dependent upon oxidant-mediated mitochondrial damage, a decrease in mitochondrial membrane potential, and caspase-9 activation (121). Further, it has been shown that TauNHCl is taken up into the cells and is concentrated in the mitochondria, where it induces opening of the permeability transition pore and mitochondrial swelling (127). Identical activity is seen upon treatment of isolated mitochondria with TauNHCl and is blocked by the permeability transition pore inhibitors bongkrekic acid and cyclosporin A, as well as by the sulfhydryl-reducing agent tris(2-carboxyethyl)-phosphine (121). The data suggest that TauNHCl causes apoptosis through direct damage to the mitochondria (128). Stimulated neutrophils release large quantities of taurine that are rapidly chlorinated by the reaction with HOCl, generating TauNHCl (127). TauNHCl causes direct mitochondrial oxidative damage leading to caspase-9-dependent and caspase-8-independent apoptosis (128). The ability of TauNHCl to cause mitochondrial damage (swelling and depolarization) was inhibited by a thiol antioxidant, indicating that the TauNHCl acts through an oxidative mechanism (121). The formation of TauNHCl switches cell death from necrosis, which can cause more extensive tissue damage, to apoptosis, which is generally believed to be more physiologically beneficial (127). Consequently, some interesting clues have been demonstrated: (i) apoptosis mediated by TauNHCl involves intracellular oxidation and activation of caspase-9. (ii) TauNHCl is taken up by Burkitt lymphoma cells and concentrates in the mitochondria. (iii) TauNHCl induces mitochondrial swelling. (iv) TauNHCl induces loss of mitochondrial membrane potential that precedes apoptosis (121).

Taurine increased the activities of SOD, GPX and CAT in B16F10 mouse melanoma cells. Taurine also reduced ROS content in a dose-dependent manner. Taken together, these results suggest that taurine decreases ROS levels by increasing the levels of the antioxidant enzymes (120). Hypotonic exposure provokes the mobilization of arachidonic acid, production of ROS, and a transient increase in taurine release in Ehrlich Lettre cells (36). The taurine release is potentiated by H_2O_2 and the tyrosine phosphatase inhibitor vanadate and reduced by phospholipase A_2 inhibitors, 5-lipoxygenase inhibitor, NADPH oxidase (NOX) inhibitor, and antioxidants (129). Thus, taurine efflux in Ehrlich Lettre cells involves modulation by ROS. Vanadate delays the inactivation of volume-sensitive taurine efflux in NIH3T3 cells (36). It is suggested that increased tyrosine phosphorylation of regulatory components of NADPH oxidase leads to increased ROS production and a subsequent delay in inactivation of the volume-sensitive taurine efflux pathway and that NADPH oxidase or antioxidative capacity differ between NIH3T3 and Ehrlich Lettre cells (129). H_2O_2 has no effect on taurine release from NIH3T3 cells when added under isotonic conditions (36). Multiple PLA_2 isoforms, and NOX are involved in the activation of volume-sensitive taurine release. Inhibition of NOX leads to a rapid inactivation of the volume-sensitive taurine efflux pathway (129). Taurine affects architecture and fluidity of

membrane, and modulates the binding affinity and capacity of Ca^{2+} to phospholipids (36). Hence, shift in the cellular taurine pool in connection with cell volume adjustment will inevitably have an impact on cell function (129). Taurine is accumulated in mammalian cells via the high-affinity taurine transporter TauT and released via a volume-sensitive taurine efflux pathway that is sensitive to pH, the membrane potential, and modulated by ROS, permeability transition pores, and protein tyrosine kinase (36).

