Disturbed homocysteine metabolism is associated with cancer

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Abstract
Hyperhomocysteinemia/Homocysteinuria is characterized by an increased level of toxic homocysteine in the plasma. The plasma concentration of homocysteine is 5–15 μmol/L in healthy individuals, while in hyperhomocysteinemic patients, it can be as high as 500 μmol/L. While increased homocysteine levels can cause symptoms such as osteoporosis and eye lens dislocation, high homocysteine levels are most closely associated with cardiovascular complications. Recent advances have shown that increased plasma Hcy is also a fundamental cause of neurodegenerative diseases (including Alzheimer’s disease, Parkinson’s disease, and dementia), diabetes, Down syndrome, and megaloblastic anemia, among others. In recent years, increased plasma homocysteine has also been shown to be closely related to cancer. In this review, we discuss the relation between elevated plasma Hcy levels and cancer, and we conclude that disturbed homocysteine metabolism is associated with cancer. Future clinical perspectives are also discussed.

Introduction
Homocystinuria is an inborn error in the metabolic pathways of sulfur-containing amino acids and is characterized by an increase in the level of toxic homocysteine (Hcy) in the serum¹. Mutations in cystathionine beta synthase (CBS), an enzyme present at the branch point between the trans-sulfuration and remethylation pathways, are the basic cause of homocystinemia. The term “hyperhomocysteinemia” is also used to describe the elevated Hcy serum level due to other genetic (CBS-independent) and environmental factors². In a normal, healthy individual, the serum Hcy level is between 5–15 μM, but it can increase to 50 μM in mild cases and to 500 μM in severe cases of homocystinemia (de Koning, Werstuck et al. 2003). This hyperhomocysteinemic condition is closely related to many disease conditions (Table 1). It is believed that increased homocysteine levels lead to various cardiovascular complications (Table 1)³⁴. If the Hcy level is left uncontrolled, patients ultimately die of stroke⁵. Further studies have also revealed that elevated plasma Hcy level is one of the key factors associated with neurodegeneration, diabetes, Down syndrome, neural tube defects, and megaloblastic anemia (see Table 1)³⁴. Hyperhomocysteinemia has also been connected to various other clinical complications, including ectopic lens, scoliosis, megaloblastic anemia, knocked knees, long limbs, and arachnodactyly, among others (Table 1)⁴. Recent advances have proven that there is a close link between hyperhomocystinuria and cancer (see Fig. 1). First, higher levels of plasma homocysteine have been observed cancer patients, and venous thromboembolism (VTE) is the second most common cause of death in cancer patients. Second, several polymorphisms in the enzymes involved in the Hcy detoxification pathways (the trans-sulfuration and remethylation) have close clinical ties to several cancer types¹³–²³. Third, folate, which is pivotal for cell proliferation, has an inverse relation with Hcy. Fourth, Hcy has also been proposed as a potential tumor biomarker for a variety of cancers²⁴. In this review, we have systematically discussed these important key events in detail and revealed that defects in Hcy metabolism may lead to cancer. Future clinical perspectives have also been described.
I (a) Low folate levels help build plasma Hcy

Homocysteine is a sulfur-containing, nonprotein, toxic amino acid found in the pathway for the interconversion of two amino acids: methionine and cysteine. Homocysteine is metabolized via two different pathways: remethylation and trans-sulfuration. When there is an excess of cellular methionine, the trans-sulfuration pathway plays a crucial role in Hcy metabolism, converting Hcy to cystathionine via CBS, which requires pyridoxal 5’-phosphate as a co-factor. When the cellular methionine level is low, Hcy is remethylated back to methionine in a betaine- or folate-dependent reaction. In the betaine-dependent pathway, the enzyme betaine-homocysteine S-methyltransferase (BHMT) catalyzes the incorporation of a methyl group from betaine into homocysteine to form methionine. In the folate-dependent pathway, Hcy acquires a methyl group from N-5-methyltetrahydrofolate with the help of 5-methyltetrahydrofolate-homocysteine methyltransferase (MTR) (also known as methionine synthase). Methionine synthase requires vitamin B12 for its functionality, and the reaction also involves recycling of tetrahydrofolate (from N-5-methyltetrahydrofolate), which may eventually be used for nucleotide biosynthesis. Methionine synthase, therefore, couples the folate and Hcy metabolism pathways (Fig. 2). Since the generation of tetrahydrofolate depends on the input of exogenous folate for folate metabolism (as outlined in Fig. 2), low folate levels ultimately result in substrate limitation for methionine synthase, thereby affecting the remethylation pathway. Thus, low folate levels result in a high plasma Hcy concentration and vice versa.

Many factors that affect the folate level have also been found to disturb the Hcy level. For instance, diets deficient in folate, cobalamin, and vitamin B6 and use of antifolate drugs (including anticonvulsants and other neurological drugs) directly increase the plasma Hcy level. Drugs that elevate the Hcy level (e.g., laxatives, diuretics, birth control pills, anti-inflammatory drugs, immune suppressants) also reduce the folic acid levels. Other conditions, including alcohol consumption, smoking, diabetes, and psoriasis, among others, are responsible for reducing the plasma folate level by affecting the folate level. Therefore, it is important to take folate supplements to restore the depleted Hcy pool.

