REVIEW ARTICLE

N-Acetyl Cysteine and Glutathione in Health and Cancer—Pharmacogenomics, Research, and Clinical Practice: Hypothesis and Review

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ABSTRACT

Context • Glutathione (GSH) is a major intracellular antioxidant capable of scavenging free radicals and detoxifying electrophiles from endogenous and exogenous sources via the free thiol group. GSH plays an important role in a multiple cellular process, including cell differentiation, proliferation, and apoptosis. Pharmacogenomics has demonstrated its important role as a key element in cellular health.

Objective • The study intended to examine the benefits of using GSH pharmacogenomics as a therapy to prevent side effects and interactions with antineoplastic agents in the diagnosis and treatment of malignancies.

Design • The research team performed a narrative review using the Google scholar and PubMed electronic databases.

Conclusions • In summary, the involvement of GSH in the carcinogenesis and drug resistance of tumor cells is clear and well understood, but further studies, aimed at understanding the GSH-driven molecular pathways, might be crucial to designing new therapeutic strategies to fight cancer progression, overcoming chemoresistance, using in combination with immunotherapies, and preventing or minimizing their negative side effects. (*Altern Ther Health Med.* 2022;28(7):169-177).

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Glutathione (GSH) is a major intracellular antioxidant capable of scavenging free radicals and detoxifying electrophiles from endogenous and exogenous sources via the free thiol group. As the most important antioxidant produced by the human body, it's the principal detoxifier of every cell in the body. It prevents cellular damage caused by diverse oxidative species. According to Hyman,[1] it's the most important molecule needed to stay healthy and prevent premature aging, cancer, heart disease, dementia, and many more degenerative conditions. Pharmacogenomics, the study of how genes affect a person's response to drugs, has demonstrated GSH's important role as a key element in cellular health.

Named initially by Hopkins in 1921, GSH is technically a peptide that consists of three amino acids—cysteine, glutamic acid, and glycine.[2] Its importance lies in the fact that it exists within the cell wall of almost every single cell, and unlike other antioxidant molecules, the body can produce it on its own.

N-acetyl cysteine (NAC), or N-acetylcysteine, is analogous to GSH and is a precursor of it. Several studies have indicated that NAC prevents the induction and maintenance of DNA damage[3] and works as cytoprotective agent.[4] It promotes anti-angiogenesis through angiostatin production, resulting in endothelial apoptosis and vascular collapse in tumors.[5]

Many preclinical studies have been performed with both molecules, and a few human clinical studies have been published. Pharmacogenomic analysis with GSH S-transferase (GST), a phase 2 detoxification enzyme, has been done on the metabolism of tyrosine kinase inhibitors.[6] These studies have allowed special analyses and studies in different therapeutic areas, particularly in oncology. More

clinical studies are needed, and medical use of the combination of NAC and GSH should be evaluated.

The current study intended to examine the benefits of using GSH pharmacogenomics as a therapy to prevent side effects and interactions with antineoplastic agents in the diagnosis and treatment of malignancies.

METHODS

Procedures

The research team performed a narrative review using the Google scholar and PubMed electronic databases.

RESULTS

Role and Importance

Traverso et al[7] wrote an excellent review on the role of GSH in cellular activity, its cytoprotective effects against carcinogenesis, and the sensitivity of tumors to the cytotoxic effects of anticancer agents. GSH plays an important role in the cellular process in multiple ways, including cell differentiation, proliferation, and apoptosis. Disturbances in GSH homeostasis are involved in the etiology and progression of many human diseases, including cancer.

GSH deficiency or a decrease in the GSH/glutathione disulfide (GSSG) ratio leads to an increased susceptibility to oxidative stress and has been implicated in the progression of cancer. GSH is responsible for many functions, including the following[8]: (1) acts as an assistant molecule for some important enzymes; (2) helps with leukotriene production; (3) aids the liver and gallbladder in detoxifying fats; (4) is involved in methylglyoxal detoxification during metabolism; (5) promotes production and protection of T-cells; and (6) contributes to apoptosis.

