Vitamin Bs, One Carbon Metabolism and Prostate Cancer

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Abstract: Somatic genetic and epigenetic alterations have been suggested to be crucially involved in development and progression of cancers including prostate cancer (PCa). Epigenetic alterations such as chemical modification of chromatin associated proteins and DNA methylation can largely affect gene expression that may be important for early normal organ development, and also produces favorable conditions for cancer cell formation, growth, and survival. Aberrant DNA methylation (hyper- or hypo-methylation) may lead to chromosomal instability, and transcriptional gene silencing for tumor suppressors or overexpression for oncogenes. Vitamin Bs play important roles in one carbon metabolism that provides critical metabolites for DNA methylation, DNA repair and nucleic acid synthesis. Nutrition uptake and circulating levels of these vitamin Bs as well as genetic polymorphisms of related key enzymes in the one carbon metabolism pathway may govern bioavailability of the metabolites, and therefore to affect the phenotypic changes (e.g., cellular malignancy) via genetic and epigenetic alterations. This article will summarize recent new findings from laboratory, epidemiological or clinical trial studies regarding influence of vitamin B and one carbon metabolism on PCa development or progression.

Keywords: One carbon metabolism, folate, folic acid, DNA methylation, vitamin B6, vitamin B12, SNP, prostate cancer.

INTRODUCTION

Prostate cancer (PCa) is the second leading cancer killer next to lung cancer in men of many highly developed countries including USA, UK and others. Although it was reported that prostate cancer incidence and mortality rates were slightly but significantly declined (http://www.cancer.gov/cancertopics/types/prostate), US National Cancer Institute estimated there are 192,280 new diagnosed prostate cancer cases and 27,360 death in year 2009. In UK, approximately 10,200 men will die from this cancer each year according to the recent statistics (http://info.cancerresearchuk.org/cancerstats/types/prostate/). While effective surgical and radiation treatments exist for clinically localized PCa, hormone-refractory metastatic PCa remains essentially incurable and most men diagnosed with metastatic disease will succumb over a period of months to years [1, 2]. Androgen ablation is the most common therapy for advanced PCa. In most cases, tumor re-emerges and can proliferate independently of androgens. In the U.S., there are 1.5 million men living with PCa who are at risk for recurrence and PCa death. Because of its high incidence, high morbidity and mortality, and especially long latency of significant clinical manifestation, PCa has been recommended as one of ideal target cancers for chemoprevention strategies. It is possible that proper diets and physical activities may eventually reduce at least 50% incidence of many types of cancer including PCa [3-6]. Total medical expenditure for PCa treatment in USA could exceed several billion dollars per year, successful prevention of PCa would mean largely reduction of a financial burden for individuals and community [7, 8]. This review will first discuss about how certain essential micronutrients (i.e., vitamin B2, B6, B12, folate, and choline) involved in one carbon metabolism may have influence on cancer risk and then focus on their effect more specifically on PCa risk.

Potential Mechanisms of One Carbon Metabolism in Cancer Development or Progression

Genomic DNA has been the center relating to the causes of many diseases including cancer. Obviously, DNA sequence mutation and aberrant epigenetic alterations have been suggested to be attributed to PCa development and progression. However, predisposed factors that can induce the somatic changes in the DNA that may lead to PCa remain to be revealed. Many vitamin Bs, especially folate or vitamin B9, may participate in one carbon metabolism that are involved in DNA synthesis, maintenance and repair as well as methylation modification. Therefore, they would be assumed to pertain to oncogenesis and progression of PCa and other cancers.