Table 1 Homocysteinemia and its associated disorders

| Complication             | Associated diseases                      | References |
|--------------------------|------------------------------------------|------------|
| Cardiovascular diseases  | Thromboembolism                          | 130, 131   |
|                          | Coronary artery                          | 132        |
|                          | Atherosclerosis                          | 133, 134   |
|                          | Vascular dementia                        | 135, 136   |
|                          | Congenital heart defects                 | 137, 138   |
|                          | Stroke                                   | 139, 140   |
| Neurodegeneration         | Alzheimer’s                              | 6          |
|                          | Parkinson                                | 141, 142   |
|                          | Schizophrenia                            | 143, 144   |
|                          | Dementia                                 | 145, 146   |
|                          | Depression                               | 147, 148   |
| Diabetes                 | —                                        | 149, 150   |
| Down’s syndrome           | —                                        | 151        |
| Megaloblastic anemia      | —                                        | 152        |
| Other diseases            | Neural tube defects                      | 55         |
|                          | Nonsyndromic oral cleft                  | 153        |
|                          | Ectopic lentis                           | 6, 130, 140|
|                          | Scoliosis                                | 154        |
|                          | Knocked knees                            | 154        |
|                          | Long limbs                               | 154        |
|                          | Arachnodactyly                           | 154        |
| Cancer                   | Refer to Fig. 3                          |            |

Fig. 1 Association between hyperhomocysteinemia and various cancer types
I (b) Low plasma folate levels lead to cancer predisposition

Folate is not only involved in nucleotide biosynthesis but also required for the conversion of deoxyuridine monophosphate (dUMP) into thymidine monophosphate. Under normal conditions, thymidylate synthase (TYMS) converts dUMP into thymidine monophosphate using 5,10-methylenetetrahydrofolate (derived from folate) as a methyl group donor. If folate is limiting, dUMP accumulates because its key methyl donor, 5,10-methylenetetrahydrofolate, is absent. These conditions lead to an imbalance in the deoxyribonucleotide pool, and, consequently, there is excessive incorporation of uracil into DNA instead of thymine; this defect is normally repaired by the enzyme uracil DNA glycosylase, which removes the misincorporated uracil from the DNA strand. When the folate concentration is disturbed (due to increased Hcy levels) the DNA glycosylase fails to cope with the DNA repair burden. This situation leads to chromosomal damage, which may then lead malignant transformation in cells. Furthermore, excision repair of uracil residues 12 base pairs apart can lead to double strand breaks, which may increase DNA instability due to relaxed DNA supercoiling and chromosomal remodeling, both of which can cause an increase in malignant transformation. Chromosomal aberrations are also associated with inappropriate differentiation and morphology of lineage-specific cells, features often associated with tumors.

Low plasma folate levels are also linked to cancer via DNA methylation. DNA methylation is an epigenetic modification that is critical for normal genome regulation and development. Indeed, it is Hcy that is recycled to methionine with the help of methionine synthase. DNA methylation is carried out with the help of a methyl donor, S-adenosyl-l-methionine (SAM), which is obtained from methionine via an ATP-dependent reaction catalyzed by S-adenomethyl synthetase. DNA methylation is jointly carried out by three types of DNA methyltransferases (DNMTs)—DNMT1, DNMT3a, and DNMT3b on SAM (Fig. 3). Since SAM is generated from 5-methyltetrahydrofolate (5′-MTHF) as shown in Fig. 3, low folate levels limit the substrate availability for methionine synthase, thereby resulting in DNA hypomethylation. DNA hypomethylation leads to decondensation of pericentromeric heterochromatin and the activation of retrotransposon elements. Global genomic hypomethylation has been found in many types of cancer, including prostate metastatic tumors, chronic lymphocytic tumors, and hepatocellular carcinoma. Regional hypomethylation of DNA sequences is also often observed during the early stages of tumorigenesis and in abnormal nonneoplastic tissue, such as hyperplasia.
I (c) Cancer patients have high plasma Hcy levels

As mentioned in the earlier sections, there is an inverse relation between plasma Hcy and folate. In cancer patients, the plasma folate level is expected to be low because tumor cells must draw folate from the blood for de novo purine synthesis.44,45 Interestingly, as shown in Fig. 1, hyperhomocystinuria is associated with several types of cancer. It is also clear from the information in this figure that the causative relationship between homocysteine toxicity and cancer is independent of the organ/tissue and the type of cancer. Table 2 shows that all cancer types in the advanced stage exhibit high plasma Hcy levels, while there was no significant change in plasma Hcy levels in early stage cancer. Furthermore, once patients are subjected to surgery or chemotherapy, there is also a sharp increase in the plasma Hcy level, leading to a higher frequency of thromboembolic events. Because most commonly used clinical chemotherapeutic agents (such as alkylating agents, antimetabolites, methotrexate, hormones, and antagonists) are anti-folate drugs, their use causes a decrease in the plasma folic acid concentration. In another development, it has also been shown that older cancer patients are at a higher risk of developing hyperhomocysteinemia than are younger patients.