Taurine did reduce formation of dimers of 2-amino-3-methylimidazo(4,5-f)quinoline (azo-IQ). Nitryl chloride (NO_2Cl), generated by incubation of NO_2^-, with HOCl, elicited a small amount of NO_2-IQ, which was prevented by 0.5 mM taurine, events which may contribute to prevention of colon cancer (74). Of note, apoptosis induction by RAW macrophages is inhibited by taurine. Murine fibroblasts transformed by oncogene expression (ras, src) or methylcholanthrene treatment were sensitive for apoptosis induction by RAW 264.7 macrophages, whereas parental cells and revertants were insensitive. Signaling by ROS and RNOS seems to represent a signaling principle for the selective elimination of potential tumor cells by macrophages (123). MPO induces apoptosis in src- or raxs-transformed fibroblasts, but not in parental nontransformed fibroblasts. MPO-mediated apoptosis induction is inhibited by taurine. This pattern of inhibition allows conclude that transformed cell dismutates $O_2^{\bullet-}$ to H_2O_2, which fosters HOCl formation by myeloperoxidase. In a second step, HOCl interacts with $O_2^{\bullet-}$ to yield the highly reactive apoptosis inducing $^{\bullet}OH$ (130). The effects of cytotoxic monochloramine on the development of gastric cancers induced by N-methyl-N'-nitro-N-nitrosoguanidine were investigated in Wistar rats. Treatment with both ammonium acetate and sodium hypochlorite significantly increased the incidence of gastric cancers, while the concomitant use of taurine with ammonium acetate and sodium hypochlorite significantly attenuated the enhanced gastric carcinogenesis. Spectrophotometric examinations revealed that taurine scavenged monochloramine. These findings suggest that Helicobacter pylori-associated gastric carcinogenesis may be mediated by monochloramine (131).

Vincristine (VCR) is a widely used chemotherapeutic agent for the treatment of acute leukemia and solid tumors, but not acute myeloid leukaemia (132). It has been hypothesized that resistance of myeloblasts to VCR is related to MPO and production of HOCl. It has been investigated the relationship between VCR degradation and MPO expression and serum HOCl concentrations in pediatric patients with acute limphoblastic leukemia, lymphoma and solid tumors (133). Patients' sera were incubated with VCR alone or in the presence of taurine. Taurine inhibited VCR degradation in the sera of the chemotherapy group. The effects were consistent for both acute limphoblastic leukemia and the lymphoma/solid tumor cases. Inhibition was also observed by taurine pretreatment suggesting a major contribution of HOCl to overall VCR degradation *in vitro* in leukemic patients. In cell free systems equimolar taurine effectively inhibits HOCl oxidation of VCR. Taurine can be metabolized in the serum via GGT to form 5-glutamyl-taurine (14). Addition of 10 μM taurine as a pretreatment for 30 min would allow sufficient time to degrade a significant proportion of this compound in the serum (134). This in turn would reduce its effectiveness as a scavenger of HOCl. *In vitro* studies with taurine have shown that its reaction with HOCl to form taurine-chloramine is still sufficiently oxidizing to inactivate some proteins (23). In an interesting research, three human leukemia cell lines (CEM/CCRF, HL-60, U937) and 15 bone marrow samples from children with acute myeloid leukaemia were studied. VCR was degraded by increasing concentrations of HOCl in cell-free systems and this activity was inhibited by taurine (133). Metabolic profiles of vinblastine-sensitive and -resistant T-lymphoid leukemic cell lines (CCRF-CEM and CEM/VLB100 respectively) have shown evidence for a significantly lower taurine content in the CEM/VLB100 resistant line when expressed relative to that of its drug-sensitive parental counterpart. These data suggest differences in the nature and relative involvements of taurine biosynthetic pathways between the two cell lines, a phenomenon that may be related to their differing sensitivities towards chemotherapeutic agents such as adriamycin which promote the generation of cytotoxic ROS *in vivo* (135). Tamoxifen caused a significant rise in the mitochondrial lipid peroxidation, protein carbonyl content and $O_2^{\bullet-}$ generation. Pre-treatment of mice with taurine (100 mg/kg) markedly lowered all these levels. It also restored decreased enzymatic and non-enzymatic antioxidants of mitochondria (99).

Taurodeoxycholic acid (TDCA) is reported to be one of the major bili acids (136). Bili acids are known to induce oxidative stress and DNA damage (137). Bile acids are equally signaling molecules in cell metabolism and signal transduction (136). TDCA significantly increased NOX5-S expression, H_2O_2 production, and cell proliferation in esophageal adenocarcinoma cells. TDCA-induced increase in NOX5-S expression and cell proliferation may depend on sequential activation of ERK2 MAP kinase in esophageal adenocarcinoma cells. Bile salts may induce upregulation of cyclooxygenase-2 and c-myc expression and activate MAPK and NF-kappaB pathways, thereby increasing cell proliferation and decreasing cell apoptosis. Finally, it was found that TDCA-induced upregulation of NOX5-S expression and increase in cell proliferation depends on activation of TGR5 receptor (a bile acid receptor) in esophageal adenocarcinoma cells (137). Hydrophobic bile acids induce oxidative stress in gastrointestinal cancer resulting in a compensatory upregulation of thioredoxin reductase (TR) mRNA, one of the key components in the complex anti-oxidant defense system within eukaryotic cells. The activation of at least parts of the redox signaling system is potentially related to the cytotoxicity and the stimulation of the cell death machinery induced by toxic bile acids. TR mRNA was upregulated after treatment with taurochenodeoxycholic acid in colon and gastric cancer cells (138).

