

# Importance of the trans-sulfuration pathway in cancer prevention and promotion

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**Abstract** The trans-sulfuration pathway is a biochemical mechanism that links methionine metabolism to the biosynthesis of cellular redox-controlling molecules, like cysteine, glutathione, and taurine. While there is some knowledge about the metabolic intermediates and enzymes that participate in trans-sulfuration, little is known about the physiological importance of this mechanism. Deficiencies within the trans-sulfuration pathway induces (i) the generation of reactive species of oxygen (ROS) and halogens (RHS), (ii) homocyst(e)ine accumulation, and (iii) the synthesis of proinflammatory molecules by macrophages, and contribute to humans pathologies like atherosclerosis and tumor development. In this review we outline the role of this biochemical pathway in tumor development and analyze current findings on the role of trans-sulfuration in mammalian physiology. The potential relationship between chronic inflammation, and tumor and atherosclerotic development are discussed.

**Keywords** Trans-sulfuration pathway · Cysteine · Glutathione · Taurine · Chronic inflammation · Tumor cell · Atherosclerosis · Reactive species

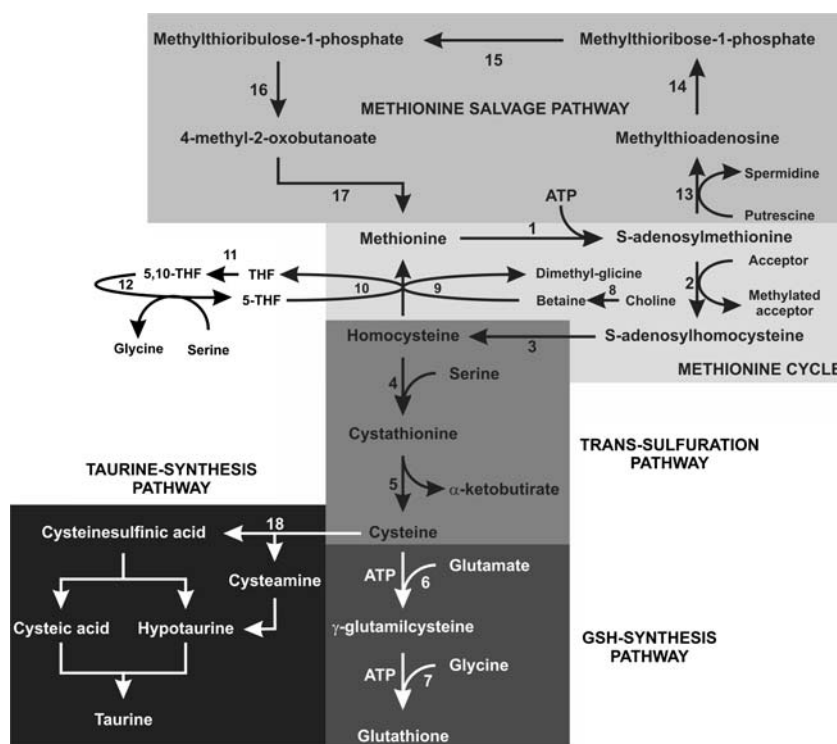
## Introduction

Methionine is an essential amino acid that influences cellular metabolism. It is a proteinogenic amino acid and an essential precursor for the synthesis of glutathione ( $\gamma$ -glutamyl-cysteinyl-glycine; GSH), a molecule that participates in spermine and spermidine synthesis and is the major source of methyl groups for biological molecules. Methionine is also necessary for cysteine biosynthesis, a process carried out through a specialized biochemical pathway called the trans-sulfuration pathway [1]. 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 [2].

Methionine metabolism can be divided into five major but interdependent biochemical pathways: (i) the methionine salvage pathway, (ii) the methionine cycle, (iii) the trans-sulfuration pathway, (iv) the GSH synthesis pathway, and (v) the taurine synthesis pathway (Fig. 1) [1, 3, 4]. The methionine salvage pathway is important for methionine conservation in cells that synthesize large amounts of polyamines for the cell cycle [1]. In the salvage pathway, methylthioadenosine phosphorylase (MTAP; E.C. 2.4.2.28) (Fig. 1, reaction 14) is one of the key enzymes. Deficiencies in the salvage pathway are responsible for many kinds of tumors, including non-small cell lung cancer, leukemia, glioma, rectal adenocarcinoma, and melanoma [5–8].