Some General Descriptions of One Carbon Metabolism and Related Pathways [9-11]

Since folate may enhance cancer cell growth, methotrexate, an antifolate agent that inhibits dihyrofolate reductase (DHFR) activity, has been used to treat cancer. DHFR is the enzyme to convert dihydrofolic acid, an inactive form of folate, to bioactive tetrahydrofolic acid (THF). Folic acid, an oxidized vitamin B9, is rarely found in dietary foods but usually in supplements or food fortification. The naturally occurring folates are mainly reduced methyl and formyl folylpolyglutamates with similar nutritional properties to that of folic acid. However, the bioavailability of natural folate from foods could be as low

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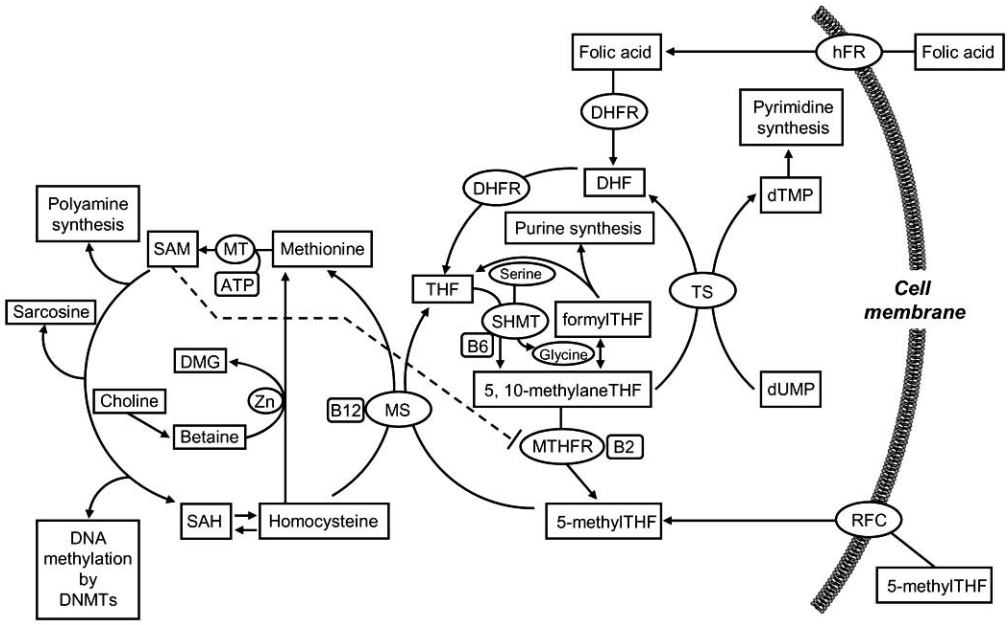

Fig. (1). Overview of one-carbon metabolism and related pathways. hFR, human folate receptor; RFC, reduced folate carrier; DHFR, dihydrofolate reductase; DNMTs, DNA methyltranferases; MS, methionine synthase; MAT, methionine adenosyltransferase; MTHFR, methylenetetrahydrofolate reductase; SHMT, serine hydroxymethlytransferase; TS, thymidylate synthase; DHF, dihydrofolate; DMG, dimethylglycine; dTMP, deoxythymidine monophosphate; dUMP, deoxyuridine monophosphate; SAH, *S*-adenosylhomocysteine; SAM, *S*-adenosylmethionine; THF, tetrahydrofolate.

as 50 % of synthetic folic acid [12]. The dietary folylpolyglutamates can be cleaved by a folylpoly-glutamate carboxypeptidase to monoglutamate form and absorbed at the mucosal epithelial cell brush border of the proximal small intestine. 5-methyltetrahydrofolic acid (5-methyl THF) will be the main form from dietary folate and synthetic folic acid absorbed in small intestine to enter the hepatic portal vein and in systemic blood stream. Although folic acid can be converted to 5-methyl THF, if excessive dose of folic acid is orally administrated high quantities of untransformed folic acid will appear in the hepatic portal vein and the circulating blood system [13-15]. It has been suspicious that this may delay the usage of folic acid in one carbon metabolism. In addition, a potential implication in malignancy development is that as suggested by a study there is correlation of high concentrations of untransformed synthetic folic acid but not 5-methyl THF in circulation with a reduction of natural killer cell cytotoxic activities [16]. This may imply that excessive folic acid could reduce host immune defense against tumor formation.

