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*Review*

# The Role of Folic Acid in DNA Methylation and Breast Cancer

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**Abstract**

Folate and folic acid (FA) are two forms of vitamin B9, a B-complex nutrient essential for the human body. Folate is the natural form of vitamin B9 and is found in foods such as citrus fruits, leafy green vegetables, and beans. In contrast, FA is the synthetic form and is commonly found in supplements and added to fortified foods. The metabolism of folate and FA plays a crucial role in DNA synthesis and methylation; therefore, understanding the mechanism through which a decrease in folate and FA consumption affects the development of breast cancer (BC) is important. DNA hypermethylation can inhibit the transcription of tumor suppressor genes, while DNA hypomethylation may have the same effect and activate oncogene transcription. However, some genetic variants exist, such as rs1801133 and rs1801131 in the *MTHFR* gene and rs1051266 in the *RFC* gene. The *MTHFR* gene encodes an enzyme that facilitates the utilization of folate to support essential bodily functions, while the *RFC* gene is responsible for transporting folate into cells and acts as an anion exchanger. Both genes intervene in the transport and absorption of FA and are related to an increased risk of cancer. Studies investigating the relationship between FA and BC often rely on *in vitro* and *in vivo* models; however, the findings may not fully translate to humans due to significant physiological and metabolic differences across species. This article explores how changes in FA metabolism due to malabsorption defects, a deficient diet or genetic variants may impact methylation processes and their relationship with BC.

**Keywords:** folic acid; folate; DNA methylation; breast cancer

## 1. Introduction

The British hematologist Lucy Wills discovered folate in 1930 while investigating macrocytic anemia in pregnant women in India. She identified an antianemic substance, which she coined "the Wills factor". In 1941, the biochemist Herschel Kenworthy Mitchell isolated folic acid (FA) for the first time from spinach and named it "folic" from the Latin *folum*, meaning "leaf". In 1945, FA was chemically defined, and its industrial production began [1–3].

FA, or vitamin B9, is essential for the biosynthesis of nucleotides, amino acids, neurotransmitters and S-adenosylmethionine (SAM). This vitamin belongs to the folate group, which includes its metabolically active form, 5-methyltetrahydrofolate (5-MTHF), as well as folinic acid. The low bioavailability of FA disrupts DNA repair and methylation processes, contributing to carcinogenesis [4,5].

Among the cancers related to FA deficiency are prostate, gastric, liver, lung, glioblastoma, leukemia and breast cancer (BC) [6]. This study focused on BC, which is the most virulent form of neoplasia, a disease process characterized by uncontrolled cell proliferation leading to the formation of a mass or tumor [7]. According to the World Health Organization (WHO), the incidence of BC worldwide in 2020 was 47.8 per 100,000 women, while the mortality rate was 13.6 per 100,000 women worldwide [8].

Epidemiological studies investigating the correlation between folate intake and breast cancer risk have shown inconsistent results. A dose-response meta-analysis of observational studies conducted before April 2019 included 39 studies on folate intake and 12 studies on plasma folate levels. The analysis revealed that folate intake was inversely correlated with breast cancer risk when comparing the highest and lowest intake categories (OR = 0.85, 95% CI = 0.79–0.92). Additionally, the dose-response analysis demonstrated a linear correlation between folate intake and breast cancer risk. Higher folate intake was associated with a reduced risk of breast cancer in premenopausal women (OR = 0.80, 95% CI = 0.66–0.97), but not in postmenopausal women (OR = 0.94, 95% CI = 0.83–1.06). In contrast, plasma folate levels were not found to correlate with breast cancer risk (OR = 0.98, 95% CI = 0.82–1.17). While folate intake appears to be negatively associated with breast cancer risk, its practical clinical significance remains unclear and warrants further investigation. Moreover, additional folate supplementation should be approached with caution [9].

