Gene-Diet Interaction: DNA Repair Gene, Folate Status and Breast Cancer Risk

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Abstract

Folate levels have been inversely associated with breast cancer risk. Because folate deficiency can cause DNA damage such as uracil misincorporation, single-strand breaks, and double-strand breaks, genetic polymorphisms in base excision repair and double-strand break repair genes may lead to variation in DNA repair proficiency and modify the effect of folate on breast cancer risk. We present two examples of interaction between plasma folate levels and DNA repair genetic variants in a nested case-control study within the Nurses' Health Study (712 case-control pairs). Compared with the reference group of non-carriers in the lowest quartile of plasma folate, the reduction in risk (66%) was statistically significant among XRCC1 194Trp carriers in the highest quartile (multivariate odds ratio, 0.34; 95% confidence interval, 0.16–0.72).

The inverse association between XRCC1 194Trp and breast cancer risk was attenuated by lower plasma folate status. The inverse association between plasma folate level and breast cancer risk was stronger among 194Trp carriers (P, trend = 0.01) than non-carriers (P, trend = 0.09). We also observed that the positive association between the XRCC2 188His allele and breast cancer risk was significant only in women in the lowest plasma folate quartile (carriers versus non-carriers; multivariate odds ratio, 2.04; 95% confidence interval, 1.05–3.97), and this excess risk was abolished among those with higher plasma folate levels. Moreover, the inverse association between plasma folate level and breast cancer risk was stronger among XRCC2 188His carriers (P, trend = 0.004) than non-carriers (P, trend = 0.09). Although none of the statistical tests for interaction was significant, these data give some support to the hypothesis that genetic variations in DNA repair genes modify the relation between plasma folate level and breast cancer risk.

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Breast Cancer

Breast cancer is the most common cancer and the second leading cause of cancer death among women in the United States. Epidemiological studies have shown that familial breast cancer constitutes only about 5-10% of total breast cancer, and only 15-20% of familial clustering of breast cancer is attributable to the strongly predisposing BRCA1 and BRCA2 mutations. Therefore, most of the genetic variants that contribute to the risk of developing sporadic breast cancer remain unknown. Common genetic variants, especially in combination, or interacting with environmental factors, may account for a larger portion of predisposition to breast cancer than the high-penetrance genes discovered to date.

DNA Repair in Breast Cancer

Deficient DNA repair capacity has been suggested as a predisposing factor in familial and sporadic breast cancer.[1,2] Reduced DNA repair capacity among breast cancer cases has been detected in mutagen (X-rays, bleomycin, BPDE) sensitivity assays based on human peripheral blood lymphocytes[2-6] and in host cell reactivation assays with BPDE or UV-induced damage.[7,8] The wide range of carcinogens used in these assays suggests that defects in global DNA repair capacity, rather than a single substrate-specific DNA repair pathway, underlie cancer risk. The spectrum of p53 gene mutations in breast cancer suggests the involvement of multiple genotoxic compounds and DNA repair abnormalities in breast cell mutagenesis.[9]

The low activity of intrinsic DNA repair mechanisms, including nucleotide excision repair capacity and MGMT capacity, in breast tissue has been reported in several studies. It also has been proposed that breast epithelium lacks the redundant systems of double-strand break repair that are present in other tissues. Thus, defects in DNA repair would be expected to have greater impact in breast tissue.[10]

The XRCC1 Gene

The XRCC1 protein is involved in the base excision repair (BER) pathway. BER is responsible for repair of a wide variety of non-bulky exogenous and endogenous base damage and single-strand breaks. Although XRCC1 has no known enzymatic activity, three distinct domains are sites of interaction with DNA polymerase (amino acids 1–183), poly(ADP-ribose) polymerase (BRCT-I, amino acids 384–