Folic acid and 5-methylTHF enter through cell membranes via their folate receptor and reduced folate carrier for intra-cellular one carbon metabolism (Fig. **1**). 5-methylTHF, directly from circulation or provided by the activity of the methylenetetrahydrofolate reductase (MTHFR), a key enzyme in the pathway, donates a methyl group to homocysteine to form methionine catalyzed by another critical enzyme methionine synthase (MS/MTR). Vitamin B12 (cobalamin) acts as a co-factor for MS enzyme. Potentially MS associated vitamin B12 could be oxidized to cob(II)alamin, resulting in MS inactivation. Therefore, methionine synthase reductase (MTRR) is required to reactivate MS by reducing cob(II)alamin with a methyl group transfer from *S*-adenosylmethionine (SAM). Methionine is subsequently adenylated by methionine adenosyltransferase (MAT) from ATP to form SAM, a universal methyl group donor in many important cellular methylation reactions including DNA methylation. SAM may inhibit MTHFR enzyme in a negative feedback control manner. Tetrahydrofolate (THF), another product of MS, can receive a carbon unit from serine to form 5, 10-methylene-THF by serine hydroxymethyl transferase and subsequently to 5-methyl-THF by MTHFR. Vitamin B6 and B2 are required as co-factors for serine hydroxymethyl transferase and MTHFR activities, respectively. Moreover, Betaine and choline can transfer their methyl group to homocysteine to generate methionine and then SAM. Thus, they may have effects on one carbon metabolism. In brief, 5-methylTHF is involved in methyl group transfer through SAM to DNA. 5, 10-methylene-THF can be used for the synthesis of deoxythymidylate and forms 10-formyl THF by the enzymes methenyltetrahydrofolate cyclohydrolase and formate-tetrahydrofolate ligase for purine synthesis. The latter two folates are important for making building blocks for DAN synthesis or repair.

One Carbon Metabolism and DNA Synthesis and Maintenance

One of the functions of folate is to maintain the steady –state level of deoxynucleotide pool for DNA synthesis and maintenance. A methyl group of 5, 10-methylene-THF is transferred to deoxyuridylate to generate deoxythymidylate (dTMP) by the enzyme thymidylate synthase (TS) in an irreversible rate-limiting reaction. This folate–dependent dTMP synthesis is critical for maintaining fidelity of DNA specific sequence. Insufficient supply of dTMP or thymidylate stress could result in increased incorporation of

uracil and potentially increase mutation rates of DNA. Thymidylate stress may be caused by folate deficiency and folate metabolism defects as well as use of antifolate agents such as methotrexate and fluorouracil. Once uracil incorporation occurs, it will be removed in a base excision repair process by a DNA glycosylase. During the repairing process, DNA re-synthesis is required and will reintroduce dUTP into genomic DNA, and possibly further induce genomic DNA instability such as double-strand breaks, deletions, chromosomal breaks, and loss of heterozygosity [17-22]. Similarly, folate may also affect mitochondrial DNA stability and mutations [23].