Cancer is a multifactorial disease, since it involves the interaction of genetic factors, epigenetic factors and environmental factors, including lifestyle and reproductive, hormonal and dietary influences. Among the epigenetic factors is methylation, which involves the addition of a

methyl group at carbon 5 of cytosine through the enzyme DNA methyltransferase (DNMT), converting cytosine into 5-methylcytosine, which affects the binding of transcription factors [10]; this can serve as a biomarker since it is related to the presence and progression of BC [11]. For example, folate deficiency can alter DNA methylation by disrupting the conversion of S-adenosylhomocysteine (SAH) to SAM in the methionine cycle. Under low folate conditions, SAM levels decrease while SAH levels increase, resulting in global DNA hypomethylation of newly synthesized DNA and potentially leading to increased expression of proto-oncogenes. Conversely, folate deficiency can also be associated with DNA hypermethylation in specific gene regions, such as in the underexpression of gene promoters, particularly in tumor suppressor genes [5].

The aim of this study was to focus on the alteration of FA metabolism caused by malabsorption defects, poor diet, or genetic variants, its impact on methylation and its relationship with BC. A systematic search was conducted, with the topic and objective clearly defined. Keywords such as folic acid, folate, DNA methylation and breast cancer were used. Mainly original research articles in English published within the last five years were considered. Only meta-analyses, reviews, and textbooks with basic concepts from previous years were included. A database was created with articles that met these criteria. The search was performed via the PubMed and Google Scholar databases.

## 2. Bioavailability of Folic Acid

FA is a synthetic form of folate that contains glutamate, and it is the most bioavailable form for humans and is more available even than natural polyglutamates [12]. FA is water soluble; furthermore, it is active as tetrahydrofolate (THF) and 5-MTHF, which is the primary form found in blood [9]. FA has several functions, including nucleotide synthesis, DNA repair, red blood cell formation, epigenetic regulation (DNA methylation), and fetal brain development [4,13].

FA is bioavailable in the body and is converted by the enzyme dihydrofolate reductase (DHF reductase) to dihydrofolate (DHF) and tetrahydrofolate (THF), which function as cofactors in the one-carbon (1-C) pathway. Structurally, FA is composed of a pteridine ring linked via a methylene group to a para-aminobenzoic acid (PABA), to which one or more glutamate residues (FA as a supplement in the form of monoglutamate) are attached via an amide bond. Fig. 1 shows the reduction of FA that occurs during absorption and its chemical structure [1,14,15].

The tolerable upper intake level (UL) of folic acid established by the Scientific Committee on Foods is maintained for all population groups. These levels are 1000 µg/day for adults, including pregnant and lactating women; 200 µg/day for children aged 1–3 years; 300 µg/day for 4–6 years; 400 µg/day for 7–10 years; 600 µg/day for 11–14 years; 800 µg/day for 15–17 years; and 200 µg/day for infants aged 4–11 months. These UL apply to the combined intake of FA, calcium salts of l-methyltetrahydrofolate (6S) and calcium salts of l-5-methyltetrahydrofolate, under the authorized conditions of use [16].

