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DNA microarray technology in nutraceutical and food safety

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Abstract

The quality and quantity of diet is a key determinant of health and disease. Molecular diagnostics may play a key role in food safety related to genetically modified foods, food-borne pathogens and novel nutraceuticals. Functional outcomes in biology are determined, for the most part, by net balance between sets of genes related to the specific outcome in question. The DNA microarray technology offers a new dimension of strength in molecular diagnostics by permitting the simultaneous analysis of large sets of genes. Automation of assay and novel bioinformatics tools make DNA microarrays a robust technology for diagnostics. Since its development a few years ago, this technology has been used for the applications of toxicogenomics, pharmacogenomics, cell biology, and clinical investigations addressing the prevention and intervention of diseases. Optimization of this technology to specifically address food safety is a vast resource that remains to be mined. Efforts to develop diagnostic custom arrays and simplified bioinformatics tools for field use are warranted.
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Current developments in genomics and new developments in genetic technologies, such as DNA microarrays, have encouraged the transition of nutrition research from epidemiology and physiology to molecular biology and genetics (IHGSC, 2001; Venter et al., 2001; Waterston et al., 2002). The product is a new sub-discipline referred to as nutrigenomics by some. Nutrigenomics includes the study of the genome-wide influences of nutrients at the transcription level. In the past, the effects and mechanisms of dietary components on health and disease have been investigated using functional assays or studies based on single genes or single physiological outcomes of measure. However, it has been increasingly obvious that to understand the effects of nutrition on health and disease, molecular studies are indispensable (Muller and Kersten, 2003). The balance of many metabolic processes involving hundreds or thousands of genes, proteins and metabolites regulates almost all of the biological functions. For example, lipid homeostasis is achieved by the intricate coordination of several organs, involving hundreds of genes, many signal transduction pathways, and a large number of biomolecules, including transcription factors, receptors, hormones, apolipoproteins, and enzymes. Assays based on single genes or single physiological end points are not optimal to provide us sufficient and thorough information to understand the mechanisms that underlie the beneficial or adverse effects of dietary nutrients or components. The power of novel

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genomics tools such as DNA microarrays are getting increasing attention because of their ability to interrogate a large fraction of the genome in a single assay. Like most tools of discovery, DNA microarray assays offer unlimited strength to those who know what to do with it. For example, attempts to use the DNA microarrays to accurately predict the proteome may not be highly successful because all mRNAs may not translate to proteins (Gygi et al., 1999). Current light showing that the significance of mRNA biology extends well beyond the ability of an mRNA to encode a specific protein, adds further versatility to DNA microarrays as an investigative tool. The study of translation and post-translational modification of proteins are addressed by another set of tools related to proteomics. Off-shoots of proteomics include for example metabolomics, metabolic profiling of a cell or tissue. Each of these approaches are fortified with their own specific merits and limitations and should be used depending on specific requirements of the investigator. For example, compared to proteomics where identification of an unknown candidate protein could well be a very extensive process, the identification of "candidate genes" in a microarray study is an instant process. Only by combining information from nutrigenomics, nutrition-related proteome (proteomics), nutrition-relevant metabolite production (metabolomics) and appropriate bioinformatics, can one gain a comprehensive understanding of nutrition-modulated homeostasis and toxicology. A challenging goal of nutrigenomics is using nutritional systems biology to identify a system of biomarkers that can predict the beneficial or adverse effects of dietary nutrients or components. These biomarkers can be used as predictive or screening tools to promote health and prevent disease.

Dietary nutrients or components may be viewed to act like signals, which are received by the nutrient sensors in the biological system. Transcription factors respond to nutrients and may therefore be viewed as nutrient sensors. After receiving nutrient signals, transcription factors modulate DNA transcription of specific genes and therefore alter the pattern of gene expression in response to the nutrients. Patterns of gene expression, protein expression, and metabolite production as influenced by certain dietary nutrients or components are considered as "dietary signatures" (Muller and Kersten, 2003). The latter two levels, proteome and metabolome, are beyond the scope of this article. This review will focus on a brief comparison of various tools for gene expression analysis with the spotlight on DNA microarrays. The DNA microarray technology lends itself to modifications that would suit it for field applications. Combined with the fact that the assay process is highly automated and swift, DNA microarrays seems to a have a significant future in diagnostics. The application of DNA microarray to nutrigenomics, toxicology, and food safety, including genetically engineered foods and the detection of food-borne pathogens, is discussed.