One Carbon Metabolism and DNA Methylation

Epigenetic alterations including changes in DNA methylation and chemical modifications of histones and other chromatin associated proteins can affect higher orders of DNA structures and therefore gene expression. Abnormal epigenetic alterations may be involved in human cancer development. For example, in prostate cancer aberrant DNA methylation, hypo- or hyper-methylation, can be found from early stages of neoplastic formation through various malignancy progression steps to life-threatening metastatic stages [24-26]. The epigenetic changes on genomic DNA may inactivate tumor suppressor genes and enhance the expression of oncogenic or growth stimulation related genes. The most known DNA methylation in mammalian cells occurs at carbon position 5 of cytosines of the CpG dinucleotide sequence during a post-replication stage of DNA synthesis. This CpG methylation reaction is catalyzed by DNA methyltransferase 1 (DNMT1) in order to maintain the preexisting cellular DNA methylation patterns in DNA replication. There are two other DNMTs, i.e., DNMT3A and DNMT3B, that are used in cells for de novo methylation of previously unmethylated CpG sites. These three DNMTs are all important for the formation and maintenance of genomic DNA methylation. The methylation reaction carried out by DNMTs requires the presence of SAM as the methyl group donor. The product of demethylation of SAM is *S*-adenosylhomocysteine (SAH) which, in turn, is a potent inhibitor of DNMTs. On the other hand, SAH can alleviate the allosteric inhibition of MTHFR by SAM. Deficient amounts of SAM may be difficult for maintaining DNA methylation and are the results of a low supply of necessary precursors or cofactors in one carbon metabolism pathway such as methionine, folate, vitamin B2, 6 and 12, and choline. Furthermore, many other cellular methylation reactions, e.g., protein modification, and phosphatidylcholine sysnthesis from phosphatidylethanolamine may require SAM and limit its supply for DNA methylation [27]. Other potential competiting source for SAM is sarcosine formation catalyzed by glycine-N-methyl transferase by transferring a methyl group of SAM to glycine. Increased sarcosine may be related to prostate cancer progression with enhanced invasion ability [27, 28]. Moreover, cellular synthesis of the polyamines such as spermidine and spermine needs methyl group donation from SAM. These alkylamines are important for multiple cellular processes including cell growth regulation and DNA stability [29] and largely produced in normal and cancerous prostatic epithelial cells [30]. An in vitro cell culture study [30] showed that human and mouse prostate cancer cell lines with high polyamine synthesis rates were more sensitive to folate deletion that led to imbalanced nucleotide and SAM pools than that of cell lines producing low polyamines. In rodent models, diets deficient of methionine, choline, vitamin B12 or folate can induce global DNA hypomethylation as well as specific gene hypermethylation, and potentially facilitate cancer development. Intriguingly, as shown in animal models the methylation alterations in preneoplastic lesions by folate/methyl-deficient diets seem to be relatively organ specific as found in liver or colon, but not in other organs examined including spleen, thymus, kidney, and pancreas [31].

Specific Gene Expression Changes and Epigenetic Alterations in Restricted Conditions of Vitamin Bs/Other Methyl-Donor Nutrients and Cancer Susceptibility

Dietary deficiency/restriction of methyl donor nutrients such as folate, other vitamin Bs, methioine/choline or combinations of the above in human cells or in rodents has been shown to affect SAM and nucleotide pools, therefore causing both epigenetic alterations and genetic damages [17, 19-21, 32, 33] which may be involved in carcinogenesis of liver and colon as well as other tissues/organs [32-37] as seen in animal models. Reduced methyl donor nutrients can decrease SAM formation and enhance accumulation of SAH. Increased SAH may inhibit DNMTs more effectively and potentially increase global DNA hypomethylation that may enhance oncogene expression, and genetic instability. More specifically, it has been shown that such deficiencies were able to induce hypomethylation of c-myc, c-fos and c-Ha-Ras genes in normal rat liver tissues even after refeeding of regular diets for 3 weeks, although the epigenetic events were eventually reversible [38]. This observation seemed to suggest that methyl–deficient diet could promote liver carcinogenesis by inappropriate activation of the proto-oncogenes. Further studies indicated that gene amplification, hypomethylation of CpG sites and overexpression of c-myc gene were detected in carcinoma tissues of the liver during chemically induced hepatocarcinogenesis with a methyl-deficient diet [38-40]. A study [41] examined the methylation status of the c-myc and c-Ha-ras and plasma folic acid concentrations in 21 patients with advanced gastric cancer. It showed that patients who had hypomethylation of these genes had lower plasma folic acid than that of those who had normal methylation. Using ICR mouse as a model to investigate chemoprevention on chemically induce colorectal cancer, the study [42] showed that folic acid could reduce incidents of cancer formation. It also found that an increased expression of c-myc was associated with lower circulating FA levels and promoter hypomethylation in tumor tissues. It has been well documented [43] that c-myc can target multiple biochemical pathways and act as a genome structure modifier causing genome instability, thus its overexpression is highly relevant to the initiation and promotion of tumor formation including PCa [44-46]. A recent study [47] suggested that gene amplification is not sufficient to explain the frequent myc overexpression in PCa. However, so far there are no studies showing any hypomethylation state of c-myc gene detected in prostate tissues. However, it has recently been shown [48] that ectopic over-expression of c-myc can cause hyper-