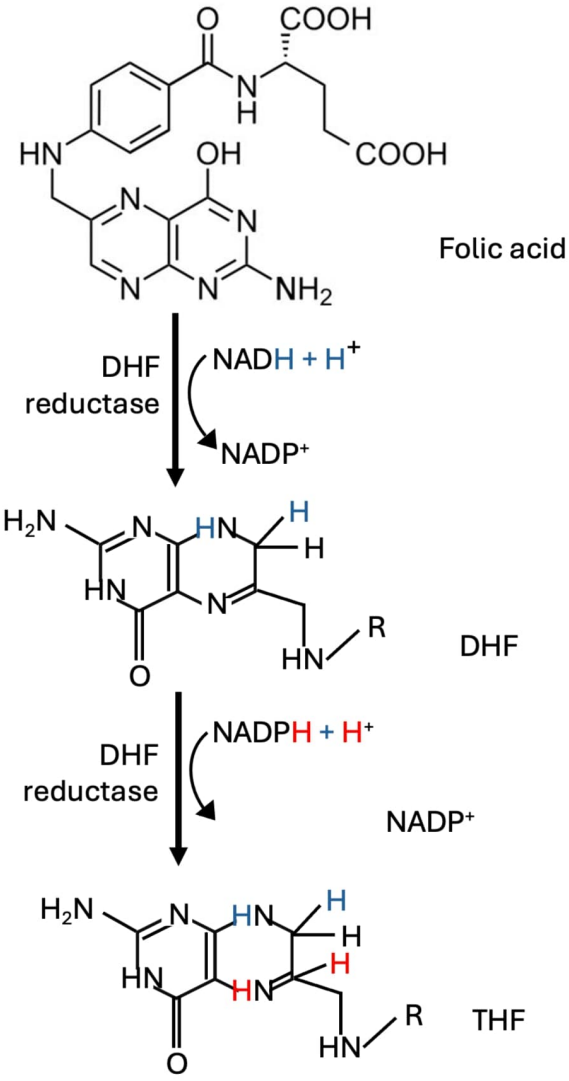


**Fig. 1. Folic acid reduction during absorption.** NADP, nicothiamide adenine dinucleotide phosphate (oxidized); NADPH, nicothiamide adenine dinucleotide phosphate (reduced); DHF, dihydrofolate; THF, tetrahydrofolate. Figure was created by the first author with BioRender© (BioRender Inc, Toronto, Canada).

## 3. Sources of Folic Acid

Folate is the most bioavailable form of vitamin B9 for humans; it is found in foods naturally as well as in synthetic forms such as FA, and it is used as a dietary supplement [13,17–19]. However, the National Health and Nutrition Survey 2014 recommends that the Mexican population obtain FA from natural foods [18]. Table 1 (Ref. [18,19]) shows those natural foods rich in FA and the amount contained in each.

**Table 1. Natural foods rich in FA [18,19].**

| Foods | | Suggested portion (gr) | Amount of FA (µg) |
|---|---|---|---|
| Vegetables | Celery | 152 | 48.5 |
| | Cooked broccoli Cambray onion | 92 | 46 |
| | Cilantro | 75 | 46.1 |
| | Raw chayote | 120 | 74.4 |
| | Cooked cauliflower Asparagus | 102 | 93.5 |
| | Cooked spinach | 125 | 55 |
| | Raw spinach | 90 | 131 |
| | Cooked squash blossom | 90 | 131.1 |
| | Raw squash blossom | 120 | 232.8 |
| | Lettuce | 134 | 54 |
| | | 132 | 76 |
| | | 141 | 184.1 |
| Fruit | Strawberry | 166 | 39.8 |
| | Kiwi | 132 | 43.3 |
| | Orange | 242 | 45.7 |
| | Papaya | 140 | 53.2 |
| Grain | Cooked amaranth | 330 | 185 |
| | Puffed rice | 17 | 58.2 |
| | Instant oatmeal | 77 | 54.8 |
| | Pasta | 25 | 44 |
| | Wheat bran | 23.3 | 24.4 |
| Meat (liver) | Pork | 100 | 110 |
| | Lamb | 100 | 220 |
| | Chicken | 100 | 590 |
| | Veal | 100 | 240 |

gr, grams; FA, folic acid.

Folate is found naturally in a variety of foods such as dark green leafy vegetables, fruits, fruit juices, nuts, beans, peas, shellfish, eggs, dairy, meat, poultry and grains. Foods with the highest concentration of folate include spinach, liver, asparagus and Brussels sprouts [20].

In contrast, FA, the synthetic form of folate, is commonly found in multivitamins, prenatal supplements and specific FA supplements. Regarding bioavailability, approximately 85% of FA is absorbed by the body when consumed with food, whereas its bioavailability approaches 100% when taken on an empty stomach [20].

## 4. Recommended Intake of Folic Acid

According to the National Institutes of Health of the United States of America, the recommended intake of FA depends on the age of the person, and emphasis is placed on not exceeding the maximum dose (Table 2) [20].

It is important to ingest the recommended amount because folate deficiency causes congenital malformations, carcinogenesis [13], atrial tachycardia, ischemic and hemorrhagic stroke in newborns [21], visual impairment, maculopathy, diabetic retinopathy [22], depression [23], neural tube defects, anencephaly and spina bifida [24].

In contrast, an intake of greater than 400 µg in women increases the risk of BC; because folate can affect endothelial function and promote cell growth, therefore a daily intake of between 200 and 320 µg of FA is recommended to decrease the risk of BC [25]. In addition, in a previous study, mice that were given 2.5 times the recommended amount of FA had increased anxiety and impaired motor coordination and memory [26].