There is no clear explanation for why the Hcy levels vary between early and late stage cancer. However, we speculate that cells in the early stage might not secrete Hcy, as it facilitates the proliferation process of cancer cells.48 Studies have shown that increased homocysteine levels lead to increased cellular proliferation in Caco-2 cell lines. This enhanced proliferation can be reversed by folate supplementation in the culture medium or by supplementation with its downstream metabolites, such as methionine, choline, and betaine. It is possible that these metabolites can inhibit the methyltransferases involved in the conversion of Hcy to methionine, thus reducing the availability of methyl groups for DNA and RNA synthesis.
| Gene                                      | Polymorphisms | Amino acid change | Cancer type                                | OR values       | References |
|-------------------------------------------|---------------|------------------|--------------------------------------------|-----------------|------------|
| Methylenetetrahydrofolate reductase (MTHFR) | 677C- > T     | A226V            | Endometrial carcinoma                      | 1.10            | 155        |
|                                           |               |                  | Esophage squamous cell carcinoma (SCC)     | 1.47            | 156        |
|                                           |               |                  | Breast cancer                              | 1.00 / 1.12     | 15, 99, 157|
|                                           |               |                  | Acute lymphocytic leukemia (ALL)           | 0.99 / 2.33     | 87         |
|                                           |               |                  | Prostate cancer                            | 0.78            | 158        |
|                                           |               |                  | Colorectal cancer                          | 1.78 / 1.00     | 85, 159-161|
|                                           | 1298A- > C    | E443A            | Prostate cancer                            | 0.58            | 79         |
|                                           |               |                  | Acute myeloid leukemia                      | 0.33 / 1.00     | 87, 162    |
|                                           |               |                  | Endometrial cancer                         | 0.88            | 155        |
|                                           | 1793G- > A    | R1793E           | Colorectal cancer                          | 0.17            | 163        |
|                                           |               |                  | Acute myeloid leukemia                      | 1.00            | 162        |
| Methionine synthase reductase (MTRR)      | 66A- > G      | I22M             | Leukemia                                   | 1.00*           | 164, 165   |
|                                           |               |                  | Colorectal cancer                          | 2.07 / 2.77     | 18, 165, 166|
|                                           |               |                  | Gastric cancer                             | 0.74 / 1.39     | 167, 168   |
|                                           |               |                  | Breast cancer                              | 4.45            | 169        |
| Methionine synthase (MTR)                 | 2756A- > G    | D919G            | Head and neck carcinoma                    | 1.10            | 170        |
|                                           |               |                  | Colorectal cancer                          | 1.03 / 1.04     | 161, 170, 171|
|                                           |               |                  | Lung cancer                                | 1.34            | 172        |
|                                           |               |                  | Hepatocellular carcinoma                   | 1.01            | 170, 173   |
|                                           |               |                  | Cervical cancer                            | 0.27            | 14         |
|                                           |               |                  | Glioblastoma multiforme                    | 1.00*           | 174        |
|                                           |               |                  | Breast cancer                              | 1.00*           | 99         |
|                                           |               |                  | Squamous cell carcinoma                    | 1.00*           | 98         |
|                                           |               |                  | Gastric cancer                             | 1.06 / 1.35     | 168, 170, 175|
|                                           |               |                  | Pancreatic cancer                          | 1.08 / 1.35     | 170, 176   |
| Methylenetetrahydrofolate dehydrogenase (MTHFD1) | 1958G- > A  | A653G           | Gastric cancer                             | 2.05            | 102        |
|                                           |               |                  | Leukemia                                   | 0.80            | 177        |
|                                           | 401G- > A     | R134K            | Gastric cancer                             | 1.43            | 102        |
|                                           |               |                  | Leukemia                                   | 0.89            | 177        |
|                                           |               |                  | Ovarian cancer                             | 0.97            | 178        |
|                                           |               |                  | Squamous cell carcinoma                    | 1.07            | 179        |
| Betaine-homocysteine methyltransferase (BHMT) | 742G- > A   | R239Q           | Breast cancer                              | 0.98 / 0.12     | 111, 180   |
|                                           |               |                  | Uterine carcinoma                          | 0.64            | 181        |
|                                           |               |                  | Ovarian cancer                             | 1.01            | 182        |
|                                           |               |                  | Colorectal adenoma                         | 1.09            | 183        |
as 5-MTHF. However, advanced-stage cancer cells might secrete Hcy because a very high Hcy concentration might also be cytotoxic to the cancer cells. Therefore, it may be important for proliferating cells to maintain an optimum Hcy concentration. This speculation, however, requires further experimental validation.

I (d) Cancer patients develop thromboembolisms due to Hcy toxicity

One major symptom of hyperhomocysteinemia is the formation of venous thromboembolism (VTE). VTE is the most frequent complication and second most common cause of death among cancer patients. Advanced-stage cancer patients develop both hyperhomocysteinemia and VTE. Alternatively, in early cancer patients (without homocysteinuria), VTE is absent. Indeed, the advanced-stage cancer patients have a greater risk for developing VTE, with a frequency of 5–15% (in comparison, the risk for the normal population is 0.1%). Postchemotherapy cancer patients (who are known to be at risk for homocystinuria) account for 13% of the total pool of VTE patients. In postsurgery patients, their susceptibilities to embolism and thrombosis are increased three-fold and two-fold, respectively. Use of central venous catheters and hormonal adjuvant therapy (e.g., Tamoxifen) also predisposes patients to VTE due to increased plasma Hcy levels. Thus, there is a close link between cancer and Hcy-induced development of VTE.