11. LIPOIC ACID

Alpha-lipoic acid (5-[(3R)-dithiolan-3-yl]pentanoic acid) is an essential cofactor for many enzyme complexes, i.e.: pyruvate dehydrogenase complex (139).

Only the R-enantiomer is biologically significant (Figure 5). ALA is found in a varity of foods, notably kidney, heart and liver meats as well as spinach, broccoli and potatoes (18). It is essential for aerobic life and a common dietary supplement, tipically at doses in the range of 100-600 mg/day (139). Although is not a typical antioxidant ALA works like that because it is able to regenerate (reduce) antioxidants, inhibiting autoxidation of GSH, vitamin C and vitamin E, maintaining a healthy cellular redox state (18). As a result, ALA can increase the GSH intracellular concentrations, decreasing intracellular ROS levels (23).

ALA works as a non-specific scavenger of ROS used for the treatment of a variety of diseases, including heavy metal poisoning. In fact, pro-oxidant effect of $Cd^{2+}$and Hg^{2+} was reversed by the mitochondrial antioxidant ALA in HepG-2 cells (140). However, the effects of ALA or its reduced form, dihydrolipoic acid (DHLA), on cancer chemoprevention has seldom been studied (141,142). Regarding, tetrachlorohydroquinone (TCHQ) is a toxic metabolite of pentachlorophenol that was proven to be a tumor promoter. DHLA can inhibit DMBA/TPA-induced skin tumor formation through its anti-inflammatory and anti-oxidizing functions. DHLA significantly inhibited tumor incidence and tumor multiplicity in DMBA/TCHQ-induced skin tumor formation. Thus, administration of DHLA prevented ROS generation, cytotoxicity, genotoxicity, and apoptotic cell death in cells treated with TCHQ (141).

On the other hand, levels of induced ROS in rat hepatoma AS-30D cells along with an increase in the incidence of apoptotic cell death were prevented by antioxidative agents as ALA (143). In addition to its protective effect against oxidative stress (144), ALA induces apoptosis in different cancer cells types (141,143). The results show that ALA inhibits the growth of FaO and HepG2 cell lines as indicated by the reduction in cell number, the reduced expression of cyclin A and the increased levels of the cyclin and cyclin-dependent kinases inhibitors. ALA-induced apoptosis is associated with p53 activation, increased expression of Bax, release of cytochrome c from mitochondria, caspases activation, decreased levels of survivin, induction of pro-apoptotic signaling like JNK, and inhibition of anti-apoptotic signaling (i.e. PKB/Akt) pathways (145). Pretreatment of neuroblastoma cells with ALA protected neural cells from bupivacaine-induced injury, involving a mechanism of activation of the PI3K/Akt signaling pathway (146). ALA scavenges ROS followed by an increase in apoptosis of 7721 human hepatoma cell line. Apoptosis induced by ALA is dependent upon the activation of the caspase cascade and the mitochondrial death pathway. Additionally, ALA induces increases in caspase-9 and caspase-3 but had no significant effect on caspase-8 activity (147). In conclusion, current findings provide evidence that ALA induces apoptosis in hepatoma cells, and suggest that it may support useful in liver cancer therapy (140,143,145,147).