Studies have found at least 60 diseases that have low GSH levels as a symptom, including breast cancer. While much research remains to be done, completed studies have found that GSH protects healthy cells from free radicals that can cause damage to cells and induce malignancy. GSH also may assist the body in preventing cancer by playing a role in apoptosis, as mentioned previously. GSH may play both protective and pathogenic roles by preventing cancer and by destroying cancer cells, respectively.[9]

Achieving proper levels of GSH can improve the ability to fend off infection, inflammation, and diseases such as cancer. As per Minich and Brown,[10] many foods can increase GSH levels, including asparagus, avocados, spinach, green beans, cucumbers, and papaya. Demirkol et al[11] measured the levels of biological thiols and found them in various fruits and vegetables, such as cabbages, red grapes, blackberries, apples, and peaches.

By incorporating more sulphur-rich foods into the diet, humans are less likely to suffer from a GSH deficiency. Other ways to boost GSH levels include increasing vitamin C intake, eating foods rich in selenium, supplementing with whey protein, and simply getting enough sleep. These nutrients also can improve nutritional status for cancer patients.[12] Herbs such as turmeric and milk thistle also can help to naturally increase GSH levels.[13]

Regarding NAC supplementation, a study from Greece[14] with 100 individuals demonstrated that NAC can restore exercise performance and reduce oxidative stress in individuals with low GSH levels. According to Demirkol et al,[11] cysteine is a semi-essential amino acid produced from methionine and serine and found in most high-protein foods, such as chicken, turkey, yogurt, cheese, eggs, sunflower seeds, and legumes.

Use in Diagnosis and Therapeutics

GSH and NAC supplementation have been shown to provide many benefits, such as improving athletic performance,[14-16] protecting against aging,[17] and preventing and decreasing the symptoms of chronic fatigue syndrome,[18,19] diabetes mellitus,[20-22] liver disease,[23-26] neurological conditions,[27-29] pulmonary diseases,[30-32] renal diseases,[33-35] immune diseases and human immunodeficiency virus (HIV),[36-40] and cancer.[41-45]

With respect to muscle cachexia and immune deficiencies, recent studies have revealed important clues about the role of cysteine and GSH in the development of skeletal-muscle wasting.[19] Evidence suggests that: (1) the cystine level is regulated primarily by the protein catabolism of the normal postabsorptive skeletal muscle; (2) the cystine level itself is a physiological regulator of nitrogen balance and body cell mass; (3) the cyst(e)ine-mediated regulatory circuit is compromised in various catabolic conditions, including old age; and (4) cysteine supplementation may be a useful therapy if combined with disease-specific treatments, such as antiviral therapy in HIV infection. The last is an important application of NAC in HIV patients.

Diabetes causes a significant decrease in the precursors—cysteine and glycine—needed to synthesize GSH in the body. Patients with uncontrolled type 2 diabetes almost always have a severe GSH deficiency. GSH isn't important as a supplement for diabetics only but is vital for a long and healthy life regardless of a person's health situation. The ability of a cell to resist the damage caused by oxidative stress is determined by the capacity of an array of antioxidants. The most abundant and important of these defenses is GSH.

GSH helps in healing damaged cells; preventing disease, such as cancer and neurological disorders; and even reversing the aging process. Some evidence exists that supplementing with GSH precursors and acetyl GSH can help patients suffering with hyperglycemia to restore their GSH synthesis, lower their oxidative stress levels, and prevent or heal oxidative damage to their cells.[20-22]

Several human studies have highlighted the benefits of NAC and GSH to the liver, particularly in the context of a nonalcoholic fatty liver.[23-25] In addition, animal studies have supported NAC's ability to protect the liver from alcohol and other toxins as well as chronic liver conditions.[26] Taken together, this evidence supports the use of NAC for liver health.