The mechanism underlying the cancer-related thrombosis induced by elevated Hcy is complex and not well understood. However, it has been thought to result from endothelial disturbances caused by the formation of Hcy-mediated free-radicals. Hcy is a pro-oxidant, and the formation of Hcy-Hcy dimers and Hcy-protein adducts that help to generate free radicals are well established. Hcy can also form a more highly reactive compound called homocysteine thiolactone. Homocysteine thiolactone has been known to form covalent adducts with

### Table 2 continued

| Gene                  | Polymorphisms | Amino acid change | Cancer type             | OR values | References |
|-----------------------|---------------|-------------------|-------------------------|-----------|-----------|
|                       |               |                   | Liver cancer            | 0.98      | 184       |
| 595G->A               | G199S         |                   | —                       |           |           |
| 716G->A               | Q239R         |                   | —                       |           |           |
| 1218G->T              | Q406H         |                   | —                       |           |           |
| Cystathionine β-synthase (CBS) | 833T->C     | I278T             | —                       |           |           |
|                       | 699C->T       | Y233Y             | —                       |           |           |
|                       | 1080C->T      | A360A             | —                       |           |           |
|                       | 572C->T       | T191M             | —                       |           |           |
|                       | 139C->T       | S466L             | —                       |           |           |
|                       | 502G->A       | V168M             | —                       |           |           |
|                       | 797G->A       | R266K             | —                       |           |           |
|                       | 1150A->G      | K384E             | —                       |           |           |
|                       | 341C->T       | A114V             | —                       |           |           |
|                       | 919G->A       | G307S             | —                       |           |           |
| TCN 2                 | 776G->C       | R259P             | Colorectal adenoma      | 0.753     | 183       |
|                       |               |                   | Colorectal cancer       | 1.137     | 185       |
|                       |               |                   | Glioblastoma           | 1.028     | 174       |
|                       |               |                   | Primary central nervous system lymphoma | 1.338 | 186 |
|                       |               |                   | Ovarian cancer         | 1.389     | 182       |
| TYMS                  | TS 3' UTR     |                   | Esophageal cancer      | 0.73      | 187       |
|                       |               |                   | Stomach cancer         | 1.12      | 187       |
|                       | TSER          |                   | Breast cancer          | 1.09      | 187       |

*Papers that reported no association have been given the value of 1.00*
lysine or arginine residues in proteins, resulting in the formation of insoluble toxic protein aggregates or amyloids. The deposition of such aggregates in the blood or heart may, therefore, impede normal heart function and physiology. Furthermore, modification of hemostatic proteins (via N-homocysteinylation or S-homocysteinylation) has also been reported to impede NO metabolism, which may cause biotoxicity in endothelial cells. Hcy also inhibits thrombomodulin and Protein C-dependent inactivation of Factor Va, therefore, blood coagulation is enhanced in the presence of Hcy. Furthermore, Hcy limits the secretion of nitric oxide (NO), leading to increased platelet aggregation and decreased antithrombic activities in the endothelial cells.

I (e) Allelic polymorphisms in sulfur metabolism genes and associated risk of cancer

Various case control and cohort studies have shown that mutations and polymorphisms exist in genes involved in homocysteine metabolism (MTHFR, CBS, MTRR, MTR, MTHFD, BHMT, TYMS, TCN 2). Polymorphic alleles of these genes were found to be linked with neural tube defects and/or vascular thromboembolism, which are symptoms of hyperhomocysteinemia. Recent studies have shown that these polymorphisms are also closely associated with different cancer types (Table 2). For instance, MTHFR has ~6375 polymorphisms, consisting of 650 deletions, 05 multiple base substitutions, 140 repeat variations, and 5580 SNPs. Two common polymorphisms, a 677C>T transition at codon 222 (Ala222Val) and a 1298A>C transversion at codon 429 (Glu429Ala), have been reported to be associated with various cancer types, including endometrial carcinoma, esophageal squamous cell carcinoma (SCC), colon cancer, acute lymphocytic leukemia (ALL), and prostate cancer. In addition to 677C>T and 1298A>C, there is a third polymorphism, 1793G>A, whose frequency is very low (~4.6% or less) and which is confined to colorectal cancer. The 677C>T polymorphism affects the protein’s catalytic activity and the 1298A>C polymorphism affects its regulatory function. Homozygotes (677CC, ~60%) are more frequent than heterozygotes (677CT, ~31%), but this pattern is reversed in the case of 1298 (1298AC, ~53% and 1298AA, ~31%). The 677TT and 1298CC homozygotes were found to have reduced prostate cancer risk, as the frequencies are very low (9 and 11%, respectively). The risk factor associated with the 677C>T polymorphism has been found to depend on the type of cancer, as it confers a higher risk for endometrial carcinoma, esophageal SCC, and prostate cancer, while it has little or no effect on the risk for colon cancer and acute lymphoid leukemia. The variable behavior of 677C>T in different cancer types indicates that the environment or genetic background might help to dictate the activity of the polymorphism. In this context, a number of factors have been proposed, including folate status, methionine, and the effects of alcohol consumption, to be the risk factor connecting the 677C>T polymorphism to colorectal cancer. Another possibility is that the 677C>T polymorphism is dominant negative in some cancer types but not in others based on the functional effect of the polymorphism.

