Allelic Loss of the Gene for the GPX1 Selenium-Containing Protein Is a Common Event in Cancer1–3

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ABSTRACT Selenium has been shown to reduce cancer incidence in animal models and more recent data indicate that it may be protective in humans as well. However, little is known about the mechanism by which selenium prevents cancer. Cytosolic glutathione peroxidase (GPX1), a selenium-containing antioxidant enzyme, has been implicated in the development of cancer of the head and neck, lung, and breast, in part because of allelic loss at the GPX1 locus. The study of allelic loss at the GPX1 locus in colon cancer was investigated by examining loss of heterozygosity (LOH) in DNA extracted from both tumor and adjacent histopathologically normal tissue obtained by laser capture microdissection. Tissue samples were obtained from 53 colon cancer patients. Two highly polymorphic markers, alanine codon repeats and a proline-leucine polymorphism (198P/L) present in the GPX1 gene, were used to examine LOH at this locus. Analysis of both polymorphisms identified LOH at GPX1 in a significant percentage of colorectal cancer (42%). These results indicated that LOH at the GPX1 locus is a common event in cancer development and that GPX1 or other tightly linked genes may be involved in the etiology of this disease. J. Nutr. 135: 3021S–3024S, 2005.

KEY WORDS: • selenium • selenoproteins • glutathione peroxidase • cancer

One of the most promising candidate chemopreventive agents is the essential trace element selenium. Decades of animal studies have consistently demonstrated that supplementing the diet of rodents with low, nontoxic amounts of selenium is effective in reducing cancer incidence in response to a broad range of carcinogens in most organ systems examined (1). In humans, several groups reported an inverse correlation between dietary intake of selenium and cancer incidence at several sites, including lung, colon, and prostate (2–6). Human supplementation studies have been few, but the reduction in cancer incidence as a consequence of consuming selenium as a supplement at levels obtainable from over-the-counter products indicated that selenium was effective in reducing cancer incidence in lung, colon, prostate, and liver (7,8). Because of this accumulative body of evidence, large chemopreventive human studies have been initiated (9). However, the mechanism by which selenium may reduce cancer incidence remains unknown.

It is likely that many of the effects of selenium are mediated through its role as a constituent of selenium-containing proteins. Twenty-five selenoproteins were discovered in the human genome (24 in the mouse) and all contain selenium as the amino acid selenocysteine (Sec)3 (10,11). Sec is incorporated cotranslationally during selenoprotein synthesis in response to in-frame UGA codons in the mRNA for these selenoproteins. Sec insertion requires dedicated translation factors including a Sec tRNA and elongation factor in addition to the RNA element in the 3′-untranslated portion of the mRNA that directs Sec incorporation in response to all in-frame UGA codons (12,13). In mammalian cells, this process is highly regulated and responsive to selenium availability, both at the levels of RNA stability and translation. Whether individual selenoproteins or selenoproteins as a group are involved in the health benefits associated with selenium remains unknown.

Selenium is an essential micronutrient shown to reduce colon cancer incidence and preneoplastic aberrant crypts foci in animal models (14–17). In human studies, data have indicated that selenium levels are inversely associated with cancer mortality and incidence. A prospective case-control study indicated a statistically significant inverse association between toenail selenium levels and the risk of colon cancer (5). A significant inverse association between selenium levels and the incidence of large adenomatous polyps, after adjustment for...
Materials and methods

Cancers, head and neck (22,24,25). In the case of head and neck cancers, including those occurring in lung, breast, and gastrointestinal tract (20).

Results

Genetic analyses of the GPx-1 locus in colon cancers and adjacent tissue. Laser capture microdissection was used to obtain tumor and nontumor cells from paraffin-embedded samples obtained during the resection of colon tumors. Paraffin-embedded colon cancer blocks were randomly chosen from the Gastrointestinal Cancers Tissue Bank housed in the Jesse Brown Veterans Affairs Medical Center, Chicago, IL. All tissues studied had a confirmed histopathological diagnosis of colon cancer. Standard hematoxylin and eosin-stained sections from each lesion were reviewed to verify the diagnosis. Patients included African Americans and Caucasians, 49–97 y old, whose tumors were evenly represented across Dukes stages. To obtain samples representing tumor and histopathologically appearing normal tissue from the same colon, both tumor cells and normal cell were selected by laser capture microdissection from the same slide for genetic analysis. Representative photos taken before and after laser capture microdissection are shown in Figure 1.