## 5. Folic Acid Metabolism

FA is inactive in the body and, to be functional, must be converted to 5-methyl-tetrahydrofolate (5-$CH_2$-THF), which is absorbed in the jejunum and duodenum and transported through the blood via folate binding and transporting proteins to the 1-C metabolic pathway [14,27]. During absorption, FA is reduced to dihydrofolate (DHF) by the enzyme dihydrofolate reductase (DHFR); subsequently, DHF is converted to its active form, THF, an acceptor molecule of the 1-C pathway. Next, transmethylation is mediated by the enzyme serine hydroxymethyltransferase (SHMT), in which a methyl group is transferred from serine to THF, forming 5,10-methylene-tetrahydrofolate (5,10-$CH_2$, THF), which can enter the blood circulation. The latter molecule can be reduced to 5-MTHF (5-$CH_2$-THF) by the enzyme methylene tetrahydrofolate reductase (MTHFR) in a process requiring riboflavin. 5-MTHF contributes to the recycling of homocysteine to methionine in a process cat-

**Table 2. Recommended intake of FA in different age groups according to the National Institute of Health of the United States of America [20].**

| Age | Recommended intake amount (µg) | Upper limit amount (µg) |
|---|---|---|
| <6 months | 65 | No data |
| 7 to 12 months | 80 | No data |
| 1 to 3 years | 150 | 300 |
| 4 to 8 years | 200 | 400 |
| 9 to 13 years | 300 | 600 |
| 14 to 18 years | 400 | 900 |
| >19 years | 400 | 1000 |
| Pregnant women | 600 | No data |
| Breastfeeding women | 500 | No data |

µg, micrograms.

alyzed by methionine synthase, and the generated methionine can be converted to S-adenosyl methionine (SAM), causing 5-MTHF to return to THF (Fig. 2) [14,26–30].

SAM is a cellular metabolite and the main donor of methyl groups in nucleic acids and proteins [28], and it participates in DNA methylation via S-adenosyl-homocysteine (SAH), which is a nonessential amino acid derived from methionine. Finally, homocysteine can be remethylated to the methionine cycle or converted to cysteine by transsulfuration [14,27].

## 6. Role of Folic Acid in Epigenetic Processes

Epigenetics is the study of the mechanisms involved in the regulation of gene expression that do not involve a change in the sequence itself; the most studied epigenetic modifications are histone acetylation and DNA methylation [31]. Acetylation is a process in which the positive charge of lysine is neutralized, which decreases the interaction between histones and DNA; this change alters the three-dimensional structure and allows DNA accessibility, which promotes the binding of transcription factors to DNA [32].

Methylation is a mechanism of gene expression regulation that occurs mainly in CpG islands; it involves the addition of a methyl group at carbon 5 of cytosine, converting it into 5-methylcytosine (Fig. 3) by means of methyltransferase enzymes: DNMT1 (maintenance), DNMT2 (conservation), DNMT3A and DNMT3B (*de novo*), DNM3L (cofactor of DNMT3A and DNMT3B) and ten-eleven-translocation (TET) (demethylation) [33–38].

Approximately 1% of the DNA bases are 5-methylcytosines, and 70 to 80% are located within CG dinucleotides. Methylation prevents gene transcription via the prevention of transcription factor binding. There are two types of methylation patterns: hypermethylation and hypomethylation. Hypermethylation represses the transcription of tumor suppressor genes, whereas hypomethylation activates the transcription of oncogenes [37–40].

FA plays a role in methylation because FA deficiency alters the metabolism of 1-C, which affects remethylation and transsulfuration [5]. Hypermethylation results in the donation of more methyl groups in promoter regions, inhibiting the expression of tumor suppressor genes such as *BRCA1*, *BRCA2*, and *ESR2*. Hypomethylation occurs during the remethylation of SAH to SAM in the methionine cycle, and SAM decreases as SAH increases, which results in reduced methyl group donation and, consequently, the overexpression of oncogenes, such as *HER2*, *PI3KCA*, *MYC* and *CCND1* [5,41].