The many mental neurological disorders and diseases have an interesting relationship to GSH. When a disease or disorder is present, GSH and other elements are below

normal levels. Some of these others are either synergistic with GSH, or they are cofactors of GSH.[27-29]

GSH is the primary antioxidant in the lungs. Because of NAC's ability to break up mucus and its powerful antioxidant effects in terms of boosting GSH, NAC has been studied as a natural remedy for lung and respiratory conditions, such as COPD, bronchitis, and pulmonary fibrosis.[30-32]

In oncology, GSH and NAC have been demonstrated to be effective by providing potent antioxidant protection, assisting detoxification mechanisms, and supporting immune function.[40-45]

Oral and Parenteral Administration

GSH is a delicate molecule and is only effective in the form of reduced L-GSH. Most oral products aren't stable enough to produce the same results as parenteral IM or IV GSH therapy. A compounding liposomal oral product is available that is effective and proven to stimulate the immune system.[40]

GSH can be given as a IV push immediately after a Myer's Cocktail, administered through the same injection site. GSH is a powerful antioxidant indispensable for detoxifying heavy metals and chemicals. Treatment starts at doses from 200 mg to 1000 mg, and the dosage may be increased according to the patient's condition and response. It can be given up to three times a week, but for general wellness, fatigue, and peak performance, the frequency is usually once a week or twice a month.

Chen et al[45] has demonstrated that the pro-oxidative, anticancer mechanism of a combination of pharmacologic Vitamin C and GSH, administered together, provides no additional benefit compared to Vitamin C alone. An antagonism exists between ascorbate and GSH in treating cancer, and therefore, IV Vitamin C and IV GSH shouldn't be administered to cancer patients on the same day.[46]

NAC—molecular formula: $C_5H_9NO_3S$—is an acetylated derivative of cysteine, a sulfur-containing amino acid. As an antioxidant precursor to GSH, NAC has been used as a prodrug in the clinical treatment of a paracetamol overdose for over 30 years, and more recently, it has also been applied as a mucolytic in the treatment of chronic obstructive pulmonary disease, cystic fibrosis, and contrast-induced nephropathy.[47] NAC is widely available in many countries, including the USA, Canada, and Australia, as an inexpensive off-the-shelf nutritional supplement commonly marketed as a potent antioxidant for brain function. Increasingly, it's being explored as an adjunctive therapy for many psychiatric conditions.[48]

NAC has been approved by the US Food and Drug Administration (FDA) since 1963. The adverse effects experienced with the use of NAC are somewhat dependent on the route of administration.[49] The pharmacokinetics and pharmacodynamics of NAC were investigated in a phase I clinical study with 26 volunteers, with a six-month oral administration of NAC. The major reported side effects were gastrointestinal symptoms, including intestinal gas, diarrhea, nausea, and fatigue, with the highest nontoxic dose being 800 mg/m^2/day.[50] In another clinical trial, oral administration of NAC at doses up to 8000 mg/day was reported to cause no significant adverse reactions in patients infected with HIV.[51]

In contrast, severe anaphylactoid symptoms, such as flushing, pruritus, angioedema, bronchospasm, and hypotension, were reported after intravenous administration of NAC. These symptoms are likely attributable to the transient, high plasma concentrations of NAC and were most prevalent immediately after the initial loading infusion; the symptoms subsided rapidly after administration was discontinued.[52]

Nevertheless, severe systemic reactions are uncommon. Considering the poor oral absorption of dietary GSH, orally administered NAC has been found to be more efficient than direct GSH administration and as effective as intravenously administered NAC.[53] Compared with cysteine, the acetyl moiety of NAC reduces the reactivity of the thiol functionality, rendering NAC less toxic and less susceptible to oxidation to disulfide and easier for absorption and distribution.[54] NAC is rapidly and almost completely absorbed after oral administration in both animals and humans; one study showed that only 3% of radioactive-labeled NAC was excreted in feces.[55] Thus, NAC is a better source of cysteine compared to parenteral administration of cysteine.