The methionine cycle plays an important role in cell physiology in a broad sense. It is the place where S-adenosylmethionine (SAM) biosynthesis occurs in a reaction catalyzed by methionine adenosyltransferase

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**Fig. 1** A simplified metabolic chart of biologically important sulfur compounds. The five discrete, interconnected pathways are indicated by different shades of gray. The enzymes required in each pathway are (1) methionine adenosyltransferase (MAT), (2) general methyltransferase, (3) adenosylhomocysteinase, (4) cystathionine  $\beta$ -synthase, (5)  $\gamma$ -cystathionase, (6)  $\gamma$ -glutamylcysteine synthase, (7) glutathione synthase, (8) choline dehydro-

genase plus betaine aldehyde dehydrogenase, (9) betaine homocysteine methyltransferase, (10) methionine synthase, (11) serine-hydroxymethyltransferase, (12) methylenetetrahydrofolate reductase, (13) spermidine synthase, (14) methylthioadenosine phosphorylase, (15) *S*-methyl-5-thioribose-1-phosphate isomerase, (16) methylthioribulose 1-phosphate dehydratase, (17) tyrosine transaminase, and (18) cysteine dioxygenase

(MAT, EC 2.5.1.6; Fig. 1, reaction 1) [9]. 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 [9]. The methionine cycle is also responsible for (i) converting betaine into dimethyl-glycine, which regenerates methionine from cysteine in a reaction catalyzed by betaine homocysteine methyltransferase (EC 2.1.1.5; Fig. 1, reaction 8), and (ii) converting 5-methylenetetrahydrofolate (5-THF) into tetrahydrofolate (THF; Fig. 1, reaction 10) by methionine synthase (EC 2.1.1.13), which produces methionine from homocysteine (Fig. 1) [3, 10].

The biochemical mechanism of trans-sulfuration is less well characterized than the methionine salvage and cycle pathways. However, the trans-sulfuration pathway is necessary for cellular physiology because it connects the methionine metabolic pathways to the generation of cysteine, glutathione (GSH), and taurine (Fig. 1) [11, 12]. Tarver et al. first demonstrated that cysteine could be synthesized from methionine in 1939

[12], and called it the trans-sulfuration or cystathionine pathway [11]. This pioneer work served as the base for further characterization of the trans-sulfuration pathway using isolated hepatocytes from the mammalian liver [13]. The first step of trans-sulfuration involves the formation of cystathionine from homocysteine and serine, in a reaction catalyzed by cystathionine synthase (EC 2.5.1.48; Fig. 1, reaction 4). Once formed, cystathionine is cleaved by cystathionase (cystathionine  $\gamma$ -lyase; EC 4.4.1.1), releasing free cysteine (Fig. 1, reaction 5). 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 [11, 14, 15], where approximately 50% of the cysteine used for GSH anabolism is derived from methionine that was synthesized from the trans-sulfuration pathway [16, 17]. When homocysteine levels are low, cysteine flux through the trans-sulfuration pathway becomes down-regulated in order to conserve homocysteine for the methionine cycle [18]. Trans-sulfuration activity in the

liver is markedly impaired or absent in fetuses, newborns, cirrhotic patients, and patients with homocyst(e)inemia [19]. The trans-sulfuration pathway is also blocked in some liver tumor cell lines, including HepG2 and HuH-7 cells [20]. While the mechanism of this blockade is currently unknown, these cells are unable to form GSH from methionine but can synthesize large amounts of homocyst(e)ine [20]. It is demonstrated that approximately 50% of all tumors are incapable of proliferating when methionine is replaced by homocysteine, resulting in cell cycle arrest, strict inhibition of mitosis, and eventual death [21–26]. Tumor cells export large amounts of homocyst(e)ine when placed in culture medium that contains high methionine, suggesting that there is an anomaly in methionine metabolism [2]. This absolute requirement for methionine by tumor cells is known as “methionine dependency” or “methionine stress” [1, 2]. The molecular mechanism of methionine dependency is currently unknown, but may be triggered by high transmethylation rates [27] that result in the hyperproduction of *S*-adenosylhomocysteine (AdoHcy) and homocysteine [1]. While the influence of methionine-dependence on trans-sulfuration is unknown, the accumulation and export of homocysteine probably impairs cysteine biosynthesis possibly affecting the function of major enzymes in the trans-sulfuration pathway. An understanding of how the major enzymes