## 7. Studies Related to Folic Acid Intake and Breast Cancer

In 2005, Kotsopoulos and colleagues [42] were the first to investigate the effects of dietary and developmental folate deficiency on mammary tumor progression in a Sprague–Dawley rat model. The rats were divided into two groups: a control group supplemented with 0.2 mg FA/kg and an experimental group supplemented with 8 mg FA/kg during the initiation and promotion phases of tumorigenesis [42]. These findings indicated that folate deficiency and supplementation did not significantly affect the initiation of mammary tumors. However, moderate folate deficiency significantly inhibited tumor progression during the promotion phase, reducing the tumor occurrence, incidence, volume and weight of mammary adenocarcinomas. However, folate supplementation (at a level four times the baseline requirement) had no significant effect on cancer progression [42]. This study suggests that moderate folate deficiency may have a protective effect against breast tumor progression, but folate supplementation has no clear adverse effect on breast carcinogenesis. Furthermore, this work suggests that the timing and dose of folate intervention are crucial factors in its modulatory effect on carcinogenesis [42].

In 2014, Deghan Manshadi *et al.* [43] studied the potential mammary tumor-promoting effect of FA supplementation in female Sprague–Dawley rats and divided them into control and experimental groups of 44 rats each. The diets provided contained approximately 4000 kcal/kg, which

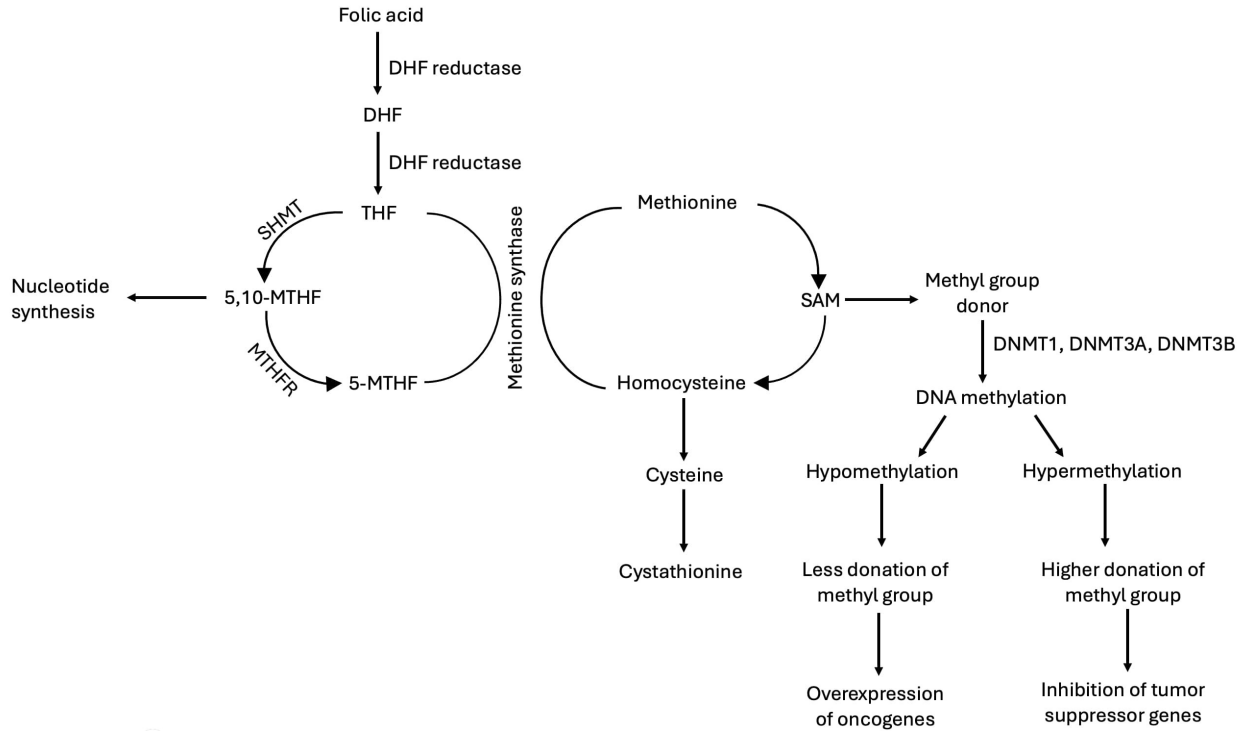


**Fig. 2. FA metabolic pathway during absorption in small intestine (adapted from Ref. [29]).** DHF, dihydrofolate; THF, tetrahydrofolate; SHMT, hydroxymethyltransferase; MTHFR, methylene tetrahydrofolate reductase; SAM, S-adenosyl methionine; DNMT, demethyltransferase. The figure was created by the first author with BioRender©.