Regarding the proper dosage, between 600 and 1800 milligrams of NAC daily seems to be effective against many conditions; 600-milligram capsules taken two to three times a day is the recommended dosage to start. Some evidence has shown that 2000 milligrams is safe for most adults. Higher doses may be needed to treat certain chronic and degenerative diseases, including COPD, impaired glucose control, and cancer. Commercial NAC is available in 500-mg tablets and capsules, 600-mg tablets and capsules, 750-mg capsules, and 1000-mg tablets.[56]

Assessing SNPs or Measuring GGT

A single-nucleotide polymorphism (SNP) is a substitution of a single nucleotide that occurs at a specific position in the genome, where each variation is present to some appreciable degree within a population, >1%.

Cystathionine beta synthase (CBS) is a gene that converts homocysteine into cystathionine.[57] This enzyme acts in a chemical pathway and is responsible for using vitamin B_6 to convert homocysteine and serine into a molecule called cystathionine. Another enzyme then converts cystathionine to the amino acid cysteine, which is used to build proteins or is broken down and excreted in urine. Additionally, other amino acids, including methionine, are produced in this pathway.

The information regarding the CBS pathway activity is still very new, and not everything is well known or understood with regard to what is taking place biochemically in individuals with said gene mutations. Time, further research, and empirical observations are needed to gauge the situation more fully.

The CBS pathway is the gateway into several essential biochemical processes. The biochemical pathways that follow and are linked to CBS are transsulfuration and GSH synthesis. GSH is among the most important endogenously-produced antioxidants in every cell of the body. GSH activity in cells is critical for normal detoxification and defense mechanisms in every cell.[57]

In the CBS pathway, the sulfur amino acids are removed if they are excessive. However, under certain circumstances, abnormalities can ensue, causing an excessive pooling of sulfur groups. As a result of normal cystathionine reactions, ammonia is generated; GSH gets made, and hydrogen sulfides are converted into sulfites and then into sulfates.[58]

Many scientific peer reviews have focused on decreased CBS pathway activity, hyper-homocysteinemia, and homocystinuria.[59-62] Elevated levels of homocysteine have been implicated in both cancer and cardiovascular disease. In these regards, one mechanism that may exist for lowering elevated levels of homocysteine in some individuals is taking supplemental NAC.

However, based upon recent, empirical observations by numerous clinicians and researchers, another scenario has emerged: certain individuals may be predisposed toward elevated or upregulated CBS pathway activity. CBS pathway upregulations can result in a higher production of ammonia and urinary sulfates as well as decreases in GSH synthesis, and/or possibly imbalances in GSH's reduction/oxidation (redox) ratio. This situation can cause diseases such as cancer and cardiovascular calcifications.[58,59,62,63]

The enzyme γ-glutamyl transferase (GGT) cleaves C-terminal glutamyl groups from amino acids and transfers them to another peptide or to an amino acid.[64,65] It's important in GSH metabolism, amino-acid absorption, and protection against oxidant injury. Although GGT is found in many tissues, the main source of serum activity is the liver, primarily the biliary epithelium; thus, GGT is used mainly as a sensitive indicator of cholestasis.[66]

GGT plays a key role in the gamma-glutamyl cycle, a pathway for the synthesis and degradation of GSH, as well as drug and xenobiotic detoxification. Other lines of evidence indicate that GGT can also exert a pro-oxidant role, with regulatory effects at various levels in cellular signal transduction and cellular pathophysiology.[67]

Serum GGT may predict many diseases as a cumulative biomarker of various environmental chemicals. Cellular GGT is prerequisite for metabolism of GSH conjugates, and GSH is a critical biomolecule for conjugation of diverse chemicals. Supporting this concept, serum GGT within its normal range has been shown to have clear dose-response associations with a variety of chemicals, such as lead, cadmium, organochlorine pesticides, and dioxin.[68]

These associations of serum GGT with environmental chemicals could have an enormous impact on public health because it would indicate that exposure to mixed chemicals at very low levels may not actually be safe. Even though study of serum and/or cellular GGT is at a beginning stage, epidemiological findings suggest that serum GGT might be useful in studying oxidative-stress-related issues in both epidemiological and clinical settings.[69] In clinical practice, it's an excellent indicator of GSH deficiency and useful for diseases monitoring.