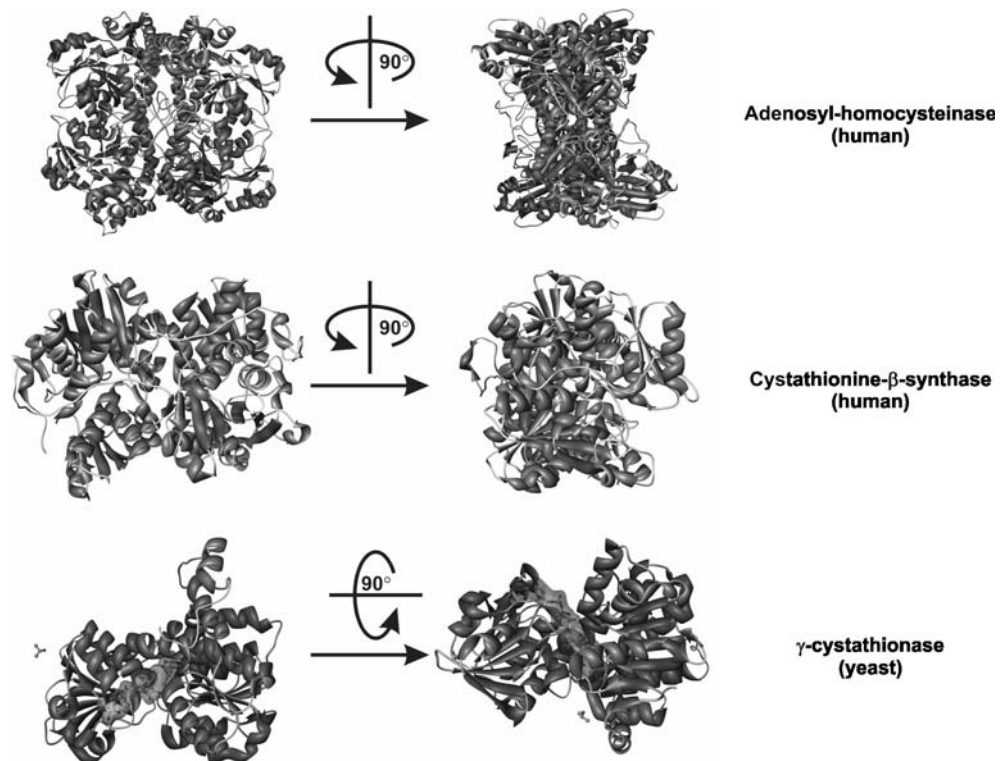
of the trans-sulfuration pathway control the flux of sulfur-containing molecules and cellular redox homeostasis is necessary in order to understand the role of this biochemical mechanism in tumor growth and development.

### The first enzyme of the trans-sulfuration pathway: adenosylhomocysteinase

Adenosylhomocysteinase (*S*-Adenosylhomocysteine hydrolase or AdoHcyase; EC 3.3.1.1) is a cytoplasmic enzyme that catalyzes the reversible hydrolysis of AdoHcy into adenosine and homocysteine (Fig. 1, reaction 3) [28, 29]. AdoHcyase is a tetrameric protein with a molecular weight of 45–50 kDa, in which each subunit contains one molecule of tightly bound  $\text{NAD}^+$  [28, 30], as determined by X-ray crystallography (Fig. 2) [31, 32]. Three-dimensional characterization of mammalian AdoHcyase showed that the four  $\text{NAD}^+$ -binding domains are located near the center of the tetramer while the catalytic domains are located far from the center [32].

The thermodynamic equilibrium of the reaction favors AdoHcy synthesis *in vitro* [33], but the hydrolysis of AdoHcy into adenosine and homocysteine by AdoHcyase prevails under physiological conditions because both reaction products are rapidly removed

**Fig. 2** Three-dimensional models of tetrameric human adenosyl-homocysteinase (Protein Data Bank accession number 1LI4), cystathionine  $\beta$ -synthase (Protein Data Bank accession number 1JBQ), and monomeric yeast  $\gamma$ -cystathionase (Protein Data Bank accession number 1N8P). A pyridoxal-5'-phosphate molecule is shown in the active site of yeast  $\gamma$ -cystathionase. The models are shown at a 90° angle to illustrate their distinctive characteristics



[29]. Once formed, adenosine is either deaminated by adenosine deaminase (EC 3.5.4.4) or enters the purine nucleotide pool through the action of adenosine kinase (EC 2.7.1.20). Homocysteine can enter the trans-sulfuration pathway and be metabolized into cystathionine (Fig. 1, reaction 4) or be re-methylated into methionine (Fig. 1, reactions 8 and 10) [29]. The existence of multiple routes for the metabolism of AdoHcy (SAM) precursors and its metabolic products, adenosine and homocysteine, is consistent with the requirement for efficient product removal to prevent AdoHcy accumulation. AdoHcy accumulation can interfere with AdoMet-dependent methyltransferases by product inhibition, which results in epigenetic alterations [29]. The AHCY gene, which encodes AdoHcyase, was mapped to the human chromosome 20q13.1 [34], and many alleles are associated with hypermethioninemia and coronary heart disease [35, 36].