ALA also induces ROS generation and a concomitant increase in apoptosis of human lung epithelial cancer H460 cells (148). Inhibition of ROS generation effectively inhibits ALA-induced apoptosis, indicating the role of ROS, especially hydroperoxides and $O_2^{\bullet-}$, in the apoptotic process (141). In H460 cells, apoptosis induced by ALA was found to be mediated through the mitochondrial death pathway, which requires caspase-9 activation (148). Likewise, the mitochondrial respiratory chain inhibitor rotenone potently inhibits the apoptotic and ROS-inducing effects of ALA, supporting the role of mitochondrial ROS in ALA-induced cell death (149). ALA induces down-regulation of mitochondrial Bcl-2 protein through peroxide-dependent proteasomal degradation, and overexpression of the Bcl-2 protein prevents the apoptotic effect of ALA. Together, these findings indicate a novel role of ALA in apoptosis induction and its regulation by Bcl-2, which may be exploited for the treatment of cancer and related apoptosis disorders (148). ALA is also able to affect apoptosis in HT-29 human colon cancer cells, as an important parameter dysregulated in tumour development (150). Exposure of cells to ALA or DHLA increased caspase-3 activity and was associated with DNA fragmentation (142). Increased mitochondrial $O_2^{\bullet-}$ production was preceded by an increased influx of lactate or pyruvate into mitochondria and resulted in the down-regulation of the anti-apoptotic protein Bcl-xL. Mitochondrial $O_2^{\bullet-}$ generation and apoptosis induced by ALA and DHLA could be prevented by the $O_2^{\bullet-}$ scavenger benzoquinone. Moreover, when the lactate/pyruvate transporter was inhibited ALA- and DHLA-induced mitochondrial ROS-production and apoptosis were blocked. In contrast to HT-29 cells, no apoptosis was observed in non-transformed human colonocytes in response to ALA or DHLA addition. In conclusion, this study provides evidence that ALA and DHLA can effectively induce apoptosis in human colon cancer cells by a mechanism that is initiated by an increased uptake of oxidizable substrates into mitochondria (150).

During cancer radiation treatment, normal skin invariably suffers from the cytotoxic effects of gamma-radiation and ROS, which are generated from the interaction between radiation and the water molecules in cells (151). Depletion of antioxidants, such as CAT, SOD, GPX, GST and GR, has been observed in irradiated cells and tissues (152). Irradiation of fibroblasts significantly increased ROS, nitric oxide, and lipid peroxidation; all of these factors substantially decreased with ALA treatment (151). ALA treatment restored antioxidant levels, reducing cell injury and protecting cells against irradiation-induced cytotoxicity (151). Production of both radiation- and H_2O_2-induced cyclooxygenase 2 upregulation, and prostaglandin 2, was markedly inhibited by ALA in PC-3 prostate cancer cells (153).

Lastly, ALA has been used with efficacy and safety at integrated treatment based on a pharmaconutritional support, combined with other antioxidants, and drugs, all given orally, in a population of advanced cancer patients (154). Finally, the addition of antioxidants, including ALA, enhanced significantly the progression through the cell cycle, namely from G0/G1 to S phase, of peripheral blood mononuclear cells isolated from cancer patients, thus providing evidence of their potential role in the functional restoration of the immune system in

advanced cancer patients (155). This antioxidant treatment was found to be effective both on ROS levels and GPX activity. Moreover, this treatment was able to reduce serum levels of interleukin-6 and TNFalpha (156).

12. SUMMARY AND PERSPECTIVE

This review examines the regulation of cellular antioxidant capacity, and the protective effects of some SCNEA. The dual (proapoptotic and antiapoptotic) responses of tumor cells to ROS arise from its concentration-dependent ability to induce proliferation and apoptosis pathways (3). Although cells possess antioxidant systems to control the redox state, which is important for their survival, excessive production of ROS can be induced and gives rise to the activation of events that lead to death or survival in several cell types (72). Truthfully, ample evidence supports that ROS production, by numerous anticancer agents, is responsible for apoptosis induction in different types of cancer (157). SCNEA depletion brings about cells at risk of oxidative damage (6). A control of the cellular redox environment is essential for the survival of many malignant cell types. Therefore, the ability of cells to maintain normal SCNEA contents is essential for their functions and survival (14). The reduced cancer risk and lack of toxicity associated with intake of these molecules suggest that specific concentrations of these compounds may produce cancer chemopreventive effects without causing significant levels of toxicity. The non-nutritive dietary constituents which possess antimutagenic property equally appear to be promising chemopreventive agents (43). In fact, recent findings indicate that function of GSH and ALA to increase ROS scavenging can be used by cells as a therapeutic strategy to treat cancer (72). These findings might be of clinical relevance and might help improve the chemotherapeutic use of multiple anticancer drugs.