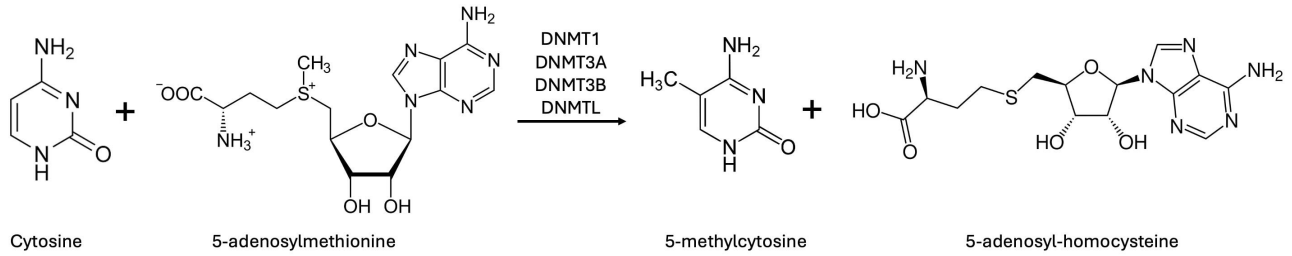


**Fig. 3. Methylation reactions of cytosine in somatic cells, it involves the addition of a methyl group at carbon 5 of cytosine, converting it into 5-methylcytosine by means of methyltransferase enzymes: DNMT1 (maintenance), DNMT2 (conservation), DNMT3A and DNMT3B (*de novo*), DNM3L (cofactor of DNMT3A and DNMT3B) and ten-eleven-translocation (TET) (demethylation).** Figure was created by the third author with BioRender©.

translates to 0.5–1 mg of FA in 2000 kcal, equivalent to the recommended daily allowance (RDA) of 0.4 mg dietary folate in humans consuming 2000 kcal/day. FA supplementation at 2.5 and 5 times the RDA (i.e., 5, 8 or 10 mg FA/kg for 12 weeks) significantly promoted mammary tumor progression in rats. The tumors in the supplemented groups were greater in weight, volume, and area, particularly those in the group that received 2.5 times the RDA. In rats that received all three supplemented levels of FA, mammary tumors presented significantly greater final tumor weights and volumes ($p$ = 0.001) and notably larger final sentinel tumor areas than those in animals that received the control diet. Another important finding was that FA supplementation increased the expression of HER2, a human epidermal receptor implicated in BC progression. This could be related to the ability of folate to affect DNA methylation, a process that regulates gene expression [43].

Conversely, in an *in vitro* study in which the MCF-7 cell line was treated with 4 and 8 mg/L FA, the number of apoptotic cells increased by 12–14%, and 32% of all apoptotic cells exhibited active caspase-3, which indicates the significant involvement of the caspase-dependent apoptotic pathway. These findings suggest that FA can promote cell death in less aggressive BC cells [42]. This study also revealed that high FA concentrations increase DNMT1 expression in MCF-7 cells, which may contribute to tumor suppressor gene hypermethylation. The authors propose that FA may influence the levels of SAM, a key

methyl donor in methylation processes, thereby altering DNA methylation and gene expression. This study suggested that high concentrations of folic acid could contribute to the downregulation of tumor suppressor genes, which could promote BC progression. This is relevant, as FA supplements are commonly recommended for the prevention of neural tube defects and other therapeutic uses, but their effects on cancer progression are complex and context specific. FA or folate may have dual effects: at lower concentrations, it may have protective effects, but at higher concentrations (particularly in its synthetic form), it may aggravate tumor progression by silencing key tumor suppressor genes [44].