methyation of certain putative tumor suppressor genes in human prostate cells. The increased hypermethylation may be due to enhanced expression of DNMT3a and b induced by overexpressed c-myc. As discussed below, epidemiologic studies indicate that the nutrients for one carbon metabolism may affect PCa formation and progression, yet until now there are related studies as to whether these nutrients have any effects on c-myc gene's epigenetic status or overexpression in PCa.

Other specific genes affected by inadequacy of one carbon metabolism-related nutrients have also been described in animal models. Since aberrant activities in the Wnt pathway play an important role in human colorectal cancer, studies [49] designed to understand if some key genes in the Wnt pathway may be altered by inadequacy of one carbon metabolism related nutrients. It was demonstrated that only when mice were placed under mild folate deficiency with depletion of vitamin 2, 6 and 12, colonic mucosa showed significant decreases of Apc, beta-catenin and cyclin D1 in the Wnt pathway but not with the folate deficiency treatment alone. The decrease in Apc expression seemed to be correlated with DNA single strand breaks within the Apc mutation cluster region. Additional studies [50] showed a similar finding that the combined 4 vitamin B depletion amplified DNA strand breaks in p53 gene exons 5-8 region and p53 expression which occurred in a lesser extent by mild folate depletion alone. MDM2, a downstream effector of p53, was also largely decreased by the combined depletion treatment. Furthermore, in rats with a long term mild folate deficient diet, two DNA repair enzymes, O^6-methylguanine-DNA methyltransferase (MGMT) and 8-oxoguanine-DNA glycosylase (OGG-1) were found up-regulated in liver but not in colon [51]. A DNA damage repairing product, 8-oxo-7,8-dihydroguanine was also found to be increased in circulating lymphocytes. Again, no global genomic methylation was observed in this study.

Recently, a methyl-deficient diet i.e., a choline and methionine deficient (CMD) diet was used to examine its influence in epigenetic events in the prostate of treated mice for about 7 months [52]. Two genes, insulin-like growth factor 2 (Igf2) and H19, with potential oncogenic functions were used as surrogate markers in the study. After 7 months treatment, about 45% of mice developed liver tumor but no any apparent pathologic changes in the prostate. However. increased expression of these two genes in the prostate was observed after 3 months of the treatment, which became irreversible with repletion of choline and methionine after 4 months of the deficiency treatment. Interestingly, there were no methylation changes in the promoters of these two genes with the CMD. Using chromatin immunoprecipitation, a significant decrease in repressive histone methylation (i.e., dimethyl-H3K9) was associated with the promoter regions of the two genes. The authors concluded that this gene locus is more prone to histone methylation modification by CMD than DNA methylation. However, it is not clear whether this is an organ specific phenomenon and whether it can be applied to human prostate.