NAC, alpha lipoic acid, and GSH are all sulfur donor supplements, meaning their building blocks are sulfur-containing amino acids.[70] In individuals whose bodies use up these amino acids too rapidly, as is the case when certain CBS variants are present, sulfur sensitivity can occur. As such, and with 28% of the population carrying at least one copy of CBS C699T SNP,[71] some people will have reactivity to NAC and the sulfur family of supplements. This is important to know in clinical practice whenever its testing becomes more available.

Clinical experience of Current Research Team

One member of the research team is a hematologist, oncologist, and integrative medicine practitioner, and another is a natural medicine practitioner and scientist. Together they have had positive experiences in providing NAC and GSH for different conditions, such as anemias, chronic fatigue syndrome, autoimmune diseases, and cancer. The research team has developed some protocols for use of NAC and GSH in treatment of a variety of diseases (Table 1).

To be more effective and avoid chemoresistance for cancer patients, the team usually gives high doses of vitamin C on the day prior to chemotherapy and then IV GSH on the day after chemotherapy, as recommended by Traverso et al.[7] In patients that refuse or who aren't candidates for chemotherapy—as suggested by Gonzalez et al[72] and following the bio-energetic theory of carcinogenesis that he proposed, identifying cancer as metabolic disease[73]—the research team recommends mitochondrial correction as a plausible, nontoxic therapeutic approach for cancer. In those patients who have received a high dose of vitamin C intravenously on day 1 followed by IV GSH on day 2—with oral vitamins and supplements including vitamin E, mixed tocopherols with tocotrienols—an upregulation in the expression of the GPX1 gene has been observed, as suggested by Min et al.[74]

Research and Clinical Practice

Agarwal et al[5] tested a hypothesis in an animal model that NAC can reduce vascular maintenance via angiotensin-induced, endothelial cell apoptosis, with immunohistochemical staining for angiostatin in MDA-MB-435 experimental tumor sections. In those breast xenografts treated with saline versus NAC, they found that NAC-treated animals showed a 37% increase in angiostatin compared to the saline controls, causing vascular collapse, anti-angiogenesis, and endothelial apoptosis.

The role of GSH in regulating cancer development and growth has been very well reviewed and postulated by Traverso et al.[7] In many normal and malignant cells, an increased GSH level is associated with a proliferative response