476), and DNA ligase III (BRCT-II, amino acids 573–592). More recently, XRCC1 was also reported to interact with polynucleotide kinase and APE1. This suggests that XRCC1 acts as a nucleating factor in BER by bringing different components together at the site of action to promote the efficiency of the repair machinery. A number of SNPs in XRCC1 have been identified. These polymorphisms may alter BER proficiency and, in turn, predispose to breast cancer. We found a marginally significant reduction in the risk of breast cancer among XRCC1 194Trp carriers.[11] As compared with non-carriers, women with at least one 194Trp allele had a multivariate odds ratio of 0.79 (95% Confidence Interval or CI, 0.60 –1.04). The inferred haplotype harboring the 194Trp allele was more common in controls than in cases (6.6 *versus* 5.3%, P value 0.07).

The XRCC2 Gene

Repair of DNA DSB is essential to the maintenance of genomic integrity. Homologous recombination (HR) and non-homologous end joining (NHEJ) are two distinct mechanisms in the repair of DSB in mammalian cells. In the HR pathway, the strand exchange is catalyzed by RAD51 and facilitated by RAD52 through direct interaction. Five RAD51 paralogs facilitate the formation of RAD51 foci in two distinct complexes, XRCC3-RAD51C and RAD51B-RAD51C-RAD51D-XRCC2. Hamster cells deficient in XRCC2 or XRCC3 exhibit defects in Rad51 focus formation, a decrease in HR induced by DSB, hypersensitivity to radiation, increased spontaneous chromosome aberrations, and increased chromosome missegregation, implying critical roles of XRCC2 and XRCC3 in HR. Deficiency in BRCA1 or BRCA2 showed similar phenotypes, suggesting potential roles of XRCC2 and XRCC3 of HR in the development of breast cancer. We did not detect an association between the XRCC2 R188H polymorphism and breast cancer risk.[12] Carriers of XRCC2 188His had an OR of 1.10 (95%CI, 0.85-1.42).

Gene-Environment Interactions

The study of gene-environment interactions between genetic variation in DNA repair genes and environmental exposures is of particular interest. Unlike the genetic polymorphisms in metabolic pathways of environmental and dietary factors, which determine the internal or biologically active dose of exposures, the study of genetic polymorphisms in DNA repair pathways may provide new insights into the genotoxic effects of certain exposures. Some genetic variants in DNA repair genes may confer susceptibility to breast cancer only in the presence of certain exposures. This interaction analysis may offer at least one molecular mechanism underlying the associations between exposures and breast cancer risk.

Folate Status

More than one third of the folate in the American diet is provided by fruits and vegetables. Most ready-to-eat cereals are fortified with folate. Epidemiological evidence has suggested a role of inadequate folate intake in the development of breast cancer.[13] An inverse association of dietary folate with the risk of breast cancer was found in three large prospective epidemiological studies, the NHS, the Iowa Women's Health Study (NHS), and the Canadian National Breast Screening Study.[14-16] In the prospective nested case-control study within the NHS, plasma folate was inversely associated with breast cancer risk (OR, 0.73; 95%CI, 0.50-1.07, for highest versus lowest quintile; P, trend=0.06).[17]

In addition to reduced DNA methylation, the disruption of DNA integrity and repair is one potential mechanism by which folate deficiency contributes to carcinogenesis.[2] Folate deficiency reduces the methylation of dUMP to dTMP and thus induces dNTP pool imbalances,[18] resulting in excessive uracil misincorporation into human DNA during DNA replication and repair processes. Uracil in DNA is repaired by the BER pathway, which creates transient single-strand breaks following the excision of uracil by uracil DNA glycosylase.[1,19] Simultaneous repair of adjacent uracils on opposite strands can cause DSB.[6] The hypomethylation due to diminished folate status increases the sensitivity of mammalian DNA to methyl-sensitive nucleases, leading to an accumulation of DNA strand breaks.[18,20] Elevated numbers of chromosome breaks were detected in folate-deficient individuals and were reversed by folate administration.[5] With folate deficiency, uracil misincorporation and excision repair recur due to the limited thymidine pool.[21] Imbalanced nucleotide pools induced by folate deficiency were shown to impair nucleotide excision repair (NER) capacity in rat colonocytes and Chinese hamster ovary cells.[22,23] Recently, in a cancer-free population, low dietary folate intake was associated with suboptimal cellular NER capacity of the removal of BPDE-induced DNA adducts by host-cell reactivation assay in peripheral blood lymphocytes.[3,24] Folate deficiency may also impair mismatch repair (MMR). Folate deficiency may alter the DNA methylation pattern, which is important in strand discrimination in MMR.[25,26] Because of these multiple adverse effects of folate deficiency on DNA integrity and repair, we hypothesize interactions that genetic polymorphisms in DNA repair genes involved in BER, NER, DSB repair, and MMR modify the association of folate with breast cancer risk.