### The second enzyme of the trans-sulfuration pathway: cystathionine $\beta$ -synthase

The cystathionine  $\beta$ -synthase (EC 4.2.1.22; CBS) gene, situated on chromosome 21q22.3, encodes an enzyme that catalyzes the synthesis of cystathione, the second step in the trans-sulfuration pathway (Fig. 1, reaction 4) [37]. Human cystathionine  $\beta$ -synthase is a 63 kDa enzyme that contains an N-terminal heme domain that regulates enzymatic activity, a catalytic domain that binds PLP (pyridoxal 5'-phosphate), and a C-terminal domain that confers responsiveness to the allosteric activator SAM. The full-length human enzyme exists in a tetrameric state (Fig. 2) [38], in which the catalytic core resembles other members of the fold II family of PLP-dependent enzymes [39]. Thus, because of its location at a branch point for remethylation and trans-sulfuration, cystathionine  $\beta$ -synthase is a highly regulated enzyme (Fig. 1, reaction 4) [4]. The C-terminal regulatory domain inhibits activity of the full-length enzyme, but binding of SAM to this domain converts the enzyme to an activated state [40]. The activation of full-length cystathionine  $\beta$ -synthase by SAM involves a conformational change, so that the C-terminal domain can no longer play an autoinhibitory role [38]. Interestingly, point mutations in the C-terminal domain of cystathionine  $\beta$ -synthase have been identified in homocyst(e)inuric patients [4], and shown to result in diminished SAM responsiveness and varying degrees of constitutive activation [41, 42]. The consequence of cystathionine  $\beta$ -synthase activation by SAM is an increase in the removal of homocysteine through the

trans-sulfuration pathway. The heme group present in the N-terminus of mammalian cystathionine  $\beta$ -synthase may act as a redox sensor [39]. While reduction of the heme moiety is associated with decreased enzyme activity, flux through cystathionine  $\beta$ -synthase is increased under oxidizing conditions [43]. Redox regulation of cystathionine  $\beta$ -synthase activity is consistent with the reported reciprocal sensitivity of the trans-sulfuration pathway to pro- and antioxidants, which enhance or diminish homocysteine flux through the trans-sulfuration pathway, respectively [43].

Cystathionine  $\beta$ -synthase enzyme deficiencies are associated with mental retardation, elevated homocysteine levels [homocyst(e)inuria], skeletal abnormalities, elevated risk of blood clots and atherosclerosis [44], as well as neurodegenerative diseases like Alzheimers and dementia [45]. Homocyst(e)inuria is correlated with other vascular effects like atherothrombosis and endothelial dysfunction because of its auto-oxidative potential, and a related increase in the production of reactive oxygen species (ROS) [46]. Since reduced free homocysteine contains a free thiol group, this molecule can react with itself to form homocystine, or with albumin and cysteine to increase RS production [47]. Plasma levels of reduced free homocysteine are found to enhance oxidative stress [47]. In addition, chronic exposure of endothelial cells to homocysteine accelerates the rate of endothelial senescence and decreases telomere length for every population doubling [48].

### The third enzyme of the trans-sulfuration pathway: $\gamma$ -cystathionase

$\gamma$ -Cystathionase (CSE, cystathionine  $\gamma$ -lyase; EC 4.4.1.1) is a PLP-dependent enzyme that catalyzes the conversion of cystathionine into cysteine,  $\alpha$ -ketobutyrate, and ammonia (Fig. 1, reaction 5). L-cysteine is further metabolized in the liver to yield GSH or taurine, although CSE itself is capable of metabolizing cyst(e)ine and producing H<sub>2</sub>S, a gaseous neuromodulator and smooth-muscle relaxant [49].

Messerschmidt et al. resolved the three-dimensional structure of yeast CSE and showed that it exists in a tetrameric state [50]. Each monomer contains one PLP cofactor and 393 amino acids (Fig. 2). Similar to other PLP-dependent enzymes, the monomer comprises three structurally and functionally distinct regions in which the N-terminus interacts with the active site of the neighboring monomer and a large PLP-binding domain that carries most of the catalytically important residues [50].

The CTH gene on human chromosome 1p31.1 encodes the enzyme CSE [51]. Recessive mutant alleles for CTH are associated with cystathioninuria, a disease characterized by abnormal accumulation of plasma that leads to increased urinary excretion of cystathionine [51]. Screening surveys of neonatal urine samples estimated the prevalence of cystathioninuria at ~1:14,000 live births [52, 53].