In another *in vitro* study, MCF10A, MCF7 and Hs578T cell lines were treated with 0 or 100 nmol/L FA for 72 h (n = 6 replicates/treatment). FA treatment altered gene expression patterns in these cell types, with significant transcriptomic changes that varied by cell line [45]. Gene expression changes were detected in 97% of the genes in Hs578T cells, 89% in MCF10A cells, and 75% in MCF7 cells. TAGLN (transgelin) expression decreased in MCF10A and Hs578T cells but increased in MCF7 cells; as TAGLN is related to tumor transformation, these findings suggest that FA may promote a cancerous phenotype in MCF10A and Hs578T cells while potentially inhibiting it in MCF7 cells. Molecular pathway analysis revealed that in MCF10A and Hs578T cells, FA promoted pathways associated with tumorigenesis, such as cell migration, proliferation, and vascularization, whereas pathways related to apoptosis and cell differentiation were repressed, suggesting the induction of more aggressive features of cancer. In Hs578T cells, FA treatment affected several key regulators related to cancer development and progression, such as *FOXM1* and *TP53*, which are associated with cancer proliferation and aggressiveness. Although the precise mechanism of action of FA could not be determined, it was suggested that variation in the expression of folate transporters (*SLC19A1*, *SLC46A1*, *FOLR1*) across cell lines could influence FA uptake and thus the observed transcriptomic changes [45].

Although further investigation is needed, these results highlight the capacity of FA to induce cell-specific gene expression changes in breast cells, which may have implications for dietary guidelines for women with BC. Further studies are needed to elucidate the role of folate in breast tumorigenesis.

## 8. Genetic Variants in the Folic Acid Metabolic Pathway and their Relationships with Breast Cancer

Gene–nutrient interactions are fundamental to the behavior of genes that participate in the metabolic pathway of FA; variants in the *MTHFR*, *RFC* and *FR* $\alpha$, $\beta$ genes increase the risk of cancer because they deregulate the FA pathway [24,46]. These genetic variants decrease the bioavailability of folate in the organism and prevent it from performing its function [5]; for example, for the gene *MTHFR*, two variants have been observed: C677T and A1298C.

The first C677T variant (rs1801133) involves a change from alanine to valine (p.Ala222Val), which causes decreased MTHFR enzymatic activity and is related to an alteration in folate metabolism and DNA hypomethylation [47,48]. The A1298C variant (rs1801131) involves a change from glutamate to alanine (p.Glu429Ala) and reduces the enzymatic activity of MTHFR, which causes an alteration in DNA synthesis and an imbalance in methylation [48,49].

The change from C to T and A to C can decrease enzyme activity by 30–40% for the former variant and by 60–to 70% for the latter. Patients who are heterozygous for both variants have increased homocysteine concentrations and reduced DNA methylation. BC risk has been associated with Asian, but not Caucasian, women, and the *MTHFR* A1298C gene polymorphism is not a susceptibility factor for BC. Concomitant low MTHFR enzyme activity resulting from the C677T gene polymorphism and low dietary intake of folate are associated with an increased risk of BC [50].

The G80A variant (rs1051266) of the *RFC* gene, a reduced folate carrier, is related to the downregulation of the transporter and leads to folate deficiency [1]. The overexpression of folate receptors (FR $\alpha$ and $\beta$) has been observed in BC as well as in lung, kidney, endometrial and colon cancer because it increases folate uptake and thus promotes tumor cell growth [1].

Folic acid plays an essential role in the synthesis of nitrogenous bases, and in DNA repair processes, insufficient folate leads to the incorporation of uracil in place of thymine, a substitution that repair mechanisms may not effectively identify owing to folate deficiency, potentially contributing to DNA strand breaks [5,51].

## 9. Folic Acid as a Treatment for Breast Cancer

Currently, there are chemotherapies that prolong the circulation time of drugs and act in a tumor-specific way. Among these advances are nanoparticles designed to specifically target the folate receptor (FR) present in tumor cells [52,53].

There are clinical trials for cancer treatment and *in vivo* studies which found that a single administration of these nanoparticles in mice with BC can inhibit tumor growth [54]; another study found that four doses suppressed tumor growth, and no side effects were observed in mice with BC [55]. Overall, FA supplementation is associated with a lower risk of BC, and investigations into the role of folate metabolism and homeostasis as biomarkers and treatments for cancer are proposed [56].