Effects of Plasma Folate and XRCC1 Arg194Trp on Breast Cancer Risk

We observed that the XRCC1 Arg194Trp genotype modified the association between plasma folate levels and breast cancer risk in the Nurses' Health Study.[27] As compared with the reference group of non-carriers in the lowest quartile of plasma folate, the reduction in risk (66%) was significant among 194Trp carriers in the highest quartile (multivariate OR, 0.34; 95% CI, 0.16-0.72). The inverse association between the carriage of the 194Trp allele and breast cancer risk was apparent in the high plasma folate categories and was attenuated among women with lower plasma folate levels. The inverse association between plasma folate levels and breast cancer risk appeared stronger among 194Trp carriers (P for trend= 0.01) than non-carriers (P for trend= 0.09) (P for interaction=0.12). In the analysis of interactions between XRCC1 Arg194Trp and plasma folate levels, the

multivariate ORs and tests of interaction did not change materially after controlling for plasma vitamin B6, vitamin B12, homocysteine, and antioxidants, one at a time or all simultaneously. This suggests that the enhanced DNA repair of downstream BER due to the XRCC1 194Trp allele may be apparent with a low level of DNA damage but is overwhelmed by excessive uracil misincorporation and single-strand breaks.

Effects of Plasma Folate and XRCC2 Arg188His on Breast Cancer Risk

The inverse association between plasma folate levels and breast cancer risk was stronger among XRCC2 188His carriers (P for trend= 0.004) than non-carriers (P for trend= 0.09).[27] A significantly positive association of the polymorphism XRCC2 188His with breast cancer risk was limited to women in the lowest quartile of plasma folate levels (carriers versus non-carriers, multivariate OR, 2.04; 95% CI, 1.05-3.97), and this excess risk was abolished among those with higher plasma folate levels. The multivariate OR remained significant for XRCC2 188His carriers in the lowest quartile after additionally controlling for plasma vitamin B6, vitamin B12, homocysteine, and antioxidants. These preliminary data suggest that adequate folate status may attenuate the elevated breast cancer risk associated with this genetic variation. The data also imply that, among the carriers of this variant, the reduced DNA repair capacity is adequate to maintain DNA integrity given a normal amount of damage; but the increased DNA DSB due to folate deficiency may overwhelm the already partially impaired DNA repair system and increase cancer risk.

Summary

Here, two examples of gene-diet interactions are presented. Although the interactions were not statistically significant, the present study provides preliminary data to support the novel hypothesis that genetic variations in the BER and DSB repair pathways modify the relation between plasma folate levels and breast cancer risk. Our data may suggest one potential biological mechanism underlying the beneficial effect of folate in the etiology of breast cancer; that is, the adverse effect of folate deficiency on breast cancer risk may be at least partially due to increased DNA damage. The critical task in the study of any gene-diet interaction is to replicate findings in other populations. In addition, large-scale screening of DNA repair genes with breast cancer risk along with evaluation of potential interactions with folate status could contribute to our understanding of the etiology of breast cancer and provide the scientific basis for identifying individuals at high risk for breast cancer and for individualized risk management strategies.

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