1. Analysis of gene expression: an introductory overview

Northern blotting (Alwine et al., 1977) has been and continues to be the gold standard of quantitative single gene expression analysis for decades. Quantitative real-time PCR is steadily replacing Northern blotting (Buttitta et al., 2003; Depreter et al., 2002; Oberst et al., 1998). By combining a gene-specific fluorescent-labeled probe and PCR, this method can detect 10 or fewer copies of targets, or as little as 0.1 pg of total RNA. The quantitative results of gene expression can be obtained within 2–3 h. However, the number of genes to be analyzed each time is limited. Recent developments such as the differential display (Liang and Pardee, 1992) utilizes a set of oligonucleotide primers, one being anchored to the poly-A tail of a subset of mRNAs and the other being short and arbitrary in sequence so that it anneals at different positions relative to the first primer. The mRNA subpopulations defined by these primers are amplified after reverse transcription PCR and resolved on a DNA sequencing gel (Gohil et al., 1999). When multiple primer sets are used, reproducible patterns of amplified complimentary DNA fragments are obtained that show strong dependence on sequence specificity of either primer. Thus, this technique does not look for predetermined genes and has the potential to identify novel genes in the context of a given aspect of biology. The compromise is that differential display is not ideal for quantitative assays. Serial analysis of gene expression (SAGE) (Velculescu et al., 1995) represents a more powerful approach for the comprehensive analysis of gene patterns. In essence, SAGE captures

the RNAs and "rewrites" them into DNA. It excises a small 14-letter tag from each. Since it would take a long time to load tens of thousands of single tags into a sequencing machine, the method glues a lot of tags together into long molecules called concatemers. The sequencer reads these molecules, counts and analyzes them, and provides a list of genes that the concatemers belong to. The commercial application of this technology is licensed to Genzyme Molecular Oncology (http://www.genzymemolecularoncology.com/) which has a proprietary database of over seven million SAGE tags representing over 125,000 unique transcripts. Because of relatively complicated sample preparation and extensive DNA sequencing, SAGE is not yet popular. It is suitable for a quantitative measurement, but labor and skill intensive.

DNA microarrays have grown to be promising tools for the genome-wide analysis of transcripts. During the past few years, quite a few types of DNA microarrays have been developed including macroarray, cDNA microarray, high-density oligonucleotide microarrays, and microelectronic arrays (Roy et al., 2002). Nylon membranes robotically spotted with cDNA inserts or genomic fragments mostly represent macroarrays. The probe density is lower, with spacing between spots typically being 1–2 mm. The detection is usually based on radioactive or chemiluminescent labeling. This type of arrays is suitable for the simultaneous analysis of tens to hundreds of genes. cDNA microarrays utilize glass or plastic slides as support material. Up to 50,000 DNA spots that are 50–150 μm in diameter are generally spotted on an area of 3.6 cm^2 with spacing less than 300 μm. The DNA spots can be PCR fragments (200–2400 bp) or oligonucleotides (20–35 bp). This type of microarray, also recognized as spotted DNA microarray, was first developed at Stanford University (Schena et al., 1995; Shalon et al., 1996). Usually two RNA samples are labeled by two different cyanine fluorescent dyes (green Cy3 and red Cy5) during the cDNA synthesis and both samples are hybridized competitively on the spotted array. Using a dual color scanner, fluorescence of both dyes (samples) are quantified independently as parameters to estimate gene expression. The ratio of intensities of both dyes (e.g. Cy3/Cy5) reflects relative expression levels for both samples. High-density oligonucleotide arrays typically contain in situ synthesized 25-mer oligonucleotide probes utilizing the photolithography and solid-phase DNA synthesis techniques (Chee et al., 1996; Fodor et al., 1991). GeneChip™, manufactured by Affymetrix (http://www.affymetrix.com), represents the most common device in the market. The process of such microarray fabrication allows simultaneous probing of tens of thousands of genes and ESTs. Therefore, this type of microarray has the power of genome-wide gene expression analysis (Roy et al., 2002). Multiple GeneChip™ arrays are synthesized simultaneously on a large glass wafer. This parallel process enhances reproducibility of arrays. The most recently developed two-array set by Affymetrix for human genome (U133) contains greater than 1,000,000 of 18 μm oligonucleotide features, which represent over 33,000 well-characterized human genes. Finally, microelectronic arrays have been recently introduced to the market and are yet to be thoroughly tested by end-users. The NanoChip™ array (http://www.nanogen.com) is a 99-site electronically-powered microarray. Each test site is electronically connected to a computer with platinum wires. The sequence-specific oligonucleotide probes are electronically addressed to specific sites. Since DNA and RNA naturally contain negative and positive charges, the biotin-labeled RNA samples can be transported and hybridized to the complementary probes on the NanoChip™ array rapidly and precisely by electronically manipulating the charge at test sites. Instead of hours of probe hybridization, as is required in the former three types of DNA arrays, hybridization of NanoChip™ array could occur in minutes.