### The trans-sulfuration pathway and tumor protection/promotion: is there a link?

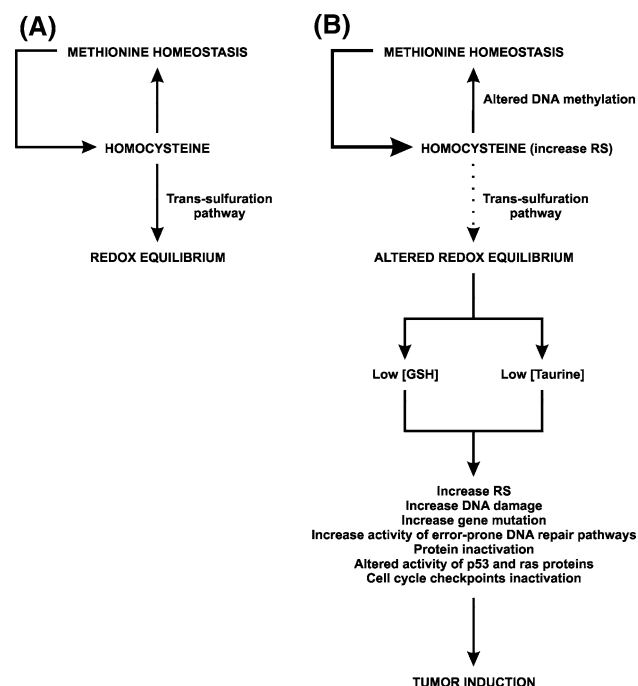
The three enzymatic steps of the trans-sulfuration pathway provide the amount of cysteine required to synthesize the cellular redox-controlling molecules, like GSH and taurine, that protect the molecular constituents of cells against reactive species (SP)-induced damages (Fig. 3A). For example, mitochondrial

and nuclear DNA are continuously and chemically damaged by both endogenous and exogenous RS, which contributes to degenerative processes such as aging and cancer [54]. It is widely accepted that RS produce a broad range of DNA damage including base and sugar modifications, base-free sites, DNA-protein cross-links, and strand breaks [55–58]. Until now, more than 20 different types of base damage have been identified following exposure to oxidative stress [59]. The most prevalent damage to purines is 7,8-dihydro-8-oxoguanine (8-oxoguanine or 8-oxoG), while the most common damage to pyrimidines is the formation of thymine glycol (Tg) [59]. Modified bases generated by RS are highly mutagenic and can induce base mispairing during DNA replication, generating mutations that affect cellular physiology [59].

### Oxidative stress and carcinogenesis

The specific mechanism by which oxidative stress contributes to carcinogenesis is largely unknown [60], but two different mechanisms are thought to play a role: (i) modulation of gene expression and (ii) induction of genetic modifications [60]. Modulation of gene expression by oxidative damage affects carcinogenesis by altering the epigenetic effects and chromosomal rearrangements. Epigenetic effects on gene expression stimulate growth signals and proliferation [60], while chromosomal rearrangements contribute to neoplastic progression [60]. In addition, a redox imbalance is shown to stimulate protein kinase and poly(ADP ribosylation) pathways, affecting signal transduction and promoting tumor development [61]. Interestingly, studies examining the effects of high homocysteine levels show that poly(ADP-ribose) polymerase (PARP) enzymes repair single strand DNA breaks (SSBs) induced by genotoxic agents or oxidative stress [62–64]. The best-studied enzyme of the PARP family, PARP-1, is characterized as an abundant nuclear protein that binds SSBs and double strand DNA breaks (DSBs) [64]. PARP-1 is involved in DNA repair, recombination, and genomic stability [65, 66] of extensive DNA breakage that is triggered by massive oxidative or nitrosative stress [67–69]. The overactivation of PARP depletes the cellular stores of NAD<sup>+</sup> and ATP [69], leading to a severely compromised cellular energetic state that inhibits apoptotic cell death and results in necrotic cell death, followed by the activation of inflammatory processes that augment tumor development.

In all related cases, the initiation potential of oxidants seems to contribute to carcinogenesis by induc-



**Fig. 3** A model showing how tumors can form from deficiencies in the trans-sulfuration pathway. The equilibrium between methionine homeostasis and the trans-sulfuration pathway balances the level of homocysteine in cells and keeps the redox homeostasis (A). Metabolic deficiencies in the trans-sulfuration pathway imbalance homocysteine levels in the cell, which increases homocysteine concentration and alters redox homeostasis, as well as the DNA methylation pattern. The low concentration of GSH and taurine leads to an increase in reactive oxygen species (ROS) generation, DNA damage, gene mutation, protein inactivation, and the altered activity of cell cycle checkpoints. All these cellular phenomena may ultimately result in tumor growth and expansion (B)

ing DNA base changes in certain oncogenes and tumor suppressor genes [70–72]. It is shown that the hydroxyl radical ( $\text{HO}^\cdot$ ) is able to activate certain oncogenes, such as K-ras and C-Raf-1. Activation proceeds through the induction of point mutations in CpG base pairs and gene deletions [72]. Point mutations in CpG dinucleotides are frequently found in certain tumor suppressor genes, such as p53 and ras, leading to inactivation [60]. Furthermore,  $\text{HO}^\cdot$  attacks cells containing mutant or absent p53, resulting in failure to arrest in G1, and reducing cell capacity to repair damaged DNA [60, 72]. This increase in replication errors can initiate additional oncogene activation and tumor suppressor gene inactivation, ultimately contributing to malignancy. Free radical-induced cytotoxicity may also initiate carcinogenesis by depleting the normal cell population and promoting the clonal expansion of more resistant cells, thus increasing the probability of mutation [60].

