Vegetables Affect the Expression of Genes Involved in Carcinogenic and Anticarcinogenic Processes in the Lungs of Female C57Bl/6 Mice

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ABSTRACT Worldwide, lung cancer is the most prevalent and lethal malignant disease. In addition to avoidance of the most predominant risk factor, i.e., tobacco use, consumption of high amounts of vegetables and fruits could be an effective means of preventing lung cancer. However, the molecular mechanisms underlying lung cancer risk reduction by vegetables are not clear. In the present study, the effect of vegetables on gene expression changes in the lungs of female C57Bl/6 mice was investigated using cDNA microarray technology. The mice were fed 1 of 8 diets for 2 wk: a control diet containing no vegetables (diet 1); a diet containing a vegetable mixture at 100 (diet 2, 10% dose), 200 (diet 3, 20% dose), or 400 (diet 4, 40% dose) g/kg; or a diet containing cauliflower at 70 (diet 5, 7% dose); carrots at 73 (diet 6, 7.3% dose); peas at 226 (diet 7, 22.6% dose); or onions at 31 (diet 8, 3.1% dose) g/kg. The vegetable mixture consisted of these 4 individual vegetables. After the mice were killed, the lungs were removed and total RNA was isolated from the lungs for expression analysis of 602 genes involved in pathways of (anti)-carcinogenesis. The results of this study suggest that individual vegetables have a higher potential of modulating genes (5 from the 8 modulated genes) in favor of lung cancer risk prevention, in comparison with the vegetable mixture (2 from the 7 modulated genes); the other gene modulations are expected to enhance lung cancer risk. The pathways involved were miscellaneous and included cell growth, apoptosis, biotransformation, and immune response. Furthermore, carrots were able to modulate most gene expressions, and most of these effects occurred in processes that favored lung cancer risk prevention. The current study provides more insight into the genetic mechanisms by which vegetables, in particular carrots, can prevent lung cancer risk. J. Nutr. 135: 2546–2552, 2005.

KEY WORDS: • vegetables • microarrays • gene expression • C57Bl/6 mice • lung

Lung cancer is the most prevalent and lethal malignant disease in the world, accounting for almost 16% of all new cancer cases. Approximately 90% of the people who develop lung cancer will die from it. No effective treatment is available; the 5-y survival rate for lung cancer patients is <15%. Thus, the need for primary prevention of lung cancer is paramount (1).

The most effective means of preventing lung cancer is avoidance of tobacco use. Cigarette smoking accounts for ~90% of cases in men and ~80% of cases in women; the remaining cases are due to occupational exposures including asbestos, arsenic, chloromethyl ethers, chromium-VI, and nickel; residential and occupational exposures to radon; and probably exposures to carcinogenic air pollutants in the general environment (2). In addition to avoiding or reducing these known causative exposures, consumption of diets high in vegetables and fruit could be an effective means of preventing lung cancer. However, report outcomes on this topic are rather conflicting. In a report jointly published by the World Cancer Research Fund and the American Institute for Cancer Research (WCRF/AICR)3 (3) in 1997, it was stated that the evidence regarding the relation between a high intake of fruit and vegetables and a decrease in lung cancer risk was convincing. However, in 2003, the International Agency for Research on Cancer reported in their Handbook on Cancer Prevention that the effect of fruits and vegetables on the risk of lung cancer was limited, rather than sufficient (4). Furthermore, Peto (5) provided evidence that dietary factors contribute very little to overall cancer incidence.