MTRR has ~9461 polymorphisms, out of which 1051 are deletions, 01 are multiple base substitutions, 315 are repeat variations, and 8094 are SNPs. Out of this pool, only one polymorphism (MTRR A66G, Ile22Met) has been studied and found to be associated with leukemia and colorectal cancer. This leukemic polymorphism has an allelic frequency of 51% in white populations. Comparison of the relationships between homozygosity and heterozygosity and colorectal cancer revealed that homozygotes (GG) have a three-fold higher risk compared with that of heterozygotes (AG). Since the leukemic allelic frequency is very high in the white population, it is important to investigate other populations to determine if genetic background affects the frequencies or functions of polymorphic MTRR.

Similarly, MTR has ~26150 polymorphisms, out of which 2643 are deletions, 06 are multiple base substitutions, 221 are repeat variations, and 23245 are SNPs. One significant polymorphism (MTR A2756G; Asp919Gly) has been documented in MTR. This 2756A>G variant is associated with head, esophageal and neck squamous cell carcinoma, colorectal adenoma, colorectal carcinoma, lung cancer, multiple myeloma, cervical cancer, uterine cancer, and glioblastoma multiforme. This polymorphism is associated with a decreased risk for colorectal cancer, and no correlation could be established between this MTR variant and the incidence of breast and upper gastrointestinal tract carcinomas, suggesting that the severity of the effect of the polymorphism might depend on the type of cancer.

MTHFD-1 has ~16991 polymorphisms, out of which 1913 are deletions, 07 are multiple base substitutions, 215 are repeat variations, and 14856 are SNPs. One polymorphism (1958G>A; Ala653Gly) associated with acute lymphoid leukemia, which is located within the 10-formyl THF synthetase domain, has been reported. No links between MTHFD variants and lung cancer risk could be established (Liu, Jin et al. 2008). Another polymorphism, (401G>A; Arg134Lys), which is in the cyclohydrolase/dehydrogenase domain, was also reported, but it is associated with a low colon cancer risk. TYMS expression is a highly regulated process that is modulated by unique tandem repeat sequences and significant polymorphisms in the 5-UTR of the thymidylate synthase enhancer region (TSER) and in the 3-UTR.
Concentrations of holotranscobalamin and higher Hcy levels are associated with different cancers, including colorectal cancer, ovarian cancer, and central nervous system lymphoma, as indicated by OR values near or equal to 1. However, most of the cancer types did not have close associations with these polymorphisms, depending on the type of cancer. In the cases of stomach, colorectal, and lung cancer, the TYMS enzyme activity and mRNA expression were found to be increased.

Transcobalamin II (TCN II) is a serum protein that transports vitamin B12 (cobalamin) from the ileum to other tissues. Vitamin B12 serves as a crucial molecule in the remethylation of methionine from Hcy and is important for the transformation of MTHF to THF. TCN-2 has ~8291 polymorphisms, out of which 1183 are deletions, 02 are multiple base substitutions, 146 are repeat variations, and 6960 are SNPs. One common polymorphism in the TCN 2 gene is a G to C substitution (776 G > C, rs1801198) that results in the replacement of a proline with an arginine. In a recent meta-analysis, it was shown that subjects with the rs1801198 GG genotype had significantly lower concentrations of holotranscobalamin and higher Hcy levels compared to subjects with the rs1801198 CC genotype. This polymorphism has also been shown to be associated with different cancers, including colorectal cancer, ovarian cancer, glioblastoma, among others. However, most of the cancer types did not have close association, as indicated by OR values near or equal to 1.

Colorectal cancer, ovarian cancer, and central nervous system lymphoma, on the other hand, have higher OR values and, therefore, exhibit significant association (Table 2). Therefore, it is thought that the TCN 2 polymorphism rs1801198 significantly alters the circulating holotranscobalamin levels. Since TCN 2 plays a vital role in vitamin B12 metabolism, it is reasonable to suspect that the rs1801198 polymorphism may affect pathological conditions related to vitamin B12 deficiency.

BHMT has ~5271 polymorphisms, out of which 484 are deletions, 01 multiple base substitution, 140 are repeat variations, and 4648 are SNPs. Out of these variations, three significant mutations (Gly199Ser, Glu239Arg, and Glu406His) and one polymorphism have been examined. The polymorphism is the G to A substitution (742 G > A, rs3733890), which replaces arginine by glutamine at codon 239. A study incorporating meta-analysis was performed to investigate the rs3733890 polymorphism and cancer susceptibility. It was shown that this polymorphism showed no statistically significant association with increased risk of various cancers, including head and squamous cell carcinoma, breast cancer, ovarian cancer, colorectal adenoma, and liver cancer. However, a negative association was observed in uterine cervical cancer (Table 2).

CBS has ~6617 polymorphisms, out of which 573 are deletions, 04 are multiple base substitutions, 1059 are repeat variations, and 4981 are SNPs. Interestingly, none of these mutations have been shown to cause any significant predisposition towards any type of cancer. This observation opens an avenue for future research studies focusing on understanding and identifying the frequencies of BHMT and CBS genotypes and their associations with and prevalence in different cancer types.