### The roles of GSH and taurine in cancer prevention or promotion

Considering the mechanisms of tumor induction, it is reasonable to assume that the trans-sulfuration pathway is necessary to maintain intracellular redox homeostasis. Alterations in this pathway increase the rate of base mispairing due to base oxidation, abasic sites, error-prone recombination events, alterations in gene expression, and chronic inflammatory processes, thus inducing tumor progression (Fig. 3B). Another mechanism that could contribute to tumor induction is the depletion of functional proteins by RS, resulting in an increase in oxidized protein level (Fig. 3B). Once formed, oxidized proteins need to be recycled by the ubiquitin system, increasing the amino acid requirement for protein synthesis, especially methionine and cysteine [4]. Assuming that cysteine levels are maintained by the breakdown of GSH [11, 73], there is a shift in the redox balance following the induction of oxidative stress, such that the ratio of GSH to GSSG (the oxidized form of GSH) is altered in favor of GSSG [11, 73].

In addition to its function as a cysteine repository, GSH is the most prevalent non-protein thiol in mammalian cells and the major low molecular-weight peptide present in eukaryotic cells [73]. 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  $\text{Ca}^{2+}$  homeostasis, regulates protein function and gene expression via thiol-disulfide exchange reactions,

modulates lymphocyte function and immune responses, and participates in the mitochondrial mechanisms that link the opening of the permeability transition pore complex to the activation of cell death [74]. Since GSH has such a wide range of functions, alterations in GSH levels are associated with numerous human diseases, including cancer, neurodegeneration, acquired immune deficiency syndrome (AIDS), aging, cystic fibrosis, liver diseases, heart attack, stroke, seizure, diabetes, sickle cell anemia, and kwashiorkor [74, 75].

Glutathione also appears to be very important for tumor development and growth. Hirono [76] was the first to show that ascites tumor cells, which are highly resistant to alkylating agents, have higher GSH levels than non-tumor cells. 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 [77–81]. Considering the homeostatic redox buffer function of GSH and its role in inactivating some carcinogens [82] and protecting cells against DNA-damaging free radicals and lipid peroxidation [83], it is plausible that tumor cells may need more GSH for their survival than other cell types. This is supported by results showing that levels of GSH and enzymes related to the GSH synthesis pathway (Fig. 1) fluctuate over a wide range of concentrations (up to 40-fold), regardless of treatment status or primary tumor origin [84]. One of the best known enzymes in GSH metabolism, glutathione-S-transferase (GSTs; EC 2.5.1.18) is shown to be associated with multidrug resistance of tumor cells [85]. GSTs belong to the family of phase II detoxification enzymes that catalyze GSH (S-glutathionylation) conjugation with different chemotherapeutic compounds to form mercapturates [86], which are easily excreted in urine, thus diminishing the therapeutic effects of antitumor drugs. In addition, GST overexpression can increase susceptibility to carcinogenesis and inflammatory disease [86], which is associated with tumor progression. 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 also been described, further highlighting the importance of GSH pathways in tumor development and growth [87, 88]. Under metastatic conditions, high levels of GSH can support (i) a rapid cell cycle, (ii) an elevated rate of DNA synthesis, and (iii) a block in apoptosis [74]. Thus, 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. A recent study by Uthus et al. [89] demonstrated that the Ames dwarf mouse has significantly increased hepatic GSH levels because of an absence of growth hormone (GH). The lack of GH in dwarf mice results in higher overall levels of tissue glutathione *S*-transferase and GSH [90], enhancing cellular detoxification and providing resistance against toxic/oxidative stress [89]. It is also reported that Ames dwarf mice live 50–64% longer than their wild type littermates [90] because of the delayed occurrence and reduced incidence of fatal neoplastic disease. These data support the hypothesis that heightened antioxidative defenses may prolong life span [90]. Thus, the Ames dwarf mouse may be more resistant to oxidative stress because it has a relatively large pool of GSH as a result of altered levels of GSH metabolites and other sulfur-containing molecules.

