A NAD(P)H:Quinone Oxidoreductase 1 Polymorphism Is a Risk Factor for Human Colon Cancer

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Abstract

Colon cancer is one of the most common cancers in North America and generally develops from colonic epithelial cells following initiation by carcinogens. We have shown that the phase II detoxifying enzyme, NAD(P)H:quinone oxidoreductase 1 (NQO1) contributes to the inhibition of carcinogen-induced colon cancer in rats at both the initiation and postinitiation stages. An inactivating polymorphism at base 609 of the NQO1 gene, 609C(=NQO1 *1) → 609T (NQO1 *2), occurs at high frequency in the human population. Thus, we carried out a case-control study to determine if this polymorphism is associated with an increased risk of developing colon cancer. A total of 298 patients with colon cancer and 349 healthy controls matched for age, gender, and ethnic origin were enrolled in the study. There was an increased incidence of the NQO1 *2/*2 genotype in patients with colon cancer, with a gender and age-adjusted odds ratio of 2.68 (95% confidence intervals, 1.14-6.28). However, the incidence of the NQO1 *1/*2 genotype was not increased in patients with colon cancer compared with controls. When the patient and control groups were stratified by tobacco and alcohol use, the incidences of the NQO1 *2/*2 genotype were increased in patients with colon cancer for tobacco and alcohol users and nonusers, suggesting that there is no interaction between the NQO1 base 609 polymorphism and tobacco or alcohol use. These results strongly suggest that NQO1 plays a significant role in preventing the development of colon cancer, and individuals with an NQO1 *2/*2 genotype are at an increased risk of developing this disease. (Cancer Epidemiol Biomarkers Prev 2006;15(12):2422–6)

Introduction

Colorectal cancer is one of the most common cancers in North America and is a major cause of mortality (1). Colon cancer is generally of a sporadic nature and develops from colonic epithelial cells following initiation by carcinogens. The growth of initiated cells is increased by promoters leading to clonal expansion, and results in progression through a series of well-defined stages from dysplastic epithelium, including the development of early lesions called aberrant crypt foci, to adenomas, malignant carcinomas, and metastatic cancer.

Dietary factors have been shown to play an important role in influencing colon cancer risk, and it has been suggested that up to 90% of colon cancers may be preventable by dietary changes (2). Animal fat increases the incidence of colon cancer, whereas fruit and vegetable intake can decrease the incidence (3). Mutagens that initiate carcinogenesis or promoters that enhance progression are present in the normal diet. However, there are also dietary factors that may prevent the initiation of carcinogenesis by inactivating or removing mutagens, or that inhibit progression by detoxifying promoters or by directly inhibiting the progression process.

Fruits and vegetables contain dithiolethiones and isothiocyanates, which have been shown to be potent cancer-preventive agents in animal models (4-6). These compounds may produce their protective effects by inducing phase II detoxifying enzymes like NAD(P)H:quinone oxidoreductase 1 (NQO1), glutathione S-transferases, and UDP-glucuronyl-transferases. These enzymes can inhibit the initiation of carcinogenesis by directly modifying and inactivating carcinogens and/or by modifying and increasing their conjugation to glutathione and glucuronic acid, leading to their excretion from cells (7). However, there is evidence that phase II enzyme inducers may also work at the postinitiation stage of carcinogenesis by inducing apoptosis in cancer cells (8).

We previously showed that in a Sprague-Dawley rat model, the dithiolethione, oltipraz, selectively induces NQO1 in the colons of these rats without inducing other phase II detoxifying enzymes (9). Using this model, we showed that selective induction of NQO1 in the rat colon prior to treatment with a carcinogen significantly inhibited the formation of aberrant crypt foci. Using the same rat model, we found that induction of NQO1 by oltipraz following treatment with a carcinogen also significantly decreased the formation of aberrant crypt foci and inhibited the formation of colon adenomas and tumors (10). Together, these results provide strong evidence that NQO1 could contribute to the inhibition of colon carcinogenesis at both the initiation and postinitiation stages. Several mechanisms may account for these effects. NQO1 may detoxify colon carcinogens and/or tumor promoters. In addition, NQO1 may stabilize p53 protein, resulting in increased apoptosis (11, 12) in carcinogen-initiated colonic epithelial cells, which prevents these cells from progressing to a neoplastic state.