Table 1. Protocols for the Use of GSH and NAC Under Clinical Conditions

Indications	Dosages/Frequency
Asthma and allergy disease	600 mg of glutathione as power-nebulizer respiratory therapy for 5-15 min daily, as needed
Autoimmune diseases	• 600 mg of glutathione IM BIW for 4 weeks • Then every 2 weeks • Plus one 600-mg capsule of NAC orally BID
Liver and kidney disease	• 1000 mg of glutathione in 50 ml of 0.9 NSS IVPB for 30 min BIW for 4 weeks • Then once every 2 weeks • Plus 2 ml of B-Complex in 0.9 NSS in a 100 ml IV for 30 to 60 min every 2 weeks and one 600-mg oral capsule NAC BID
Chronic fatigue syndrome and fibromyalgia	• 600 mg of glutathione IM BIW for 6-8 weeks • MethylAssist, which contains 1000 mcg of L-5-MTHF • 1000 mcg of methylcobalamine • 75 mg of benfotiamine • one 25-mg oral capsule of pyridoxal 5'phosphate daily • 600 mg of NAC one capsule orally BID
Mutation MTHFR and related diseases	• One capsule of MethylAssist orally QD • One 500-mg capsule of curcumin orally QD • One 2000-mg capsule of Omega 3 EPA orally QD • 600 mg of glutathione IM every 2 weeks • If needed, 2 ml of B-complex in 0.9 NSS in a 100 ml IVPB for 30 to 60 min every 2-4 weeks
Autism and ADD Disease	• 600 mg of Glutathione in 0.9 NSS in a 50 ml IVPB for 15-30 min BIW for 8 weeks • 600 mg of NAC orally BID • 2000 mg of Omega 3 EPA QD
Alzheimer's disease and dementia syndromes	• 1000 mg of glutathione IVPB for 30 min every 3 weeks • 600 mg of NAC PO BID • 2000 mg of Omega 3 EPA QD

Abbreviations: GSH, glutathione; NAC, N-acetyl cysteine; IM, intramuscular; BIW, twice a week; BID, two times a day; NSS, normal saline solution; IVPB, IV piggyback; L-5-MTHF, L-5-methyltetrahydrofolate; MethylAssist, commercial supplement; QD, once a day; EPA, eicosapentaenoic acid

and is essential for cell cycle progression. The molecular mechanism by which GSH modulates cell proliferation remains largely speculative.

A key mechanism for GSH's role in DNA synthesis relates to the maintenance of reduced glutaredoxin or thioredoxin, which is required for the activity of ribonucleotide reductase, the rate-limiting enzyme in DNA synthesis. Furthermore, in liver cancer and metastatic melanoma cells, GSH level is correlated with growth, and it has also been demonstrated that a direct correlation exists between GSH levels and cellular proliferation and metastatic activity.[75-78]

A study using GSH demonstrated in mice that it could reduce the melanogenesis and oxidative stress when exposed to UVB irradiation.[79] Eighteen female BALB/c mice were randomly divided into 3 groups: (1) a control group (n = 6)—a group without UVB irradiation and L-GSH administration; (2) a UVB irradiated group (n = 6)—a group irradiated with a UVB dose of 250 mJ/cm2 for 3 minutes; and (3) a treatment group (n = 6)—a group irradiated with UVB and treated with 100 mg/kg of L-GSH by oral gavage. Treatment was given for 14 days, and UVB irradiation was given on days 9, 11, and 13. Oral L-GSH significantly reduced lipid peroxidation and elevated superoxide dismutase activity and serum GSH level ($P < .05$). L-GSH also inhibited melanin content and tyrosinase activity significantly as compared with the UVB-irradiated group ($P < .05$).

A combined Chinese and Japanese study[80] examined co-cultured cervical carcinoma cells (CaSki cells) and human umbilical vein endothelial cells (HUVECs). These cells show higher resistance to chemotherapeutic agents than do single-cultured cells, as indicated by higher cell viability, increased expression of angiogenic proteins, and elevated level of paclitaxel metabolites under coculture conditions. The researchers demonstrated that this integrated microfluidic platform with multiple functions could facilitate the understanding of the interaction between tumors and endothelial cells, and the platform could become a tool for drug screening within an engineered-tumor microenvironment, demonstrating again the use of pharmacogenomics.

Nagana et al[81] demonstrated that researchers can measure few coenzymes but can examine the antioxidants GSSG—oxidized GSH—and rGSH reduced GSH—in tissue and whole blood using NMR spectroscopy for a better understanding of cellular metabolism and the ability to target the antioxidants with new therapeutic maneuvers.

Clinical Research

A phase I study[6] was done to investigate the pharmacokinetics (PKs) of olmutinib in three populations—Korean, Japanese, and Caucasian. The researchers evaluated safety and tolerability and performed a population PK and pharmacogenomic analysis.