In their report, the WCRF/AICR described results from

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1 Supplemental Tables 1 and 2 and the microarray data files are available as Online Supporting Material with the online posting of this paper at www.nutrition.org.
2 To whom correspondence should be addressed. E-mail: j.vandelft@grat.unimaas.nl.
3 Abbreviations used: BNIP1, BCL2/adenovirus E1B 19kDa-interacting protein 1; Cyd, cytidine 3'; CyS, cytidine 5'; CTSS, cathepsin S; FCER1G, Fc receptor, IgE, high affinity I, γ polypeptide; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; GLUL, glutamate-ammonia ligase (glutamine synthase); GSR, glutathione reductase 1; G1P2, interferon, α-inducible protein; HPGD, hydroxyprostaglandin dehydrogenase 15 (NAD); IGFBP3, insulin-like growth factor binding protein 3; LDR, LDL receptor; SELENBP1, selenium binding protein 1; SLC6A4, solute carrier family 6 (neurotransmitter transporter, serotonin), member 4; SULT1A1, sulfortransferase family 1A, phenol-prefering, member 1; TG2, transglutaminase 2; TOP2A, topoisomerase (DNA) II α 170 kDa; WCRF/AICR, World Cancer Research Fund and the American Institute for Cancer Research.
epidemiologic studies on dietary factors and risk of lung cancer (3). Prospective epidemiologic studies showed that dark green and yellow-orange vegetables, in particular, rich in β-carotene and vitamin E, are the specific types of vegetables that best protect against lung cancer. Limited evidence is available regarding the preventive potential of legumes such as beans and peas against lung cancer (3). However, vegetables contain relatively high amounts of specific micronutrients with specific anticarcinogenic potential. Observational epidemiologic studies demonstrated a significantly decreased relative risk of lung cancer between a high vegetable intake and serum levels of these micronutrients (6,7). The antioxidative capacities of these micronutrients were suggested to protect against oxidative DNA damage resulting in lower cancer risk (7–11). However, the results of the randomized controlled trials in which the effect of daily high doses of β-carotene, vitamin A, and/or vitamin E administered orally over several years on lung cancer incidence was investigated, were not supportive of this hypothesis (12–15). Instead of a protective effect, adverse effects on lung cancer development were reported. It was suggested that the high concentrations of these antioxidants resulting from supplementation had prooxidant effects that induced DNA damage and membrane instability. Furthermore, the results of these trials emphasized that it is not known whether the epidemiologic associations are specific for the micronutrients or whether the micronutrient measurements are serving as a proxy for the intake of other protective substances or even more healthy dietary habits in general. In addition, the complex mixture of numerous substances rather than a single constituent in vegetables could be responsible for the net effect.

In a previous study by our group, we investigated the effect of vegetable Introduction changes in colonic mucosal tissue from female C57BL/6 mice (16). In addition to prevention of lung cancer, epidemiologic studies provided evidence for protection against colorectal cancer incidence by vegetables. In contrast to the colon and rectum, the lungs are not a site of direct contact, i.e., the vegetables pass the gastrointestinal tract, but not the lungs as they are ingested. Furthermore, the dietary compounds that reach the lung may have been modulated by the first-pass metabolism in the liver. Therefore, the lungs are subject only to systemic exposure of vegetable-derived compounds and the mechanisms by which vegetables protect against lung cancer could therefore differ from those in the colon. The specific mechanisms by which vegetables may prevent lung cancer are not known and require elucidation. In general, the number of studies in which the effect of whole vegetables instead of a specific constituent was examined is limited. Changes in multigene expression patterns can provide information about regulatory mechanisms and broader cellular functions and biochemical pathways.

Current DNA microarray technology allows the simultaneous analysis of the expression of a large numbers of genes (17). Therefore, in the present study, we used microarray technology to gain more information about the effects of vegetables in the lungs of mice at the level of the expression of multiple genes involved in various genetic pathways associated with cancer risk prevention. Two different approaches were taken: in the first, the dose-dependent effect of a mixture of 4 vegetables (cauliflower, carrots, peas, and onions) on gene expression changes in the lung was examined; in the second approach, the role of individual vegetables present in the vegetable mixture, was investigated.

MATERIALS AND METHODS

Mice and diet. The number and type of mice used, the preparation and composition of the vegetable diets, and the treatment of the mice in the present study were described previously (16). In short, 8-week-old female C57BL mice (Charles River Laboratories) were randomly assigned to 1 of 8 different diets for 2 wk: a control diet containing no vegetables (diet 1); a diet containing a vegetable mixture at 100 (diet 2, 10% dose), 200 (diet 3, 20% dose), or 400 (diet 4, 40% dose) g/kg; or a diet containing: cauliflower at 70 (diet 5, 7% dose); carrots at 73 (diet 6, 7.3% dose); peas at 226 (diet 7, 22.6% dose); or onions at 31 (diet 8, 3.1% dose) g/kg. The vegetable mixture used consisted of the 4 individual vegetables used in diets 5–8, i.e., cauliflower (32% wet wt), carrots (30% wet wt), peas (30% wet wt), and onions (10% wet wt). The composition of the different diets is presented in Table 1.

The mice were maintained in the laboratory animal care facilities at Maastricht University under controlled environmental conditions (temperature 21 ± 1°C, relative humidity 50% ± 10, 12-h light:dark cycle). Body weights of the mice were recorded weekly. The study was approved by the Institutional Committee of Animal Experimentation at the Maastricht University.