NQO1 is a flavoenzyme that catalyzes the two-electron reduction of quinones and nitrogen oxides (13, 14). A major function of this enzyme may be to decrease the formation of reactive oxygen species by decreasing one-electron reductions and the associated redox cycling (15). It has been shown to activate some anticancer drugs (16), and it may also play an important role in cancer prevention (9, 10, 13, 17). NQO1 consists of two identical protein subunits of 30 kDa whose expression is transcriptionally controlled (13). The enzyme is induced by a wide variety of inducers (18). The induction
Table 1. Age, gender, ethnic origin, tobacco and alcohol use of patients with colon cancer and healthy controls

| Age (y) | Patients with colon cancer (n = 298) | Healthy controls (n = 349) |
|---------|-----------------------------------|--------------------------|
| Range   | 26-89                             | 29-89                    |
| Median  | 66                                | 64                       |
| Gender (%) |                                    |                          |
| Male    | 161 (54.0)                        | 181 (51.9)               |
| Female  | 137 (46.0)                        | 168 (48.1)               |
| Ethnic origin (%) |                                |                          |
| Caucasian | 280 (94.0)                      | 327 (93.7)               |
| Aboriginal | 14 (4.7)                        | 18 (5.2)                 |
| Asian   | 4 (1.3)                           | 4 (1.1)                  |
| Tobacco use (%) |                                |                          |
| User*   | 192 (64.4)                        | 206 (59.0)               |
| Nonuser | 106 (35.6)                        | 143 (41.0)               |
| Alcohol use (%) |                                |                          |
| User†   | 117 (39.3)                        | 151 (43.3)               |
| Nonuser† | 181 (60.7)                       | 198 (56.7)               |

* >4 cigarettes/d.
† ≤4 cigarettes/d.
‡ >4 drinks/wk.
§ ≤4 drinks/wk.

pathway may involve a cytosolic redox signal which alters the expression and/or interaction of transcriptional factors like Jun, Nrf, Maf, Fos, and Fra with the xenobiotic response element and the antioxidant response element (19-25). The enzyme is ubiquitous in eukaryotes but levels vary in different tissues (13, 25, 26).

An inactivating polymorphism involving a base change from cytosine to thymine at base 609 of the NQO1 gene occurs at a frequency of ~50% in the human population, with 10% having two 609T (NQO1 *2) alleles (27, 28). However, these percentages vary in different ethnic groups, with a frequency rate of 2% to 5% in Caucasians and Blacks, and 20% in Asians (28). The NQO1 *2 form of the NQO1 protein has little or no activity (27, 29), and cells with one 609C (NQO1 *1) allele and one NQO1 *2 allele have approximately half of the NQO1 enzyme activity of NQO1 *1/*1 cells, but NQO1 *2/*2 cells have no activity (27, 30). Thus, humans with an NQO1 *2/*2 genotype have no NQO1 activity, and those with an NQO1 *1/*2 genotype have reduced enzyme activity. Because NQO1 induction has been shown to inhibit carcinogenesis in animals (8, 9), individuals with NQO1 *2/*2 and NQO1 *1/*2 may be at higher risk for carcinogen-induced cancers. Clinical studies have shown an increased frequency of the NQO1 *2 allele in patients with renal (31), uterine (31), esophageal (32, 33), bladder (34), breast (35), and gastric (32, 33) cancers and both pediatric (36, 37) and adult leukemias (38, 39) compared with healthy controls. These findings suggest that the active form of NQO1 may provide protection from some carcinogen-induced cancers. In contrast, one study showed a lower frequency of NQO1 *2 in patients with bladder cancer (40) compared with controls. Other studies have reported no differences in the occurrence of NQO1 *2 in renal (41), esophageal (42), breast (42, 43), gastric (42), prostate (42, 44), and head and neck cancers (45), adult gliomas (46), and lymphomas (42, 47). The possible role of the NQO1 *2 allele in lung cancer has been studied extensively, with mixed results (42, 48-50). The inactive form of the enzyme may increase or decrease the risk, or have no effect on the risk of developing lung cancer, depending on the type of lung cancer, ethnic origin, and smoking behavior. Previous studies identified the NQO1 *2 allele as a risk factor for colorectal cancer either alone (51, 52), when associated with other genetic changes such as a K-ras (52) or a CYP1A1 mutation (53), or when associated with smoking (53). However, three other studies showed no significant differences in NQO1 genotype frequencies between patients with colorectal cancer and healthy controls (42, 54, 55). A recent meta-analysis suggested a statistically significant association of the NQO1 *2 allele with a moderately elevated risk of developing colorectal cancer (56). Thus, the role of the NQO1 polymorphism as a risk factor for colon cancer remains controversial.