They found that a single-nucleotide polymorphism in the GSTM3 gene (rs4783) and a copy number variation in the GSTM1 gene were significantly related to the area under the curve (AUC). A one-compartment model with first-order absorption adequately described the observed olmutinib data. PK parameters were dose-proportional and didn't differ by population, and food intake didn't affect olmutinib absorption. Pharmacogenomic analysis indicated that GST might be involved in olmutinib metabolism. This good phase I trial probed the benefits of GSH pharmacogenomics in cancer therapy.

An Italian study[82] examined the use of Nevirapine (NVP), used in developing countries as a first-line treatment for HIV infection but associated with common, serious, adverse drug reactions, such as liver toxicity and the most severe and rare Stevens-Johnson syndrome (SJS) and toxic epidermal necrolysis (TEN). The researchers found GSTT1 and GSTM1 null genotypes by multiplex polymerase chain reaction (PCR) in a population of 181 patients from Mozambique, for enzymes involved in the metabolism NVP. They found that GSTM1 contributes to the definition of a genetic risk profile for SJS/TEN susceptibility. The study showed an excellent application of GSH genomics that can be used as another instrument in oncology.

In another clinical study on hematopoietic stem cell transplantation (SCT) in children using busulfan,83 the researchers found that the GSTA1 diplotypes were a factor that should be included in future population pharmacokinetic models, including similar conditioning regimens, to improve the prediction of busulfan exposure after its initial dose.

A veterinarian study[84] demonstrated the use of GST-theta genotypes to predict the risk of toxicity with an antineoplastic agent, cyclophosphamide.

Regarding clinical use of GSH genotypes and the prevalence of diseases, a study was performed to determine the GSTM1 and GSTT1 polymorphisms in 154 healthy, unrelated individuals from the Javanese-Sundanese and Malay ethnic populations of Indonesia,[85] to provide a resource for improving the prognosis of possible susceptibilities in specific populations. This study contributed significant information on the variability of GSTT1 and GSTM1 gene polymorphisms worldwide, which can provide new knowledge about the relationship between ethnicity and the prevalence of certain diseases. These researchers have provided another tool to use in clinical practice.

The GST enzymes are involved in the detoxification of a range of carcinogenic compounds as well as chemotherapeutic agents. Therefore, genetic variants in the GST genes could influence survival in cancer patients. Prior studies have suggested that GSTM1 non-null haplotypes (+/-, +/+) might be associated with decreased survival for people with colorectal neoplasms who have been treated with oxaliplatin, as compared to those with the GSTM1 null (-/-) genotype. Indeed, patients with cancer and the GSTM1 (+/+) genotype who have been treated with cisplatin or oxaliplatin may have a decreased likelihood of disease-free survival and increased incidence of recurrence as compared to patients with the (-/-) genotype (HR: 2.25; 95%CI: 0.93 - 5.44, $P = .05$).[86]

Other clinical and genetic factors may also influence disease free survival and incidence of disease recurrence in patients who are treated with platinum compounds. Furthermore, GSTM1 non-null was also found to be associated with decreased response to paclitaxel and platinum compounds (e.g., cisplatin or carboplatin) in European women with histologically-confirmed ovarian neoplasms as compared to GSTM1 null (5-year survival between the GSTM1-non-null and GSTM1-null genotypes: 43.9 versus 53.7 months, $P = .001$).[87]

Although results from previous studies have been inconsistent, some functional genetic polymorphisms in GSTM1 are listed as PharmGKB VIP with moderate level 2B of clinically significant association (https://www.pharmgkb.org/ ID:1447679509).