Tissue sampling. Mice were killed by bleeding the yena cava inferior wilder and Nembutal (Sanofi Santé) anesthesia. Nembutal was administered s.c. in the neck at a dose of 60 mg/kg body weight. The lungs were removed and quickly washed in ice-cold 1X PBS, immediately frozen in liquid nitrogen, and stored at –80°C until use. For total RNA isolation, frozen lung tissue was ground to a powder in a stainless steel mortar under liquid nitrogen and homogenized in 800 μL TRIZOL Reagent (Gibco/BRL).

Total RNA isolation and cDNA probe synthesis. Total RNA was extracted according to the manufacturer’s instructions. The RNAsefree Mini Kit (Quagen) together with a DNase treatment was used to purify total RNA from salt and residual DNA. The quantity of each RNA sample was measured by a spectrophotometer and vials containing low- to 30-μg RNA. RNA integrity was determined by a Bioanalyzer (Agilent Technologies Netherlands). All samples contained intact total RNA with an rRNA ratio (28S:18S) > 1.5.

RNA pools (n = 3/diet group) were prepared by pooling equal amounts of total RNA from 2 or 3 mice. Cyanine (Cy3)- and Cy5-labeled cDNA probes were prepared using 3 μg total RNA from each pool, by the method of Hasseman et al. (18).

cDNA Microarray preparation and hybridization. Preparation of cDNA microarrays and cDNA microarray hybridizations were carried out as described previously (16). In short, cDNA microarrays were prepared at the Genome Centre Maastricht, the Netherlands and contained 6,092 mouse genes. Hybridizations for the groups containing the vegetable mixture were carried out according to a loop design with 4 microarrays, as follows: D0j→D1j→D2j→D3j→D0j (the pools for control, 10, 20, and 40% diet denoted, respectively, by D0j, D1j, D2j, and D3j with j = 1, 2, or 3; arrows join the samples put on the same array and indicate the sample labeled with Cy5). The loop was repeated 3 times, and a total of 12 arrays were used. For each of the 3 sets of pools from the individual vegetable groups, a reference hybridization design was constructed, including a flip-flop experiment (19). In this design, each vegetable group was compared with the same pool from the control group. In total, 24 cDNA microarray hybridizations were performed. Cy3- and Cy5-labeled cDNA probes were mixed according to the designs and hybridized to the cDNA microarray by the method of Hasseman et al. (18). Slides were scanned on a GMS 418 Array Scanner (Affymetrix). The images obtained (resolution 10 μm; 16-bit tiff image) were processed with ImaGene 5.0 software (Biodiscovery) to measure mean signal intensities for spots and local background.

Real-time RT-PCR. To verify the cDNA microarray results, 11 gene expression differences, representing 6 genes that were responsive to vegetables, were analyzed by real-time RT-PCR as described previously (16) (Supplemental Table 1: primer sequences).

Statistical analysis. The microarray data were analyze using ANOVA models (20) without background correction and using base-2 logarithmic transformation of the measured intensities. Due to computational limitations, the models were fit in 2 stages. The models included a normalization step, taking into account both the
global (across-genomes) and local (gene-specific) normalization (21). All pairwise differences between the diet groups were examined. This means that for every gene, the difference in intensity between 2 diet groups was calculated, thereby creating an expression difference. An expression difference for a particular gene between diet group A and B is generated by subtracting the log-transformed mean intensity of that particular gene representative for diet group B from the log-transformed mean intensity of that particular gene representative for diet group A. For each gene, the Tukey procedure was used to correct for multiple comparisons (22). To control the overall (across genes) probability of false-positive findings at ≈5%, a P-value < 0.0001 was considered to indicate significance of an individual pairwise comparison (expected P-value after Bonferroni correction).

Statistical analysis of the body weights of the mice was carried out using SPSS version 6.1.1 for Macintosh. Data were analyzed by means of ANOVA (single factor) followed by Student’s t test. A 2-sided P-value < 0.05 was considered to indicate significance.

RESULTS

Body weights. During the week before the start of the intervention (wk 0), all mice consumed the control diet. At the end of this week, body weights did not differ between the groups (Supplemental Table 2). After the intervention, body weights had increased within each group (P < 0.05), but final body weights did not differ among the groups.