A second NQO1 polymorphism has been identified at base 645 of the NQO1 gene also involving a base change from cytosine to thymine (57); however, this polymorphism occurs at a much lower frequency than the one at base 609 (58). The *1 allele (NQO1 *3) also results in a phenotype with lower NQO1 activity due to the decreased stability of the variant RNA and the decreased activity of the resulting protein (58). The role of this polymorphism in cancer has received only limited attention with one study in childhood acute lymphoblastic leukemia (37), one study in colon cancer (55), and one study in squamous cell carcinoma of the head and neck (45) showing no significant differences in the frequency of NQO1 *3 in patients compared with healthy control subjects.

Because we have shown that NQO1 may play an important role in preventing colon carcinogenesis in a rat model (9, 10), the presence of NQO1 *2 may represent a genetic factor that predisposes individuals to this disease. Therefore, we carried out a case-control study in humans to determine if this NQO1 polymorphism is associated with an altered risk of developing colon cancer. Because tobacco and alcohol use have been identified as risk factors for developing colon cancer (59-61), we also investigated whether there was a link between these risk factors and the NQO1 genotype.

Materials and Methods

This study was approved by the Research Ethics Board of the University of Manitoba. A total of 298 patients with colon cancer and 349 healthy control subjects were enrolled in a case/control study over a 5-year period. All individuals enrolled in the study provided informed consent for their participation in the study. Individuals previously or newly diagnosed with pathologically proven colon cancer were recruited from patients at CancerCare Manitoba, Winnipeg, Manitoba, Canada. Individuals for the healthy control population were recruited from volunteers that had not been diagnosed with colon cancer who attended community-based clinics in Winnipeg. All participants completed a questionnaire providing information about their age, gender, ethnic origin, and use of tobacco or alcohol. Genotyping was carried out by PCR RFLP analysis on whole blood samples obtained from all participants in the study using primers and conditions previously described (45). The frequency of the NQO1 *1/*1, NQO1 *1/*2, and NQO1 *2/*2 genotypes were compared in the patient and control groups by a logistic regression analysis and odds ratios (OR) were calculated. Participants were stratified by use of tobacco and/or alcohol and the genotype

Table 2. Genotype distribution and allele frequency of NQO1 base 609 polymorphism for patients with colon cancer and healthy controls

| Genotype       | Patients with colon cancer, n (%) | Healthy controls, n (%) | OR (95% CI) |
|----------------|----------------------------------|-------------------------|-------------|
| NQO1 *1/*1     | 201 (67.5)                       | 239 (68.5)              |             |
| NQO1 *1/*2     | 79 (26.5)                        | 102 (29.2)              | 0.92 (0.65-1.31) |
| NQO1 *2/*2     | 18 (6.0)                         | 8 (2.3)                 | 2.68 (1.14-6.28) |
| Allele frequency |                                   |                        |             |
| NQO1 *1        | 0.807                            | 0.831                   |             |
| NQO1 *2        | 0.193                            | 0.169                   |             |
distributions in these subgroups were compared. Individuals who currently or previously smoked more than four cigarettes per day were considered tobacco users, and individuals who currently or previously consumed more than four drinks of alcohol per week were considered alcohol users. Study participants were also stratified by ethnic origin and the genotype distribution in these subgroups were compared.

**Results**

The age, gender, ethnic origin, smoking, and alcohol use characteristics of study participants at enrollment are shown in Table 1. In general, the patient and control groups were well matched in terms of age, gender, and ethnic origin. The majority of participants in this study were Caucasian. The proportions of tobacco and alcohol users in the patient and control groups were similar.

The genotype distribution of the NQO1 base 609 polymorphism in the healthy control population was similar to a previous report (58). We observed an increased incidence of the NQO1 \(*2/*2\) genotype in patients with colon cancer compared with healthy controls (Table 2). The gender- and age-adjusted OR [95% confidence intervals (95% CI)] for the NQO1 \(*2/*2\) genotype was 2.68 (1.14-6.28) and this result was statistically significant (P < 0.05). The incidence of the NQO1 \(*1/*2\) genotype was not increased in patients with colon cancer compared with healthy controls, with an OR of 0.92 (95% CI, 0.65-1.31). The frequency of the NQO1 \(*2\) allele was increased in patients with colon cancer compared with healthy subjects; however, this result was not statistically significant (Table 2).