On the other hand, the GSTM1 null genotype has been associated with improved survival rates and significant reduction in hazard of death as compared to the non-null genotypes. This was also true when the GSTM1 null genotype was combined with other genotypes; women with the GSTM1 null genotype combined with the GSTT1 null, or combined with GSTP1 rs1695 A>G—GSTM1 null/AG+GG—had a lower mortality—adjusted HR = 0.31, (95% CI, 0.121 to 0.805 $P = .016$) and = .45 (95% CI, 0.243–0.846), $P = .013$, respectively. The adjusted HR in patients with the GSTM1 null/GSTT1 null/GSTP1 AG +GG genotype combination led to an even further reduction in the hazard—HR = 0.31 (95% CI, 0.120 to 0.836), $P = .02$).88

Clinical Practice

GSH levels are measured in red blood cells (RBCs), which are readily available. The turnaround time is 5 to 7 days. The level of GSH in erythrocytes is a sensitive indicator of intracellular GSH status, the overall health of cells, and of the ability to endure toxic challenges. GSH levels are thousands of times higher in cells than in plasma. Plasma GSH represents primarily the GSH that the liver has synthesized and exported.

The active form of the tripeptide, rGSH, and the ratio of rGSH:GSSH is normally about 9:1. After a blood sample is obtained, erythrocyte rGSH is very susceptible to oxidation, and the rGSH:GSSH ratio drops rapidly. Specimen handling to prevent the ex-vivo oxidation of rGSH is impractical, and direct measurement of rGSH in vivo isn't feasible outside of a research setting. However, research clearly indicates that undesirable ratios of rGSH:GSSH are associated with abnormally low levels of total cellular GSH. Therefore, it's clinically meaningful to assess the level of total erythrocyte GSH as an indicator of GSH status and metabolism.[89]

In clinical practice, GSH synthetase deficiency is a genetic metabolic disorder that affects the body's ability to produce GSH. People with GSH synthetase deficiency can have mild, moderate, or severe disease. The signs and symptoms of the deficiency may include anemia, metabolic

acidosis, frequent infections, and symptoms caused by problems in the brain including seizures, intellectual disability, and ataxia.[90] It's caused by genetic changes—pathogenic variants or mutations—in the GSS gene.

The deficiency is inherited in an autosomal recessive manner.[90] Diagnosis of a metabolic disorder such as GSH synthetase deficiency may be suspected when a doctor observes signs of the deficiency, including metabolic acidosis. A physician can order tests to confirm the diagnosis, including enzyme assays, urine analysis, and genetic testing.[91] Treatment for GSH synthetase deficiency may include using sodium bicarbonate to treat metabolic acidosis and taking vitamin supplements.[91]

Oncologists worry about risk and prognosis in some cancers, and genomic studies have been done that they can use as models for clinical practice.[92,93]

Another issue in clinical practice is the chemotherapy resistance of tumors, and Bansal and Simon[94] have provided an excellent review and determined that a combination of inhibitors of GSH synthesis and/or its use with either chemotherapeutics or targeted treatment might increase the sensitivity of such drugs and provide viable options for patients suffering from therapy-resistant tumors. One therapeutic option with GSH or NAC has been shown to facilitate drug release for a synergistic effect with cancer treatment,[95] causing metabolism and antiproliferative effects in breast cancer[43] and a synthetic lethal interaction with GSH modulators. This highlights the promising option of harnessing GSH metabolism for patient-directed therapy in cancer.[96]

Recommendations

It's highly recommended to use RBC GSH, serum GGT, and homocysteine levels as well as GSH pharmacogenomics/pharmacokinetics in clinical practices to be more targeted in the daily decision-making process regarding the therapeutic options for cancer patients. It would better, however, to design more clinical trials applicable to basic daily needs with the aim of underscoring GSH's relevance in translational research for future therapeutic treatment design.

CONCLUSIONS

In summary, the involvement of GSH in the carcinogenesis and drug resistance of tumor cells is clear and well understood, but further studies, aimed at understanding the GSH-driven molecular pathways, might be crucial to designing new therapeutic strategies to fight cancer progression, overcoming chemoresistance, using in combination with immunotherapies, and preventing or minimizing their negative side effects.

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