Gene expression. By means of microarray technology, the expression levels of 602 genes were measured simultaneously. In Table 2, genes that were differentially expressed due to the dietary treatments are listed.

In the groups consuming a mixture of vegetables, significant differences in gene expression were found for 25 diet group comparisons, representing 18 genes. Remarkably, 23 of these 25 comparisons were between the group consuming the highest vegetable mixture (40%) and one of the other diet groups, indicating that most gene expression effects occurred in the highest vegetable mixture (40%) group. In general, the relation between vegetable dose and the effect on gene expression was not linear. Based on a literature review, only 7 of the 18 differentially expressed genes are likely to play a role in processes affecting lung cancer development (Table 3, Fig. 1A). These processes include cell growth [insulin-like growth factor binding protein 3 (IGFBP3)], apoptosis [IGFBP3, glyceraldehyde-3-phosphate dehydrogenase (GAPDH), transglutaminase 2, C polypeptide (TGM2)], metabolism [topoisomerase (DNA) II α 170 kDa (TOP2A), glutamate-ammonia liggase (glutamine synthase) (GLUL)], and immune response [Fc receptor, IgE, high affinity I, γ polypeptide (FCER1G), cathepsin S (CTSS)].

In the groups consuming individual vegetables, gene expression differed in 22 diet group comparisons, representing 11 genes. Three of these 11 genes differed significantly between groups consuming the various levels of the vegetable mixture, i.e., GLUL, hemoglobin α, adult chain 1 (HBA-A1), and CTSS. Ten of the 22 significant gene expression differences occurred in the group consuming carrots, representing 6 genes [GLUL, CTSS, solute carrier family 6 (neurotransmitter transporter, serotonin), member 4 (SLC6A4), hydroxyprostaglandin dehydrogenase 15 (NAD) (HPGD), sulfotransferase family 1A, phenol-prefering, member 1 (SULT1A1), and selenium binding protein 1 (SELENBP1)]. CTSS was the only gene that was modulated by >1 individual vegetable. In addition to carrots, intake of onions affected the expression of this gene. This was the single gene effect occurring in this group. Consumption of peas caused 7 gene expression differences, accounting for 3 genes, including HBA-A1, glutathione reductase 1 (GSR), and the LDL receptor (LDLR). Intake of cauliflower modulated the expression of BCL2/adenovirus E1B 19kDa-interacting protein 1 (BNIP1) and interferon-α-inducible protein (GIP2), representing 4 gene expression differences. According to the literature review, 8 of these 11 genes might be involved in lung cancer development (Table 3, Fig. 1B).

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**Table 1**

Composition of the 8 diets

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Control diet</th>
<th>10% Vegetable diet</th>
<th>20% Vegetable diet</th>
<th>40% Vegetable diet</th>
<th>Cauliflower diet</th>
<th>Carrots diet</th>
<th>Peas diet</th>
<th>Onions diet</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>g/kg diet</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Cerelose/Dextrose</td>
<td>515</td>
<td>422</td>
<td>329</td>
<td>143</td>
<td>451</td>
<td>452</td>
<td>303</td>
<td>488</td>
</tr>
<tr>
<td>Dicacel/Cellulose</td>
<td>77</td>
<td>70</td>
<td>63</td>
<td>49</td>
<td>71</td>
<td>67</td>
<td>63</td>
<td>73</td>
</tr>
<tr>
<td>Cauliflower</td>
<td>0</td>
<td>17.5</td>
<td>35</td>
<td>70</td>
<td>70</td>
<td>70</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Carrots</td>
<td>0</td>
<td>56.5</td>
<td>113</td>
<td>226</td>
<td></td>
<td>73</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Onions</td>
<td>0</td>
<td>7.7</td>
<td>15.5</td>
<td>31</td>
<td></td>
<td>31</td>
<td></td>
<td></td>
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<tr>
<td>Other constituents</td>
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<td>402</td>
<td>402</td>
<td>402</td>
<td>402</td>
<td>402</td>
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<tr>
<td>Vitamin premix</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>3</td>
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<tr>
<td>Micronutrient premix</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>3</td>
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</tr>
</tbody>
</table>