In *in vitro* cell culture systems, it is well known that folate depletion will retard cell growth and increase apoptotic cell death in different cell origins including colon [53. 54] and prostate cells. In four human colon cancer cell lines examined [53] key genes for cell cycle control, DNA repair, apoptosis and angiogenesis were affected by the folate deficiency condition in a cell-specific manner. Three normal human colon cell lines under mild folate deficiency [54] were also studies for specific genes in cell cycle checkpoints, intracellular signaling, cell adhesion and migration. It was found that folate receptor 1, p53, p21, p16 and beta-catenin were increased by decreasing folate supplies. With decreasing folate, E-cadherin, SMAD-4 and APC were increased in cell specific manners, i.e., only in one or two of the three cell lines. p53 gene exon 7-8 strand breaks were also detected in one cell line. These cells were cultivated in the deficient medium for 32-34 days, however, there were no detectable overall global methylation changes. For prostate cells, as mentioned above, the recent available data [30] demonstrated that the growth rates of prostate cancer cells were significantly hampered under folate deficient conditions. The study convincingly showed that the growth retardation by folate deficiency was due to their high polyamine production ability. Although under the conditions the high polyamine synthesis rates increased SAH/SAM ratio and caused imbalance of nucleotide precursors in the prostate cells tested, this report did not show further specific information regarding how exactly high production of polyamines causes prostate cell growth retardation.

Epidemiological and Intervention Studies of One Carbon Metabolism-Relatd Nutrients on Prostate Cancer

Studies from epidemiologic investigation regarding the relationships of the dietary one carbon metabolism related nutrients and PCa risk do not provide conclusive results which are not unusual in many of this kind of studies. Inspiring from other related studies on the implication of these nutrients in several malignancies, including colorectal, lung, and cervix, a case-controlled study [55] using smoker subjects in the Alpha-Tocopherol, Beta-Carotene (ATBC) Lung Cancer Prevention Study in Finland was conducted to determine the associations of serum folate, B6, B12, and homocysteine with PCa risk. The authors concluded that there were no associations of these factors with the cancer risk. The limitation of the study for generalization purpose was the subjects who were smokers. An extension of the analyses from the ATBC [56] was to examine the intakes of folate, B6, B12 and methionine of the study subjects. Again, the authors concluded that the one carbon metabolism nutrients studied offer no protective effects against PCa except for vitamin B6 which may have a modest but significant protective effect. Moreover, it was found that a high intake of vitamin B12 could significantly increase PCa risk in smoker men. Similar results were also obtained in a prospective study [57] and the European Prospective Investigation into Cancer and Nutrition study [58] in which plasma folate and/or homocysteine levels did not have any association with reduction of PCa risk. Increased plasma folate and B12 may increase PCa. Then, a large cohort of 65,836 men in the American Cancer Society Cancer Prevention Study II Nutrition [59] was used to investigate folate intakes and their association with PCa incidence. In a 9-year follow-up, 5,158 men were found to have PCa,

however, the folate intake was not related to an overall PCa risk.