When the groups were stratified by tobacco and alcohol use, for both tobacco users and nonusers, there were increased incidences of the NQO1 \(*2/*2\) genotype in patients with colon cancer compared with healthy controls with ORs of 2.19 (95% CI, 0.86-5.59) and 5.43 (95% CI, 0.59-49.55), respectively, but these were not statistically significant. When the groups were stratified by ethnic origin, there was a higher incidence of the NQO1 \(*2/*2\) and NQO1 \(*1/*2\) genotypes in the Aboriginal healthy control group compared with the Caucasian control group, but these results were similar to findings reported previously for these ethnic groups (57). The NQO1 \(*2/*2\) genotype was increased in patients with colon cancer compared with the corresponding control group in both ethnic subpopulations (Table 4). The ORs for the NQO1 \(*2/*2\) genotype were 5.0 (95% CI, 0.64-39.06) for the Aboriginal population and 2.52 (95% CI, 0.94-6.76) for the Caucasian population; however, these results were not statistically significant. The incidence of the NQO1 \(*1/*2\) genotype was not increased in patients with colon cancer compared with the healthy controls for either the Aboriginal or the Caucasian populations.

**Discussion**

Using a rat model of colon carcinogenesis, we have shown that NQO1 may play an important role in preventing colon cancer (9, 10). Thus, the presence of an NQO1 inactivating polymorphism may represent a genetic factor that predisposes individuals to this disease. This study examined the role of the base 609 polymorphism in the NQO1 gene in the development of colon cancer in humans. A total of 298 patients with colon cancer and 349 healthy control subjects that were well-matched for age, gender, ethnic distribution, and tobacco and alcohol use were enrolled in this study (Table 1).

The incidences of the NQO1 \(*1/*2\) and the NQO1 \(*2/*2\) genotypes in our healthy control population were similar to previously reported values (58). We found a 2.68-fold greater incidence of the NQO1 \(*2/*2\) genotype in patients with colon cancer compared with the healthy control population, and this difference was statistically significant (Table 2). This finding is in agreement with several previous studies of the role of the NQO1 base 609 polymorphism in colon cancer risk (51-53). The ORs for the NQO1 \(*2/*2\) genotype in these studies, which

### Table 3. Genotype distribution of NQO1 base 609 polymorphism for patients with colon cancer and healthy controls stratified by tobacco and alcohol use

| Group     | Genotype distribution (%) | Patients | Controls | Patients | Controls | OR (95% CI) | Patients | Controls | OR (95% CI) |
|-----------|---------------------------|----------|----------|----------|----------|-------------|----------|----------|-------------|
| **Tobacco** |                           |          |          |          |          |             |          |          |             |
| Users (%) | NQO1 \(*1/*1\)          | 128 (66.7) | 140 (68.1) | 50 (26.0) | 59 (28.5) | 0.93 (0.59-1.45) | 14 (7.3) | 7 (3.4) | 2.19 (0.86-5.59) |
|           | NQO1 \(*1/*2\)          | 73 (68.9)  | 99 (69.2)  | 29 (27.4) | 43 (30.1) | 0.92 (0.52-1.60) | 4 (3.8)  | 1 (0.7)  | 5.43 (0.59-49.55) |
| Nonusers (%) |                           |          |          |          |          |             |          |          |             |
| Users (%) | NQO1 \(*1/*1\)          | 77 (65.8)  | 98 (64.9)  | 33 (28.2) | 49 (32.5) | 0.86 (0.50-1.46) | 7 (6.0)  | 4 (2.7)  | 2.23 (0.63-7.89) |
|           | NQO1 \(*1/*2\)          | 124 (68.5) | 141 (71.2) | 46 (25.4) | 53 (26.8) | 0.99 (0.62-1.57) | 11 (6.1) | 4 (2.0)  | 3.13 (0.97-10.07) |
| **Alcohol** |                           |          |          |          |          |             |          |          |             |
| Users (%) | NQO1 \(*1/*1\)          | 242 (42.1) | 242 (42.1) | 112 (19.7) | 112 (19.7) | 0.94 (0.75-1.19) | 21 (3.8) | 21 (3.8) | 1.02 (0.64-1.60) |
|           | NQO1 \(*1/*2\)          | 240 (41.9) | 240 (41.9) | 112 (19.6) | 112 (19.6) | 0.94 (0.75-1.18) | 21 (3.8) | 21 (3.8) | 1.02 (0.64-1.60) |
| Nonusers (%) |                           |          |          |          |          |             |          |          |             |
| Users (%) | NQO1 \(*1/*1\)          | 10 (6.8)  | 10 (6.8)  | 5 (3.2)  | 5 (3.2)  | 1.00 (0.40-2.53) | 0 (0.0)  | 0 (0.0)  | Not determined |
|           | NQO1 \(*1/*2\)          | 10 (6.8)  | 10 (6.8)  | 5 (3.2)  | 5 (3.2)  | 1.00 (0.40-2.53) | 0 (0.0)  | 0 (0.0)  | Not determined |
| Nonusers (%) |                           |          |          |          |          |             |          |          |             |