1 The control diet is the basal 20% casein reference diet.
2 In the vegetable diets, the basal diet was adjusted for cerelose/dextrose and dicacel/ cellulose resulting in similar energy densities for all diets.
3 The digestible energy of each diet was 15.59 kJ/g.
4 Other constituents (equal between diets) in g/kg diet: DL-methionine, 2; NaCl, 3; choline Cl 50%, 4; Soya oil, 50; KCl, 7; KH2PO4, 7; MgO, 2; CaHPO4 · 2H2O, 13; CaCO3, 10; cornstarch, 100; casein, 200; MgSO4 · 7H2O, 4.0.
5 Composition in mg/kg: thiamine hydrochloride, 1; riboflavin, 0.25; nicotinamide, 5; calcium pantothenate (purity 45%), 4.5; pyridoxine hydrochloride, 1.5; cyanocobalamin (purity 0.1%), 12.5; choline chloride (purity 50%), 500; folic acid, 0.25; biotin, 0.5; menadione, 0.013; all-rac-α-tocopheryl acetate (purity 50%), 3.75; retinyl acetate and palmitate (500 IU/mg), 0.5; cholecalciferol (500 IU/mg), 0.06.
6 Composition in mg/kg: FeSO4 · 7H2O, 52.8; (Fe, 10.6), MnO2, 23.9, (Mn, 15.1) ZnSO4 · H2O, 10 (Zn, 3.6); NiSO4 · 6H2O, 3.9 (Ni, 0.9); NaF, 0.6 (F, 0.3); KI, 0.06 (I, 0.005); CuSO4 · 5H2O, 4.8 (Cu, 1.2); Na2SeO3 · 5H2O, 0.09 (Se, 0.03); CrCl3 · 6H2O, 0.45 (Cr, 0.09); SnCl2 · 2H2O, 0.6 (Sn, 0.3); NH4VO3, 0.06 (V, 0.03).
### TABLE 2

Differentially expressed genes in lung mucosa of C57Bl/6 female mice fed different vegetable-containing diets

<table>
<thead>
<tr>
<th>Ac#1</th>
<th>Gene Name by NCBI (Abbreviation)2</th>
<th>Comparison3</th>
<th>cDNA microarray4</th>
<th>RT-PCR5</th>
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<tr>
<td></td>
<td><strong>Vegetable mixture</strong></td>
<td></td>
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<tr>
<td>BG079172</td>
<td>Topoisomerase (DNA) II α 170 kDa (TOP2A)</td>
<td>40-10</td>
<td>1.19 ± 0.21</td>
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<tr>
<td>C78483</td>
<td>Translation elongation factor EF-1 α-1 chain (EFH1)</td>
<td>40-20</td>
<td>0.82 ± 0.16</td>
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</tr>
<tr>
<td>BG085131</td>
<td>Fc receptor, IgE, high affinity I, γ polypeptide (FCER1G)</td>
<td>40-20</td>
<td>0.62 ± 0.06</td>
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</tr>
<tr>
<td>BG063822</td>
<td>Pleckstrin homology domain containing, family C (with FERM domain) member 1 (PLEKHC1)</td>
<td>40-20</td>
<td>-0.39 ± 0.07</td>
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</tr>
<tr>
<td>BG088394</td>
<td>Selenoprotein P, plasma 1 (SEPP1)</td>
<td>40-20</td>
<td>0.28 ± 0.05</td>
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</tr>
<tr>
<td>Al116399</td>
<td>Apolipoprotein A-II (APOA2)</td>
<td>40-20</td>
<td>0.63 ± 0.10</td>
<td></td>
</tr>
<tr>
<td>BG063515</td>
<td>Ferritin heavy chain (FTH)</td>
<td>40-20</td>
<td>0.24 ± 0.05</td>
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</tr>
<tr>
<td>BG076621</td>
<td>Heat shock 70kD protein 5 (glucose-regulated protein) (HSPA5)</td>
<td>40-20</td>
<td>-0.34 ± 0.07</td>
<td>1.10 ± 0.15</td>
</tr>
<tr>
<td>BG072171</td>
<td>Histidine decarboxylase (HDC)</td>
<td>20-10</td>
<td>0.48 ± 0.09</td>
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<tr>
<td>BG088567</td>
<td>Insulin-like growth factor binding protein 3 (IGFBP3)</td>
<td>10-C</td>
<td>0.71 ± 0.11</td>
<td>0.80 ± 0.22</td>
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<td>BG085131</td>
<td>Ferritin heavy chain (FTH)</td>
<td>40-20</td>
<td>0.24 ± 0.05</td>
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<tr>
<td>BG076621</td>
<td>Heat shock 70kD protein 5 (glucose-regulated protein) (HSPA5)</td>
<td>40-20</td>
<td>-0.34 ± 0.07</td>
<td>1.10 ± 0.15</td>
</tr>
<tr>
<td>W29976</td>
<td>Cathepsin S (CTSS)</td>
<td>40-20</td>
<td>-0.40 ± 0.06</td>
<td></td>
</tr>
<tr>
<td></td>
<td><strong>Specific vegetables</strong></td>
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<tr>
<td>W17665</td>
<td>Glutamate-ammonia ligase (glutamine synthase) (GLUL)</td>
<td>40-20</td>
<td>0.28 ± 0.05</td>
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<tr>
<td>AA109900</td>
<td>Hemoglobin α, adult chain 1 (HBA-A1)</td>
<td>40-20</td>
<td>0.45 ± 0.09</td>
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<tr>
<td>W29976</td>
<td>Cathepsin S (CTSS)</td>
<td>40-20</td>
<td>0.28 ± 0.05</td>
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<tr>
<td>BX516671</td>
<td>Solute carrier family 6 (neurotransmitter transporter, serotonin), member 4 (SLC6A4)</td>
<td>40-20</td>
<td>0.28 ± 0.05</td>
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<tr>
<td>AA396890</td>
<td>BCL2/adenovirus E1B 19kDa-interacting protein 1 (BNIP1)</td>
<td>40-20</td>
<td>0.35 ± 0.07</td>
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<tr>
<td>BG066439</td>
<td>Glutathione reductase 1 (GSR)</td>
<td>40-20</td>
<td>0.10 ± 0.01</td>
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<tr>
<td>BG063992</td>
<td>LDR receptor (LDLR)</td>
<td>40-20</td>
<td>0.45 ± 0.09</td>
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<tr>
<td>Al256565</td>
<td>Hydroxyprostaglandin dehydrogenase 15 (NAD) (HPGD)</td>
<td>40-20</td>
<td>0.35 ± 0.02</td>
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<tr>
<td>Al226890</td>
<td>Sulfolipidtransferase family 1A, phenol-prefering, member 1 (SULT1A1)</td>
<td>40-20</td>
<td>0.45 ± 0.09</td>
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<tr>
<td>Al156918</td>
<td>Selenium binding protein 1 (SELENBP1)</td>
<td>40-20</td>
<td>0.47 ± 0.08</td>
<td></td>
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</tbody>
</table>