A large prospective, nested case control study [60] by analyzing plasma concentrations of betaine, choline, cysteine, methionine, methylmalonic acid (MMA), vitamin B2, and vitamin B6 in 561 cases and 1,034 controls found that there were no associations of betaine, cysteine, methionine, or vitamin B6 with PCa risk. On the other hand, increased MMA might be inversely associated with the cancer risk and elevated choline and vitamin B2 seemed to be positively associated with the cancer risk. The population used in the above study was also used to investigate if the intakes of folate, riboflavin, vitamin B6, vitamin B12, and methionine have effects on PCa survival [61]. This probably was the first study for effects of the nutrients on the cancer survival (up to a 20 y follow-up) and found that although folate, riboflavin, vitamin B12, and methionine intakes did not have any association with PCa survival, a high vitamin B6 intake appeared to increase PCa survival in men with localized cancer. A prospective study [62] in the 1969 Busselton (Western Australia) Health survey with a cohort of 964 men (follow up time: 20,254 years) was performed to analyze the relationship of serum folate and cancer mortality or morbidity rate. The data showed that decreased serum folate levels were inversely associated with increased PCa mortality risk. A case-control study [63] with 1,294 PCa case patients and 1,451 control patients from various areas of Italy showed that high intake of dietary folate might reduce PCa risk, yet intakes of vitamin B6 and methionine had no effect on the cancer risk. Another case control study [65] was conducted with 140 prostate cancer cases and 230 age-matched clinic controls plus 250 negative prostate biopsy controls for the association of folate intake and cancer risk. The authors found that there was an inverse relationship of folate intake and the cancer risk, especially for high-grade PCa. A placebo-controlled randomized trial of aspirin and folic acid supplementation for cancer chemoprevention in the Aspirin/Folate Polyp Prevention Study [64] was conducted with up to about 10 years follow up. Secondary findings of this trial regarding PCa incidence were reported that, although aspirin showed no correlation with PCa incidence, folate supplement intake had a positive association with increased PCa risk. However, interestingly, baseline dietary folate was inversely associated with PCa risk. The authors concluded that the effect of supplemental folate on PC risk may be different from that of dietary folate, indicating a complex role of folate metabolism in PCa. In addition, a study with 2 randomized, double-blind, placebo-controlled vitamin Bs intervention trials in Norway with a total of 6837 patients having ischemic heart disease was performed between 1998 and 2005, plus two additional years follow up [66]. There were four groups receiving orally with folic acid (0.8 mg/d) plus vitamin B12 (0.4 mg/d) and vitamin B6 (40 mg/d), folic acid (0.8 mg/d) plus vitamin B12 (0.4 mg/d), vitamin B6 alone (40 mg/d), or placebo. The authors indicated that folic acid participants received were twice the recommended daily allowance but below the tolerable upper intake level of 1 mg/d. The authors concluded that the treatment with folic acid and vitamin B12 can increase cancer incidence including lung cancer and PCa and all-cause mortality. The authors reasoned that high doses of folic acid might enhance the growth of established neoplastic lesions, as well as that excessive unmetabolised folic acid in plasma may have a potential to reduce natural killer cell activity [16] with lowered cancer immunity.

In addition to the one carbon metabolism-related nutrient intakes, genes coded for the enzymes in the metabolism pathway could be another potentially important factor to influence efficiency of use of the nutrients. For example, it was suggested [67, 68] that genetic polymorphism of the MTHFR gene may have a potential implication in the relationship of dietary folate intake with PCa risk. By evaluating the C677T and A1298C polymorphisms of the MTHFR gene in genomic DNA from prostatic tissues of 81 PCa cases and 42 controls selected from patients with benign prostatic hypertrophy (BPH), the 677CT genotype of the MTHFR may be in favor of decreasing risk of PCa [67]. Another study [68] with a relatively large family-based case-control study containing 439 cancer cases and 479 sibling controls was designed to assess the relationship between two MTHFR polymorphisms, C677T and A1298C, and PCa risk and aggressiveness using genomic DNA from white blood cells. It concluded that the 677T-1298A haplotype of this gene was associated with reducing the risk of becoming more advanced disease.

Moreover, a nested case-control study [69] consisting of 223 prostate cancer cases and 435 matched controls nested in a population-based Northern Sweden Health and Disease Cohort using DNA from white blood cells was performed to determine MTHFR C677T and A1298A polymorphism frequencies and PCa risk. The results indicated that either 677C-->T or 1298A-->C polymorphism did not have any significant association with the risk of prostate cancer. Intriguingly, in conjunction with previously report [57] of this group of the investigators that showed plasma folate levels were positively associated with prostate cancer risk, this study further suggested that the increased PCa risk at high plasma folate levels might be attributable to the 677C-->T polymorphism. A hospital-based case-control study [70] with 182 PCa cases and 205 BPH control cases analyzed MTHFR C667T, A1298C, the MTR A2756C and the MTRR A66C polymorphisms in blood leukocyte genomic DNA. It was found that MTHFR667C→T is associated with PCa development and MTR 2256 A→ C may be associated with tumor aggressiveness. A study [71] including 93 PCa patients and 166 BPH cases as controls in Turkey was conducted to assess the relationship of the MTHFR C677T and A1298C polymorphisms with PCa. The findings were that the C667T had no association with the cancer, but 1298A→C was inversely associated with PCa risk. A meta-analysis study [72] based on 6 previous studies with a total of 3511 cases and 2762 control cases describing the MTHFR C677T genotypes and on 4 previous studies of a total of 838 cases and 1121 control cases describing MTHFR A1298C genotypes was performed to examine the association of the MTHFR genotypes and PCa risk. The meta-analysis concluded that only 677C→T not A1298C may have effects on reduction of PCa risk. On the contrary, two recent large studies [73, 74] produced null results. One of these two studies used 1,144 cases and 1,144 controls from the Cancer Prevention Study-II Nutrition Cohort to assess the