### Table 4. Genotype distribution of NQO1 base 609 polymorphism for patients with colon cancer and healthy controls stratified by ethnic origin

| Group       | Genotype distribution (%) | Patients | Controls | Patients | Controls | OR (95% CI) | Patients | Controls | OR (95% CI) |
|-------------|---------------------------|----------|----------|----------|----------|-------------|----------|----------|-------------|
| **Ethnic origin** |                           |          |          |          |          |             |          |          |             |
| Aboriginal  | NQO1 \(*1/*1\)          | 4 (28.6)  | 10 (55.6) | 6 (42.9) | 6 (33.3) | 2.50 (0.50-12.50) | 4 (28.6) | 2 (11.1) | 5.00 (0.64-39.06) |
|             | NQO1 \(*1/*2\)          | 2 (50.0)  | 2 (50.0)  | 1 (25.0) | 2 (50.0) | Not determined | 1 (25.0) | 0 (0.0)  | Not determined |
| Asian       | NQO1 \(*1/*1\)          | 195 (69.6) | 227 (69.4) | 72 (25.7) | 94 (28.8) | 0.89 (0.62-1.28) | 13 (4.6) | 6 (1.8)  | 2.52 (0.94-6.76) |
|             | NQO1 \(*1/*2\)          | 27 (9.6)  | 37 (11.5) | 12 (4.2) | 14 (4.3) | Not determined | 6 (2.1)  | 0 (0.0)  | Not determined |
toted 1,343 patients with colorectal cancer and 1,440 control subjects, ranged from 1.5 to 2.9. However, the current results differ from other three studies with colon cancer patients, which found no significant difference in the NQO1 genotype distribution in the patient population compared with controls (42, 54, 55). These latter studies totaled 887 patients with colorectal cancer and 1,438 controls. It is not clear why these different studies produced such different results. However, in the current study, the age, sex, and ethnic distribution of the patients and controls were very well matched, which was not the case in some of the previous studies. In addition, we did not include any patients with rectal cancer in our study.

We investigated whether there was an interaction between the use of tobacco or alcohol, the NQO1 base 609 polymorphism, and colon cancer risk. When patients and healthy controls were subcategorized by tobacco or alcohol use, there were increased incidences of the NQO1 *2/*2 genotype in patients with colon cancer compared with healthy controls for all four subgroups, with ORs that were generally similar to the results obtained for the groups as a whole (Table 3). Although these results approached significance in some cases, they were not statistically significant, likely because the number of subjects in these subpopulations were small compared with the total population. However, overall, these results suggest that there is no interaction between the NQO1 base 609 polymorphism and tobacco or alcohol use. Based on our previous studies of colon cancer prevention in a rat model (10), the current results suggest that NQO1 may play a protective role against colon cancer in humans by detoxifying dietary carcinogens or promoters or by increasing apoptosis in carcinogen-initiated colon epithelial cells. Although >90% of our study population was Caucasian, both the colon cancer patient and control populations included ~5% individuals with an Aboriginal ethnic origin. These individuals were either American Indian or Inuit. Although the number of individuals in this subgroup was small, the incidences of the NQO1 *1/*2 and NQO1 *2/*2 genotypes in the control population were 28.6% and 11.1%, respectively; ~6-fold higher than the Caucasian control subgroup or the control group as a whole (Table 4). However, these results were similar to a previous report for North American Indian and Inuit populations (57). Although the number of Aboriginal subjects enrolled in this study was too small for the results to be statistically significant, the OR for the aboriginal subpopulation was 5.00, which was higher than that for the Caucasian subgroup and the population as a whole.

These results, along with our studies of NQO1 prevention of colon carcinogenesis in rats, strongly suggest that NQO1 plays a significant role in preventing the development of colon cancer, and individuals having an NQO1 *2/*2 genotype are at an increased risk of developing this disease. Additional studies to identify interactions with other risk factors such as glutathione S-transferase polymorphisms and diet may identify subgroups within the general population that might benefit from genetic screening for the NQO1 polymorphism. Larger studies with ethnic groups having a high incidence of the NQO1 *2 allele would be of particular interest in order to determine if individuals in these groups have an even greater risk of developing colon cancer compared with the general population.

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