I (f) CBS is associated with cancer via H2S production

H2S, which is a signaling molecule, is substantially involved in vasorelaxation, acting as a neuromodulator. Recently, H2S has gained attention in cancer due to its cytotoxic and cytoprotective effects. It plays a key role in the bioenergetics of tumor cells and stimulates their proliferation, migration, and invasion. In humans, CBS normally catalyzes the condensation of serine with homocysteine to produce cystathionine and water, a pivotal reaction in the trans-sulfuration pathway. In an alternative reaction, CBS can produce H2S via β-elimination and β-replacement reactions. The β-elimination reaction involves catalysis of cysteine by CBS with corresponding H2S production, whereas in the β-replacement mechanism, the reaction between L-cysteine and 2-mercaptoethanol enables CBS to produce H2S. Various clinical studies have shown that there is CBS overexpression and, hence, increased H2S production, in many cancer types, including colon, ovarian, gastric, colorectal, prostate, gastroesophageal cancer, and endometrial cell angiogenesis. (Fiorucci, Antonelli et al.; Guo, Gai et al.; Bhattacharyya, Saha et al.; Szabo, Coletta et al.; Modis, Coletta et al.; Hellmich, Coletta et al.; Katsouda, Bibi et al.). The role of H2S in cancer has been elucidated. It is known to enhance tumor growth and increase cellular proliferation by (i) stimulating cellular bioenergetics, (ii) activating proliferative, migratory, and invasive signaling pathways, and (iii) enhancing angiogenesis in tumors. Other studies have demonstrated that HCT116 cells (a transformed cell line) have CBS upregulation and enhanced H2S production compared to nontransformed cells.

ShRNA-mediated silencing of CBS or suppression of its activity by pharmacological means (using aminooxyacetic acid) results in reduced mitochondrial function (ATP...
turnover, respiratory reserve capacity, and oxygen consumption) and impaired glycolysis. A clinical study of colon cancer showed reduced angiogenesis and increased growth in xenografts derived from colon cancer patients incubated in mice treated with aminooxyacetic acid. Alternatively, H$_2$S production induces angiogenesis in various experimental models. In another development, investigated the effects of S-adenosyl-L-methionine (SAM) on tumor bioenergetics. SAM is an allosteric activator of CBS that binds to the regulatory domain of CBS. The study revealed that SAM enhances H$_2$S production of in the HCT116 cancer cell line. Mechanistically, it is not completely known how H$_2$S helps to stimulate tumor growth. However, it has been argued that H$_2$S serves as an autocrine stimulator during tumor proliferation. Therefore, modulation of the CBS and H$_2$S levels could help limit cancer proliferation and promote its reversal.

**Conclusion**

It is clear from this review that there are compelling genetic, epigenetic and environmental factors that establish a close association between disturbed Hcy metabolism and cancer. Therefore, Hcy-elevating drugs should be restrictively prescribed to cancer patients, and clinicians should closely monitor Hcy levels after chemotherapy or surgery. To date, the effects of Hcy on the growth and proliferation of tumor cells remain poorly understood. Insight into the effects of Hcy on the growth and proliferation of cancer cells would yield novel, promising strategies to curb cancer. Nevertheless, Hcy can be used as a potential tumor biomarker for a variety of cancers.

**Conflict of interest**

The authors declare that they have no conflict.

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33. Smith, J. L., Goldsmith, G. A. & Lawrence, J. D. Effects of oral contraceptive steroids on vitamin and lipid levels in serum. *Am. J. Clin. Nutr.* 28, 371–376 (1975).

34. Grant, C. E. G. The contraceptive pill: its relation to allergy and illness. *Nutr. Health* 2, 33–40 (1983).

35. Amatayakul, K., Uttaravichai, C., Singkamani, R. & Ruckphaopunt, S. Vitamin metabolism and the effects of multivitamin supplementation in oral contraceptive users. *Contraception* 30, 179–196 (1984).

36. Hjelte, K., Brynskov, J., Hippe, E., Lundström, P. & Munck, O. Oral contraceptive and the cobalamin (Vitamin B12) metabolism. *Acta Obest. Gynecol. Scand.* 64, 59–63 (1985).

37. Obwegeser, R., Hohlagschwander, M. & Sinzinger, H. Homocysteine-a pathophysiological cornerstone in obstetrical and gynaecological disorders? *Hum. Reprod. Update* 5, 64–72 (1999).

38. Montfort, M. W. & Fajer. Structure, multiple site binding, and segmental accommodation in thymidylate synthase binding to dUMP and an antifolate. *Biochemistry* 29, 6964–6977 (1990).

39. Blount, B. C. et al. Folate deficiency causes uracil misincorporation into human DNA and chromosome breakage: implications for cancer and neuronal damage. *Proc. Natl Acad. Sci. USA* 94, 3290–3295 (1997).

40. Hay, R. K. M., Park, J.-G. & Gazdar, A. Hyperhomocysteinemia in women with advanced breast cancer: clinical and biochemical implications. *Int. J. Lab. Hematol.* 42, 1–9 (2010).

41. Ehrlich, M. DNA methylation in cancer: too much, but also too little. *Nat. Rev. Cancer* 6, 257–266 (1996).

42. Hall, L. E., Mitchell, S. E. & O’Donnell, C. J. DNA hypomethylation, cancer, the immunodepression conundrum. *Nat. Rev. Immunol.* 2, 433–443 (2002).