1 AC#: GenBank accession numbers of the cDNA fragments present on the microarrays.
3 Comparisons: For the groups consuming the vegetable mixture, 6 different comparisons per gene are possible, i.e., C-10%, C-20%, C-40%, 10%–20%, 10%–40% and 20%–40%. For the groups consuming the specific vegetables, 10 different comparisons per gene are possible, i.e., C-T1, C-T2, C-T3, C-T4, T1-T2, T1-T3, T1-T4, T2-T3, T2-T4 and T3-T4. C: control group; 10: 10% vegetable mixture group; 20: 20% vegetable mixture group; 40: 40% vegetable mixture group; T1: cauliflower group; T2: carrot group; T3: pea group; T4: onion group.
4 All pairwise differences between the diet groups were examined. This means that for every gene the difference in intensity between 2 different diet groups was calculated, thereby creating an expression difference. An expression difference for a particular gene between diet group A and B (i.e., pairwise comparison between diet group A and B, stated as A-B) is generated by subtracting the log transformed mean intensity of that particular gene representative for diet group B from the log transformed mean intensity of that particular gene representative for diet group A. Values are estimated expression difference in mean log-transformed intensity ± SE, n = 3. Statistical analyses: ANOVA, P-value after Bonferroni correction: P < 0.0001.
5 Normalized expression difference in mean log-transformed intensity ± SE, n = 3; 11 gene differences were validated. Empty cells indicate genes that were not taken into account.
Two genes involved in immune response were modulated in the groups consuming the highest vegetable dose (40%). GLUL expression was decreased in the group consuming carrots. The modulation by the vegetable mixture of TOP2A and GLUL gene expression might result in increased cell proliferation, abortive G2 cell cycle checkpoints, tumor dedifferentiation, and sensitivity to anticancer drugs. TOP2A was downregulated in the group consuming the highest vegetable dose (40%), but upregulated in the group consuming carrots. The modulation by the vegetable mixture of TOP2A and GLUL gene expression might result in increased cell proliferation; again, these results are not in line with the proposed hypothesis of cancer risk prevention by vegetables.