LOH in paired samples was assessed by examining a highly polymorphic alanine repeat region encoded within the first exon of human GPX1, shown to result in repeats of 5, 6, or 7 alanine codons (25). LOH was determined when the analysis indicated heterozygosity in the DNA of noncancerous tissue but only a single allele being evident in the DNA derived from the tumor (Fig. 2). Genotyping data are summarized in Table 1; 8 of 53 patients exhibited LOH by these criteria; of these, only 25 of 53 samples were informative (heterozygosity in noncancerous tissue), for an LOH frequency of 32%.

In addition to the alanine repeat polymorphism, a single nucleotide polymorphism in the GPX1 gene results in either a leucine or proline at codon 198 (25), and this variation was shown to increase the risk for lung (21) and possibly breast cancer (22). The identity of the amino acid at codon 198 (proline or leucine) has functional consequences with regard to level of enzyme activity in response to increasing levels of selenium provided to cells in culture (22).

Allelic loss of chromosome regions bearing tumor suppressor genes is a key event in the evolution of epithelial and mesenchymal tumors, and this event can be detected by loss of a heterozygous marker (23). Loss of heterozygosity (LOH) occurs at the GPX1 locus during the development of several cancer types, including those occurring in lung, breast, and head and neck (22,24,25). In the case of head and neck cancers, GPX1 allelic loss was shown to occur in histopathologically normal tissue adjacent to tumors, indicating that loss at this locus may be an early event in cancer evolution (24).
if their diets are supplemented with selenium (1,14–17,26) and GPX1 levels respond to selenium supplementation (27). In addition, functional polymorphisms within the human GPX1 gene are associated with increased risk of lung cancer and possibly cancers of other organs (21,22). In addition to data indicating a risk of cancer associated with specific GPX1 alleles, LOH at the GPX1 locus was shown to be a common event in cancer development (25,22,24). The previously reported data on GPX1 LOH in several tumor types is now extended to colon cancer in this study, although it still remains to be established whether it is the loss of a GPX1 allele or a tightly linked gene that promotes tumorigenesis. The GPX1 gene is located at chromosome location 3p21, and lung cancers exhibiting 3p LOH have reduced GPX1 enzyme activity and compromised oxidative defense with elevated levels of the DNA oxidation product 8-hydroxydeoxyguanosine (28). LOH at this position is also associated with higher number of relapses and shorter disease-free survival for lung cancer patients (29). Conceivably, allelic loss could unmask a recessive mutation in the remaining allele or promote cancer

by resulting in the reduction in cellular GPX1 activity, in attenuated antioxidant defenses, or alterations in affected signaling pathways. Collectively, these data indicate the possibility that lower levels of GPX1 are a risk factor for cancer and that the loss of 1 of 2 copies of the gene during tumor development plays a role in disease progression. In addition, these data indicate that GPX1 may be involved in the mech-

## Table 1

<table>
<thead>
<tr>
<th># ala codon repeats</th>
<th>Normal cells</th>
<th>Tumor cells</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(n = 53)</td>
<td></td>
</tr>
<tr>
<td>5/5</td>
<td>23</td>
<td>29</td>
</tr>
<tr>
<td>6/6</td>
<td>4</td>
<td>6</td>
</tr>
<tr>
<td>7/7</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Homozygosity</td>
<td>28 (53%)</td>
<td>36 (68%)</td>
</tr>
<tr>
<td>5/6</td>
<td>12</td>
<td>7</td>
</tr>
<tr>
<td>5/7</td>
<td>9</td>
<td>6</td>
</tr>
<tr>
<td>6/7</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>Heterozygosity</td>
<td>25 (47%)</td>
<td>17 (32%)</td>
</tr>
</tbody>
</table>

*Loss of heterozygosity was realized by the clear demonstration of heterozygosity in DNA obtained from normal cells but not in tumor cells.*
TABLE 2
Summary of the codon 198 polymorphism frequency in DNA from tumor and adjacent normal cells obtained from colon cancer patients

<table>
<thead>
<tr>
<th>GPX1 codon 198</th>
<th>Normal tissue</th>
<th>Tumor tissue</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pro/Pro</td>
<td>24 (45.3%)</td>
<td>33 (62.3%)</td>
</tr>
<tr>
<td>Pro/Leu</td>
<td>26 (49%)</td>
<td>15 (28.3%)</td>
</tr>
<tr>
<td>Leu/Leu</td>
<td>3 (5.7%)</td>
<td>5 (9.4%)</td>
</tr>
</tbody>
</table>

1 Allelic identity at codon 198 was identified by restriction enzyme digestion of a PCR product using Apa 1.

LITERATURE CITED