43. Ehrlich, M. DNA methylation in cancer: too much, but also too little. *Genetics* 153, 540–541 (2000).

44. Zhang, D., Wen, X., Wu, W., Guo, Y. & Cui, W. Elevated homocysteine level and folate deficiency associated with increased overall risk of carcinogenesis: meta-analysis of 83 case-control studies involving 35,758 individuals. *PLoS ONE* 10, e0124923 (2015).

45. Goyette, P. et al. Molecular detection of cytokeratin 19–positive cells in the peripheral blood of patients with operable breast cancer: evaluation of their prognostic significance. *J. Clin. Oncol.* 20, 3404–3412 (2002).

46. Refsum, H. et al. The Hordaland Homocysteine Study: a community-based study of homocysteine, its determinants, and associations with disease. *J. Nutr.* 136, 17315–17405 (2006).

47. Sun, C.-F., Haven, T. R., Wu, T.-L., Tsao, K.-C. & Wu, J. J. Serum total homocysteine increases with the rapid proliferation rate of tumor cells and decline upon cell death: a potential new tumor marker. *Clin. Chim. Acta* 321, 55–62 (2002).

48. Akgul, B., Milovic, V., Caspary, W. F. & Faust, D. Hyperproliferation of homocysteine-treated colon cancer cells is reversed by folate and 5-methyltetrahydrofolate. *Eur. J. Nutr.* 43, 93–99 (2004).

49. Rickles, F. R., Levine, M. & Edwards, R. L. Hemostatic alterations in cancer patients. *Cancer Metastasis Rev.* 11, 237–248 (1992).

50. GATT, A. et al. Hyperhomocysteinemia in women with advanced breast cancer. *Int. J. Lab. Hematol.* 29, 421–425 (2007).

51. Green, K. B. & Silverstein, R. L. Hypercoagulability in cancer. *Hematol. Oncol. Clin. North Am.* 10, 499–530 (1996).

52. Hest, J. A. et al. Effect of folate and B-6 on the impact of risk factors for deep vein thrombosis and pulmonary embolism: a population-based study. *Arch. Intern. Med.* 162, 1245–1248 (2002).

53. Kakkar, A., Haas, S., Walsh, D. & Encke, A. Prevention of perioperative venous thromboembolism: outcome after cancer and noncancer surgery. *Br. J. Surg.* 88, 47 (2001).

54. Zhu, H. et al. Homocysteine remethylation enzyme polymorphisms and increased risks for neural tube defects. *Mol. Genet Metab.* 78, 216–221 (2003).

55. Welch, G. N. & Loiczo, J. Homocysteine and atherothrombosis. *N. Engl. J. Med.* 338, 1042–1050 (1998).

56. Chowhan, R. K., Mittal, S., Dar, T. A., Karnal, M. A. & Singh, L. R. Ignored avenues in alpha-synuclein associated proteopathy. *ChN Neurol. Drug Targets* 13, 1246–1257 (2014).