In its turn, taurine is a conditionally essential non-proteinogenic amino acid that is required for many aspects of mammalian metabolism [91]. Taurine is required for the development and survival of mammalian cells, being the most abundant single amino acid in leukocytes (20–50 mM) [92–95]. Taurine also protects the lung from ozone, bleomycin, nitrogen dioxide, and amiodarone induced injury [96–99]. In addition, taurine can protect cells from oxidant-induced injury by forming taurine chloramine (Tau-Cl). Tau-Cl synthesis occurs through a reaction between taurine and plasma hypochlorous acid (HOCl) [100], resulting in the inactivation of this strong oxidant and cytotoxic agent. HOCl is the final product of the reaction between H<sub>2</sub>O<sub>2</sub> and chloride (Cl<sup>-</sup>) ion that is catalyzed by myeloperoxidase (MPO; EC 1.11.1.7). HOCl is also found in equilibrium with molecular chlorine (Cl<sub>2</sub>), and responsible for damaging lipids, proteins, and DNA [101, 102]. High levels of HOCl production by polymorphic variants of MPO are associated with chronic inflammation, which increases the risk of leukemia, lung cancer, and laryngeal cancer [101, 102]. Chronic inflammation induced by elevated levels of HOCl is also observed in atherosclerotic tissue [103], a common condition in hyperhomocyst(e)inuric patients. HOCl can halogenate/oxidize pyrimidine and purine bases of DNA, generating the highly mutagenic 5-chloro-2'-deoxycytidine (5-Cl-dC) [102]. 8-Chlorinated products of 2'-deoxyadenosine and 2'-deoxyguanosine, as well as semistable or unstable chloramines (RNHCl and RR'NCl) [101] are also generated from the reaction with HOCl [102]. Unstable nucleoside chloramines, such as thymidine chloramine, are shown to initiate DNA single and double strand breaks through nitro-

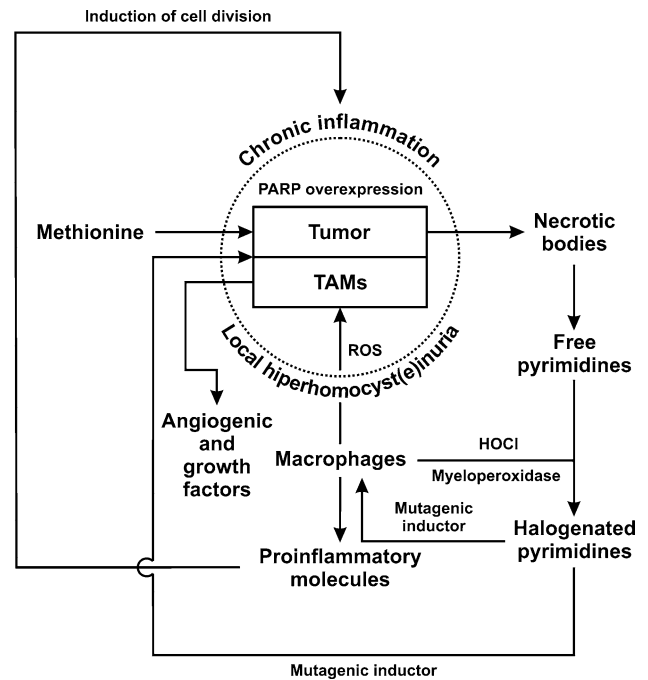
gen-centered radicals and to transfer chlorine atoms to other nucleosides [101]. While uracil concentrations are normally low in plasma, the dissolution of dead cells by necrosis, which is induced by PARP enzyme overexpression [64] and hyperhomocyst(e)inuria, liberates nucleobases and nucleosides into the extracellular environment [102]. This phenomenon increases the uracil content in inflamed tissue fluid (which is as high as 600 μM) to almost 1000-fold higher than the plasma level [102] and provides MPO with ample substrate for halogenation. Thus, all of the necessary factors for pyrimidine halogenation are provided by the inflammatory milieu [101, 102]. Furthermore, because inflammation also causes cells to proliferate, it sets the stage for thymine analogs like halogenated nucleobases to be misincorporated into newly synthesized DNA, which induces mutations [102]. The inflammation-mediated chlorination of cytosine residues in DNA may also account for several DNA alterations observed in human tumors, including alterations in methylation patterns [104].

Under normal conditions, taurine controls cell and tissue levels of Cl<sup>-</sup> and HOCl by reacting with these molecules to generate Tau-Cl [91]. In addition, Tau-Cl downregulates the immunologic response by reducing the production of proinflammatory mediators like nitric oxide, tumor necrosis factor- $\alpha$ , prostaglandin E<sub>2</sub>, and monocyte chemoattractant protein-1, which blocks the cascade effect of chronic inflammation that leads to tumor progression [91].

### Is the methionine dependency of tumor cells an adaptation?

Considering that chronic inflammation and mutagenesis are important factors for tumor development, and that metabolic deficiencies in the trans-sulfuration pathway are directly correlated with chronic inflammation (especially deficiencies that result in cellular homocyst(e)ine accumulation), it is likely that the elevated production of homocyst(e)ine by methionine-dependent cancer cells is an adaptation that has allowed tumor cells to survive and colonize a constantly changing, biological environment. In this sense, atherosclerosis and cancer share many common mechanisms related to disease development and progression, both being a consequence of complex interactions between genetic and environmental factors [105]. Recently, a number of reports have suggested that both atherosclerosis and cancer develop from a clonal proliferation of altered cells at the sight of local tissue injury, inflammation, and genomic instability