Of particular interest in the prognosis of cancer are genes involved in defense against ROS because ROS can cause DNA damage and contribute to the pathogenesis of cancer (2). In general, cell heterogeneity is a major impediment to the successful treatment of cancer, and it is conceivable that heterogeneity could limit the use of specific tools in the treatment of human cancer patients like other therapies are limited. Although it is not currently possible to predict the relative specificity of different treatments for each type of cancer, target several different signaling pathways in cancer cells is advantageous from a therapeutic standpoint since it lessens the impact of heterogeneity as a contributing factor to resistance, and this could be an important component of antitumor drug therapy (104). Continued research is needed to better understand the mechanisms of SCNEA involved in control of cell death, as well as, to determine the most rationale and effective combination of antioxidant active agents against cancer (80). Regulating oxidative stress protection can assist in limiting tumour cell proliferation and to gain resistance to conventional chemotherapies. It is deserved to investigate SCNEA to achieve the greatest clinical benefit for cancer patients (88).

A very recent study shows that, in worms, neurons respond to low levels of environmental oxygen expressing hypoxia-inducible factors (HIFs) protecting distant tissues from stress-induced cell death. HIF activity promotes cell survival, and in patients with cancer, high levels of HIF expression are linked to poor prognosis (158). This molecule and others that mediate this cell-cell signalling and can be regulated by antioxidants may be targets for cancer treatment (159). Pharmacological use of sulphur-antioxidants may require some refinement but nevertheless has a clear potential in limiting the development and/or severity of cancer (160). The field of sulfur metabolism has yielded more differences between cancer cells and normal cells than any other area of metabolism (24,66). Cystathionase deficiency has been found in 50-80% of neuroblastoma (161), 45% of hepatoblastoma (162), and 80% of acute leukemias (163). Equally, sulphur metabolism has shown a key role in kidney (164) and liver (165) of Ehrlich ascites tumor-bearing mice, as well as in PC12 pheochromocytoma cells (166).

13. ACKNOWLEDGEMENTS

Thanks are due to the Ministerio de Ciencia y Tecnología of Spain, SAF2010-17573, and Junta de Andalucía, Proyectos de Investigación de Excelencia, Convocatoria 2010, CVI-6656, Spain.

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Abbreviations: ALA: alpha-lipoic acid; BSO: L-buthionine-S,R-sulfoximine; CAT: catalase; CBS: cystathionine beta-synthase; CGL: cystathionine-gamma-lyase; CySS: cystine; DHA: dehydroascorbic acid; DHLA:

dihydrolipoic acid; DMBA: 7;12-dimethylbenz-alpha-anthracene; GA: phosphate-activated glutaminase; GCS: gamma-glutamylcysteine synthetase; GGT: gamma-glutamyltranspeptidase; GLS: glutaminase; GPX: glutathione peroxidase; GR: glutathione reductase; GSH: glutathione; GSS: glutathione synthetase; GSSG: oxidized GSH; HIF: hypoxia inducible factor; 4-HPR: retinoid N-(4-hydroxyphenyl) retinamide; HTL: homocysteine thiolactone; HSP: heat shock proteins; H_2O_2: hydrogen peroxide; JNK: c-Jun N-terminal kinase; MAPK: mitogen activated protein kinase; MGMT: O^6-methylguanine DNA methyltransferase; MPO: myeloperoxidase; MTA: 5′-methylthioadenosine; NAC: N-acetylcysteine; NADPH: nicotinamide adenine dinucleotide phosphate; NF-kappaB: nuclear factor-kappaB; NOX: NADPH oxidase; $^{\bullet}OH$: hydroxyl radical; OTC: L-2-oxothiazolidine-4-carboxylic acid; $O_2^{\bullet-}$: superoxide; PARP: poly (ADP-ribose) polymerase; PKC: protein kinase C; RNOS: reactive nitrogen oxygen species; ROS: reactive oxygen species; SAH: S-adenosyl-L-homocysteine; SAM: S-adenosyl-L-methionine; SAPK: stress-activated protein kinase; SCNEA: sulphur-containing non enzymatic antioxidants; SOD: superoxide dismutase; TauNHCl: taurine chloramine; TCHQ: tetrachlorohydroquinone; TDCA: taurodeoxycholic acid; TNF: tumor necrosis factor; TR: thioredoxin reductase; VCR: vincristine

Key Words: Cysteine, Glutathione, Homocysteine, Lipoic acid, Methionine, ROS, Taurine, Review

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