Two genes that were modulated by the vegetable mixture are involved in metabolism, i.e., TOP2A and GLUL. TOP2A encodes for an essential nuclear enzyme that induces topological changes in DNA (27). Overexpression of TOP2A might contribute to accelerated cell proliferation, abortive G2 cell cycle checkpoints, tumor dedifferentiation, and sensitivity to anticancer drugs. TOP2A was upregulated in the group consuming the highest vegetable dose (40%). GLUL plays an important role in controlling body pH and in removing ammonia from the body. GLUL expression is increased in the lungs in response to trauma or infection. It was hypothesized that reactivation of GLUL expression would have growth suppressive effects on tumor cells; however, this has not yet been resolved (28). In the present study, GLUL expression was downregulated in the group consuming the highest vegetable dose (40%), but upregulated in the group consuming carrots. The modulation by the vegetable mixture of TOP2A and GLUL gene expression might result in increased cell proliferation; again, these results are not in favor of lung cancer risk prevention.

Two genes involved in immune response were modulated in the group consuming the highest vegetable dose (40%), i.e., FCER1G and CTSS. FCER1G is one of the Fc receptors; it plays a role in effective immunity against malignant cells, probably by enhancement of Fc receptor–mediated antibody-dependent cellular cytotoxicity, resulting in tumor cytotoxicity (29). CTSS was characterized as a key enzyme in major histocompatibility complex class II–mediated antigen presentation. Increased expression of CTSS in lung tumors was associated with apoptosis, probably by induction of massive Ca2+-mediated intracellular cross-linking (26). GAPDH was upregulated and TGM2 downregulated in the group consuming the highest dose of vegetable mixture (40%). The results of the modulation by the vegetable mixture on IGFBP3, GAPDH, and TGM2 are expected to be induction of cell growth and reduction of apoptosis, changes that are not in line with the proposed hypothesis of cancer risk prevention by vegetables.
Gene expression differences in lung tissue of female C57Bl/6 mice fed different vegetable diets for 2 wk. Values are estimated differences in mean log-transformed intensity ± SE per vegetable group compared with control (set to 0); n = 3. (A) Expression profile of the genes (TOP2A, FCER1G, IGFBP3, TGM2, GLUL, CTSS, GAPDH) likely to play a role at different stages during lung cancer development modulated by different doses of a vegetable mixture consisting of cauliflower, carrots, peas, and onions compared with control (set to 0); (B) Expression profile of all differentially expressed genes (SULT1A1, SELENBP1, BNIP1, GSR, LDLR, HPGD, GLUL, CTSS) by ≥1 individual vegetables (cauliflower, carrots, peas and/or onions) compared with control (set to 0). *Different from the control, P < 0.0001 (ANOVA, P-value after Bonferroni correction).

FIGURE 1 Gene expression differences in lung tissue of female C57Bl/6 mice fed different vegetable diets for 2 wk. Values are estimated differences in mean log-transformed intensity ± SE per vegetable group compared with control (set to 0); n = 3. (A) Expression profile of the genes (TOP2A, FCER1G, IGFBP3, TGM2, GLUL, CTSS, GAPDH) likely to play a role at different stages during lung cancer development modulated by different doses of a vegetable mixture consisting of cauliflower, carrots, peas, and onions compared with control (set to 0); (B) Expression profile of all differentially expressed genes (SULT1A1, SELENBP1, BNIP1, GSR, LDLR, HPGD, GLUL, CTSS) by ≥1 individual vegetables (cauliflower, carrots, peas and/or onions) compared with control (set to 0). *Different from the control, P < 0.0001 (ANOVA, P-value after Bonferroni correction).

associated with better survival probability for lung cancer patients (30). However, increased CTSS was also associated with autoimmune diseases (31). FCER1G was upregulated, whereas CTSS was downregulated in the group consuming the highest vegetable dose (40%). CTSS was also downregulated in the groups consuming carrots or onions, which could be responsible for the observed effect in the group consuming the highest vegetable dose (40%). The net effect of the modulation of FCER1G and CTSS by the vegetable mixture on immune response is not clear because the effects on the immune system are opposite for these genes.

Summarizing the effects on gene expression changes by the vegetable mixture, 5 of the 7 modulated genes involved in lung cancer preventive mechanisms are expected to result in increased lung cancer risk.