A meta-analysis of FA intake between 153 and 400 µg revealed a decreased risk of BC compared with women who consumed less than 153 µg but not those who consumed more than 400 µg ($p < 0.05$) [57]. Zhang *et al*. [25] published another meta-analysis involving 677,858 participants and reported that an intake of between 200 and 320 µg of FA was correlated with a lower risk of BC. However, excessive folate intake also increases the risk of cancer if preneoplastic lesions are present because excess folate promotes high growth rates via the provision of thymidylate and purines during DNA synthesis [58].

Lajous *et al*. [59] conducted a case control study with healthy Mexican women (n = 1391) and individuals with BC (n = 475) to determine the relationship between folate intake and BC risk; they reported an inverse association, with greater protection against BC in postmenopausal women, whereas the risk was greater in women who did not consume FA or who consumed low concentrations.

FA deficiency during cell division can cause uracil to be substituted in the DNA sequence, resulting in chromosomal breaks; approximately one normal human cell undergoes 50 double-stranded DNA breaks per cell cycle [60,61]. Notably, methylation patterns are more common in neoplastic tissue than in normal tissue [61].

## 10. Conclusion

FA is indispensable in the maintenance of DNA stability and in the prevention of many diseases. Owing to its importance, the recommended dose (400 µg) should be consumed, and the indications of healthcare personnel should always be followed. Alterations in FA metabolism due to malabsorption, inadequate diet or genetic variants can affect methylation patterns, so several authors have proposed that methylation status is a biomarker of BC.

It is important to consider the *MTHFR*, *RFC* and *FR* $\alpha$ and $\beta$ genetic variants to understand alterations in FA metabolism that are associated with altered cancer risk profiles. For example, variants that reduce *MTHFR* activity can lead to hypomethylation and subsequent DNA instability, increasing cancer risk. Conversely, excessive FA intake, especially in the presence of preneoplastic lesions, may favor tumor growth due to its role in DNA synthesis.

Current research indicates that maintaining an optimal intake of FA is vital for reducing the risk of BC. Clinical studies suggest that moderate FA supplementation can reduce the risk of BC, whereas excessive or deficient intake could be damaging. Furthermore, the role of FA in cancer therapy, especially through targeted delivery of nanoparticles, holds promise for future treatments.

Overall, understanding the intricate relationships among FA metabolism, epigenetics, and cancer risk is essential for the development of effective prevention and treatment strategies. We noted limitations in terms of the effect of FA on BC, which is complex and may change according to context. Studies have shown contradictory results, as at low concentrations, FA could have protective effects, but at high doses, it could contribute to cancer progression. This phenomenon makes it difficult to establish clear recommendations on FA supplementation in BC patients. There are studies on FA and BC that have used *in vitro* and *in vivo* assays, but the results may not be directly applicable to humans because of physiological and metabolic differences between species. Retrospective studies in humans are recommended to observe the effects of low or high doses of FA. In addition, the relationships between genetic variants in the *MTHFR*, *RFC*, and folate receptor (FR$\alpha$ and FR$\beta$) genes and BC risk are complex because the results may differ according to diet and other lifestyle factors.

Further research is needed to refine FA intake recommendations, explore its potential as a therapeutic tool in oncology and gain a thorough understanding of the mechanism of FA and its role in cancer pathophysiology as a possible protective factor.

## Availability of Data and Materials

Not applicable.

## Author Contributions

DGSR: conception and design, acquisition of data, analysis and interpretation of data, drafting the manuscript, reviewing for important intellectual content, final approval; PCB: acquisition of data; GMAM: conception and design, acquisition of data, reviewing for important intellectual content, final approval; SRE: analysis and interpretation of data, reviewing for important intellectual content, final approval; SLJY: conception and design, reviewing for important intellectual content, final approval. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript. All authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

## Ethics Approval and Consent to Participate

Not applicable.


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## Conflict of Interest

The authors declare no conflict of interest.

## Supplementary Material

Supplementary material associated with this article can be found, in the online version, at https://doi.org/10.31083/IJVNR26221.

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