associations of 39 single nucleotide single nucleotide polymorphisms (SNP) in 9 genes (i.e., the genes MTHFR, MTR/MS, MTRR, cystathionine β-synthase (CBS), serine hydroxymethyltransferase (SHMT1), TS, DHFR, methyl-enetetrahydrofolate dehydrogenase/methenyltetrahydrofolate cyclohydrolase/formyltetrahydrofolate synthase (MTHFD1), and formyltetrahydrofolate dehydrogenase (FTHFD) involved in the one-carbon metabolism pathway with PCa risk [73]. The authors revealed that all SNPs examined did not have any association with PCa risk. The second large study [74] was a meta-analysis using data from eight case-control studies and four genome-wide association stuides as well as from a nested case-control study with the UK population-based Prostate Testing for Cancer and Treatment study to evaluate eight SNPs in seven genes including MTHFR C677T, MTHFR A1298C, MTR A2756G, MTRR A66G, MTHFD1 G1958A, solute carrier family 19, SLC19A1/ RFC1 G80A, SHMT1 C1420T, and folate hydrolase 1, FOLH1 T1561C. Similarly, the results of this study showed no indication of any significant association of these SNPs with PCa risk.

In summary, the above epidemiologic and clinical trial studies produced inconsistent or conflicting results regarding whether dietary vitamin Bs and other dietary nutrients related to in one carbon metabolism pathways can reduce or enhance PCa risk. Similarly, SNP analyses of crucial enzyme genes in the pathways also generated inconsistent conclusions. Synthetic folic acid used in the large trial studies showed it can increase PCa risk. The authors suggested that administration of excessive doses of folic acid may generate large amounts of unmetabolized folic acid which may have opposite effects on fighting cancer including PCa.

CONCLUDING REMARKS

Based on numerous laboratory studies as discussed above, it is in a general agreement that one carbon metabolism related nutrients may have influences on genetic and epigenetic regulation of gene expression that ultimately affects phenotypic outcomes of several malignancies. However, as aforementioned, very few such studies directly related to the prostate or PCa have been reported. One report [30] showed that human prostate cells with high production of polyamines had a higher response to folate deficiency in reducing cell proliferation. It warrants further investigation on the interaction of polyamine synthesis and one carbon metabolism. More recently, the same group of investigators showed that *in vitro* murine prostate cell cultures with mild folate depletion can induce genetic and epigenetic changes, which seems to support the idea that we should continuously study the effects of folate manipulation on the prostate *in vivo* and *in vitro* [75]. A high profile study [28], not directly related to the relationship of the nutrients to PCa risk, reported recently that compared to benign prostate tissues, PCa tissues can produce higher amounts of sarcosine, a product of a glycine N-methyltransferase (GNMT), that has positive effects on cancer metastasis. Because GNMT uses methyl group of SAM to make sarcosine, it is unclear if one carbon metabolism related nutrients via this sarcosine production pathway may have effect on risk of PCa progression. This could be a case, as some epidemiology studies discussed above indicated certain related nutrients are associated with PCa progression or survival. In the future, molecules such as sarcosine and GNMT should also be considered in PCa epidemiology studies. Overall epidemio-logical or clinical trial studies as discussed above seem to indicate that these nutrients and one carbon metabolism related pathways have influence on PCa development and progression, although the conclusions are not very consistent or even conflicting, suggesting requirement of more researches on effects of one carbon metabolism and related nutrients on prostate cells or PCa models at molecular, genetic, and epigenetic levels.

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