[105–107]. Atherosclerosis may begin when an injury or infection transforms a single arterial smooth-muscle progenitor of a proliferative clone, which resembles carcinogenesis [108, 109]. Atherosclerotic plaque and malignant tumor formation are associated with (i) cell proliferation regulatory pathways, (ii) alterations in adhesion molecule expression, (iii) alterations in protease expression, (iv) altered ligand-growth factor interactions, (v) altered nuclear transcription factor expression, and (vi) the production of angiogenesis modulators [105]. The infiltration of macrophages into the cellular mass [110] is another common characteristic of atherosclerotic lesions and tumors. Since Rudolf Virchow first demonstrated that the inflammatory process influences atherosclerosis and tumor development in the 19th century [111], a growing body of evidence has shown that macrophages play an important role in initiating and promoting both pathologies [110, 111]. For example, microbial and viral infections induce a chronic immunologic response that results in a persistent inflammatory process that could result in tumor development. The induction of a chronic inflammatory process by the tumor itself or its leukocyte-infiltrate may also accelerate cancer growth and metastasis [112, 113]. In both cases, the combined effects of ROS, cytokines, chemokines, as well as angiogenic factors produced by tumor-associated macrophages (TAMs) and other inflammatory cells explains the abnormal growth of healthy cells [110, 114]. Although the molecular mechanisms of macrophage-inducing tumor and atherosclerosis development remain poorly understood, it is well established that once a cellular mass becomes infiltrated by macrophages, the ability of tumor and atherosclerotic tissue to survive the immune response increases exponentially. If we consider that methionine-dependent tumors produce large amounts of homocyst(e)ine from methionine (Fig. 4), and hyperhomocyst(e)inuric individuals are at increased risk of developing atherosclerosis, it is plausible that homocysteine is one of the signals required to recruit macrophages to the site of tumor and atherosclerotic cells (Fig. 4). It is possible that tumor cells induce a local hyperhomocyst(e)inuria that recruits a subpopulation of macrophages (Fig. 4). Once established, the macrophages trigger a series of biochemical events that result in the increased synthesis of proinflammatory molecules, ROS, halogenated pyrimidines, angiogenic and growth factors, and create an optimal cellular microenvironment (Fig. 4). This may lead to the selection and stimulation of cells with the ability to evade the immune system and colonize other tissues. Evidence for a role of homocyst(e)ine in the recruitment of macrophages and induction of



**Fig. 4** Schematic model of a cancer microenvironment induced by chronic inflammation and local hyperhomocyst(e)inuria. assuming that methionine-dependent tumor cells produce high levels of homocysteine and generate a localized hyperhomocyst(e)inuria, macrophages are recruited by the release of reactive oxygen species (ROS). The combination of ROS, homocyst(e)ine and parp overexpression, induces tumor cell necrosis, releasing high amounts of pyrimidines into the tumor mass. the pyrimidine molecules are converted into halogenated pyrimidines by macrophage myeloperoxidase and HOCl. the halogenated pyrimidines are strong mutagenic substances that (i) increase the genetic diversity of tumor cells and help to select the best-adapted cells, and (ii) convert some macrophages into tumor-associated macrophages (TAMs). once established with the tumor cells, TAMs initiate angiogenic and growth factor synthesis that contributes to the expansion and maintenance of a cancer microenvironment. in addition, the induction of a constant, chronic inflammatory response by macrophages results in the liberation of proinflammatory molecules that induce necrosis and increase the velocity of tumor cell division

inflammatory processes further supports this idea [115]. Further studies will be needed to analyze the effect of local hyperhomocyst(e)inuria that has resulted from trans-sulfuration deficiencies on the development of optimal microenvironments for tumor and atherosclerosis development.

### Concluding remarks

The trans-sulfuration pathway is an important biochemical mechanism that links methionine metabolism to the production of redox-controlling molecules. While genetic and biochemical knowledge about the



trans-sulfuration pathway has been accumulated in the past few years, further research is required to assess the physiological importance of this pathway in eukaryotic cells. Deficiencies in certain components of the trans-sulfuration pathway are linked to the development of various pathologies in humans. For example, excessive homocyst(e)ine production, observed in particular trans-sulfuration pathway deficiencies, may result in a chronic inflammatory process that leads to atherosclerosis development. It is also observed that the excessive production of homocyst(e)ine may activate macrophages and induce proinflammatory molecule synthesis that is associated with the development and selection of tumor cells, and the establishment of optimal biochemical and environmental conditions for metastasis. In this sense, a study analyzing the importance of the trans-sulfuration pathway in tumor and atherosclerotic development, the recruitment of macrophages for active growing cellular masses, and the creation of microenvironments that select active growing cells is urgently required in order to effectively design new chemotherapeutic molecules or protocols.

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