For the individual vegetables, most of the significant gene expression differences occurred in the carrot-fed group. In addition to the genes already discussed GLUL (↑) and CTSS (↓), SLC6A4 (↑), HPGD (↑), SULT1A1 (↑), and SELENBP1 (↑) gene expressions were also modulated. No role for SLC6A4 in lung cancer preventive mechanisms has been described. HPGD encodes for an enzyme that metabolizes a number of prostanoid and nonprostanoid compounds. The products of the nonprostanoid compounds are generally highly reactive α,β-unsaturated aldehydes and ketones, which may cause carcinogenesis (32). The protein product of SULT1A1 plays an important role in chemical defense mechanisms against various xenobiotics but also bioactivates many dietary procarcinogens (33). It was shown that a higher activity of this enzyme in the lung is associated with a decreased lung cancer risk (34). SELENBP1 encodes for a protein that contains selenium. Little information is available about the function of this protein in lung cancer or other cancers. Chen et al. (35) found that reduced selenium-binding protein 1 expression is associated with a poor outcome in lung adenocarcinomas, possibly by increasing cell proliferation and decreasing differentiation. No information was available from our literature review about gene expression modulation in the lung due to carrot consumption. In the present study, the dose of β-carotene was expected to be comparable to physiological doses because it is present in the biological source and not supplemented in high amounts, as in other studies investigating the relation between β-carotene and lung cancer. These physiological levels of β-carotene may explain the relatively beneficial effects of carrots on gene expression changes in the mouse lung in the present study.

Five of the 6 genes affected by carrots could be involved in mechanisms protective against lung cancer; 3 (GLUL, SULT1A1, and SELENBP1) of these 5 are affected in such a way that lung cancer protective mechanisms could be expected to be induced.

In addition to the modulation of CTSS by carrots, intake of onions downregulated the expression of this gene. No other genes were affected by the onions. No information on lung cancer risk prevention by onions or other allium vegetables is available from epidemiologic studies. The present study is the first in which onions were shown to modulate gene expression in vivo.

Three genes were modulated in the group consuming peas, i.e., HBA-A1 (↓), GSR (↑), and LDLR (↑). No lung cancer preventive pathways in which HBA-A1 could play a role are currently known. GSR encodes for an enzyme that reduces oxidized glutathione. Glutathione is involved in the antioxidative defense system against endogenous and exogenous prooxidants (36). This is the first study in which vegetables induced GSR expression in the lungs, thereby probably supplementing the lungs with more reduced oxidized glutathione and possibly resulting in an improved antioxidative defense in the lungs. The protein product of LDLR is a cell surface receptor that plays an important role in cholesterol homeostasis. It was shown that tumor cell lines have higher LDLR activity than the corresponding normal cells. Furthermore, LDL uptake was shown to be higher in lung tumor tissue than in the corresponding normal tissue. The reasons for this are not clear, but it is hypothesized that cholesterol is needed for cell growth (37).

Two of the 3 genes modulated by peas may be involved in lung cancer preventive pathways; only the effect on GSR may lead to lung cancer risk prevention by inducing antioxidative defense.

Cauliflower modulated the expression of the genes BNIP1 (↑) and GIF2 (↓). Only BNIP1 is likely to be involved in lung cancer prevention. It has been suggested that BNIP1 has proapoptotic properties by interacting with BCL2 and BCL2L1 (38,39). However, the precise function of BNIP1 is unclear (38,39). Upregulation of BNIP1 by cauliflower could...
increase apoptosis, which is generally regarded as a protective mechanism against cancer by removing genetically damaged lung cells before they can undergo clonal expansion.

In this study, 13 genes that could be involved in lung cancer risk preventive pathways were modulated. From Table 3, it becomes clear that for the groups consuming the vegetable mixture, only 2 of 7 genes, whereas in groups consuming individual vegetables 5 of 8 genes were modulated in such a way that lung cancer preventing pathways could be stimulated; the other 8 gene modulations may enhance lung cancer risk. These results do not provide a strong indication of favorable vegetable-induced gene expression modulations in processes that could reduce lung cancer risk. However, it is very difficult to assess the contribution of each gene expression modulation in carcinogenesis. It seems that individual vegetables have a higher potential than a mixture of vegetables to modulate genes in processes that may reduce lung cancer risk. Furthermore, carrots modulated the expression of the most genes, and most of these effects favored lung cancer risk prevention. The current study provides more insight into the genetic mechanisms by which vegetables, in particular, carrots, can affect processes involved in lung cancer development.

LITERATURE CITED