57. Sharma, G. S., Kumar, T. & Singh, L. R. N-homocysteinylation induces different structural and functional consequences on acidic and basic proteins. *PLoS ONE* 9, e116386 (2014).
134. Harker, L. A., Harlan, J. M. & Ross, R. Effect of supplementing on homocysteine-induced endothelial injury and atheroma formation. Circ. Res. 53, 731–739 (1983).
135. Quadri, P. et al. Homocysteine, folate, and vitamin B-12 in mild cognitive impairment. Alzheimer disease, and vascular dementia. Am. J. Clin. Nutr. 80, 114–122 (2004).
136. McIvor, S. P., Dynan, K. B., Lawson, J. T., Patterson, C. C. & Passmore, A. P. Moderately elevated plasma homocysteine, methylenetetrahydrofolate reductase genotypes, and risk for stroke, vascular dementia, and Alzheimer disease in Northern Ireland. Stroke 33, 2351–2356 (2002).
137. Rosenquist, T. H., Ratatsh, S. A. & Selhub, J. Homocysteine induces congenital defects of the heart and neural tube: effect of folate acid. Proc Natl Acad USA 93, 15227–15232 (1996).
138. Hobbs, C. A., Cleves, M. A., Melnyk, S., Zhao, W. & James, S. J. Congenital heart defects and abnormal maternal biomarkers of methionine and homocysteine metabolism. Am. J. Clin. Nutr. 81, 147–153 (2005).
139. McDowell, J. F. & Lang, D. Homocysteine and endothelial dysfunction: a link with cardiovascular disease. J. Nutr. 130, 3695–3725 (2000).
140. Relfsrm, H., Ueland, P. M., Nygard, O. & Volland, S. E. Homocysteine and cardiovascular disease. Ann. Rev. Med. 49, 31–62 (1998).
141. Martignoni, E. et al. Homocysteine and Parkinson's disease: a dangerous liaison? J. Neural. Sci. 257, 31–37 (2007).
142. Lamberti, P. et al. Effects of levodopa and COMT inhibitors on plasma homocysteine in Parkinson disease patients. Mov. Discov. 20, 69–72 (2005).
143. Moustafa, A. A., Hewedi, D. H., Issa, A. M., Fryderek, D. & Missak, B. Homocysteine levels in schizophrenia and affective disorders-focus on cognition. Front. Behav. Neurosci. 8, 343 (2014).
144. Muntyan, J.-W., Kahn, R. S., Blom, H. J. & den Heijer, M. Homocysteine, methylenetetrahydrofolate reductase and risk of schizophrenia: a meta-analysis. Mol. Psychiatry 11, 143 (2006).
145. Leblhuber, F. et al. Hyperhomocysteinemia in dementia. J. Neural. Transm. 107, 1469–1474 (2003).
146. Shea, T. B. & Rogers, E. Homocysteine and dementia. N Engl. J Med 346, 2007–2008 (2002).
147. Tierney, H. et al. Vitamin B12, folate, and homocysteine in depression: the Rotterdam Study. Am. J. Psychiatry 159, 2099–2101 (2002).
148. Almeida, D. O. et al. Homocysteine and depression in later life. Arch. Gen. Psychiatry 65, 1286–1294 (2008).
149. Janula, A. et al. Homocysteine and diabetes. Wiad. Lek. (Wars., Pol. 1960) 58, 319–323 (2005).
150. Baliga, B. S., Reynolds, T., Fink, L. M. & Forseca, V. A. Hyperhomocysteinemia in type 2 diabetes mellitus: cardiovascular risk factors and effect of treatment with folate and pyridoxine. Endocr. Pract. 6, 435–441 (2000).
151. Gueant, J. L. et al. Homocysteine and related genetic polymorphisms in Down’s syndrome IQ. J. Neural. Neurosurg. Psychiatry 76, 706–709 (2005).
152. Schuh, S. et al. Homocystinuria and megaloblastic anaemia responsive to vitamin B12 therapy. An inborn error of metabolism. N. Engl. J. Med 310, 688–690 (1984).
153. Wong, W. Y. et al. Nonsyndromic orofacial clefts: association with maternal homocysteine metabolism-related genetic enzyme genes and colorectal cancer: a nested case-control study. Zhonghong Zhong Liu Za Zhi 28, 429–432 (2006).
154. Shu, Q. et al. Polymorphisms of methionine synthase and methionine synthase reductase and risk of lung cancer: a case-control analysis. Pharm. Genom. 15, 547–555 (2005).
155. Cui, L.-H. et al. Folate metabolism-related gene polymorphisms and susceptibility to primary liver cancer in North China. Med. Oncol. 29, 1837–1842 (2012).
156. Demmert, A., Simon, M., Moskau, S. & Linnebank, M. The methionine synthase polymorphism c2756A>G alters susceptibility to globulostoma multi-forme. Cancer Epidemiol. Biomark. Prev. 15, 2514–2516 (2006).
157. Zhang, F. F. et al. Genetic polymorphisms in folate metabolism and the risk of stomach cancer. Cancer Epidemiol. Biomark. Prev. 16, 115–121 (2007).
158. Suzuki, T. et al. Alcohol drinking and one-carbon metabolism-related gene polymorphisms on pancreatic cancer risk. Cancer Epidemiol. Biomark. Prev. 17, 2742–2747 (2008).
159. Zhang, H., Ma, H., Li, L., Zhang, Z. & Xu, Y. Association of methylenetetrahydrofolate dehydrogenase 1 polymorphisms with cancer: a meta-analysis. PLoS ONE 8, e69366 (2013).
160. Cui, Y., Jing, Y. & Sun, Z. Lack of association between MTHFD1 G401A polymorphism and ovarian cancer susceptibility. Tumor Biol. 35, 3835–3839 (2014).
161. da Silva, L. M. R. B. et al. MTHFD1 G1958A, BHMT G742A, TC2 C776G and TC2 A667G polymorphisms and head and neck squamous cell carcinoma risk. Mol. Biol. Rep. 39, 887–893 (2012).
162. Xu, X. et al. B-vitamin intake, one-carbon metabolism, and survival in a population-based study of women with breast cancer. Cancer Epidemiol. Biomark. Prev. 17, 2109–2116 (2008).
163. Mostowska, A., Myka, M., Lianert, M., Roszk, A. & Jagodziński, P. P. Folate and choline metabolism gene variants and development of uterine cervical carcinoma. Clin. Biochem. 44, 596–600 (2011).
164. Pavlik, P. et al. Folate and choline metabolism gene variants in relation to ovarian cancer risk in the Polish population. Mol. Biol. Rep. 39, 5553–5560 (2012).
165. Hazra, A. et al. Twenty-four non-synonymous polymorphisms in the one-carbon metabolic pathway and risk of colorectal adenoma in the Nurses’ Health Study. Carcinogenesis 28, 1510–1519 (2007).
184. Chang, S.-C. et al. Single nucleotide polymorphisms of one-carbon metabolism and cancers of the esophagus, stomach, and liver in a Chinese population. *PLoS ONE* **9**, e109235 (2014).

185. Koushik, A. et al. Nonsynonymous polymorphisms in genes in the one-carbon metabolism pathway and associations with colorectal cancer. *Cancer Epidemiol. Biomark.* **15**, 2408–2417 (2006).

186. Kurzwelly, D. et al. Genetic variants of folate and methionine metabolism and PCNSL incidence in a German patient population. *J. Neurooncol.* **100**, 187–192 (2010).

187. Gao, C. M. et al. Polymorphisms in thymidylate synthase and methylenetetrahydrofolate reductase genes and the susceptibility to esophageal and stomach cancer with smoking. *Asian Pac. J. Cancer Prev.* **5**, 133–138 (2004).