2. Comparison of DNA microarrays

DNA microarray analyses are sensitive and to a limited extent quantitative. Spotted cDNA microarray and high-density oligonucleotide microarray are the most commonly used DNA microarrays for transcriptome analysis (Fig. 1). Near-full automation minimizes manual errors and enhances the reproducibility of the assay (Call et al., 2001; Chee et al., 1996; Fodor et al., 1991; Schena et al., 1995; Shalon et al., 1996). For those working with organisms for which the genome is not fully characterized, DNA microarray may not be a very efficient tool. Lack of information related to gene sequences will compromise

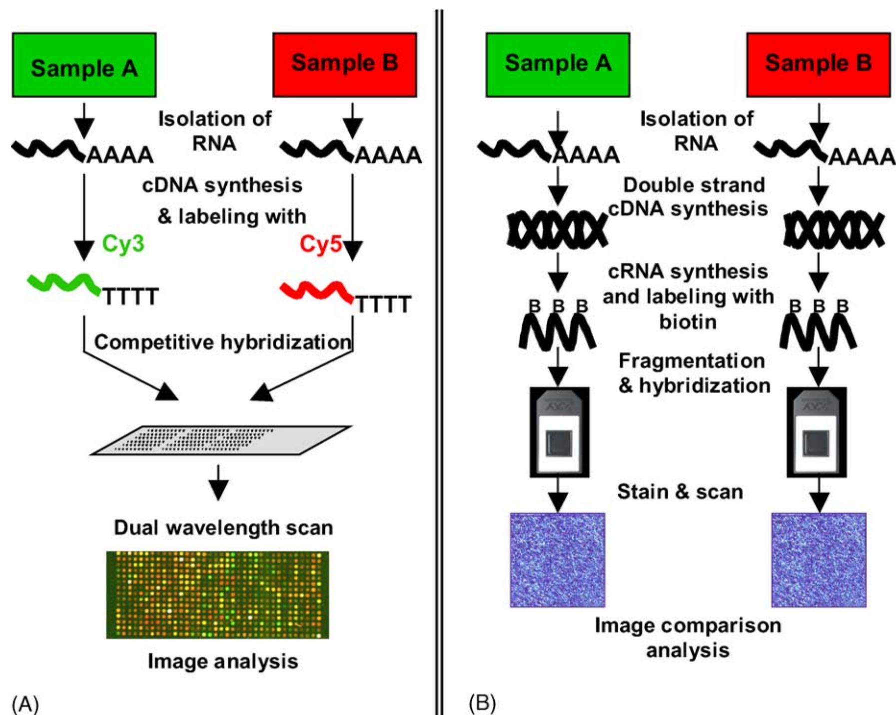

Fig. 1. Outline of approach for spotted microarray (A) vs. high-density oligonucleotide microarray (B). Sample A: control; sample B: test.

the fabrication of arrays having the power to execute genome-wide analysis of gene expression. The Integrated Molecular Analysis of Genome and their Expression (IMAGE) consortium initiated in 1993 support the study of human genes. This consortium represents one of the world's largest public collections of genes. The consortium shares high quality arrayed cDNA libraries and places sequences, maps and expression data on the clones in these arrays in the public domain. Besides human and mouse genomes, the collection now contains clones from rat, zebrafish, xenopus and rhesus macaques. All of these clones are available free of any royalties. Distributors such as the ATCC, Research Genetics and Genome Systems supply cDNA clones to individual laboratories.

The technique of in situ synthesis of oligonucleotides for high-density oligonucleotide microarrays using the photolithography technology is still limited to commercial production. It is not cost-effective to have custom high-density arrays made to serve the needs of the average laboratory. Spotted DNA microarrays, however, have been widely developed in academic, governmental and commercial laboratories. On the down side for spotted arrays, the initial setup time for this technology is usually much longer than that for using GeneChip™ high-density oligonucleotide microarrays. A standardized operation system, including hardware, software, a defined protocol, chemicals and analysis tools, has been set up for GeneChip™ arrays. However, to set up the operation for spotted DNA microarrays, one has to design and produce probes (cDNAs) or purchase oligonucleotides from vendors, assure the accuracy of probes, select a robotic device for arraying, optimize a suitable protocol from several available protocols (http://cmgm.stanford.edu/pbrown/protocols/4_human_RNA.html; http://www.microarrays.org/pdfs/HumanRNALabel.pdf; http://sequence.aecom.yu.edu/bioinf/microarray/protocol4.html), and choose a proper image analysis software. For an average laboratory, this is a mammoth undertaking. Spotted DNA microarray is suitable to study a select set of genes, but it may not be as suitable for a large scale genome-wide expression profiling, since it requires tracking, handling,

and maintaining quality control of a large number of clones (Finkelstein et al., 2002). Fabrication of custom or specialty DNA microarrays requires extensive skill. Prudent selection of genes for specialty arrays is required to avoid redundancy, alternative splicing and cross-hybridization, which may mask changes in the transcriptional level. The select battery of genes should be broader than the specific genes of interest depending on the broader aspects of biology in question. Software-assisted pathway reconstruction approaches should be particularly helpful in such efforts. Finally, it is essential to incorporate negative control DNA that is known to be absent from the test samples and unlikely to cross-hybridize with any target genes. These considerations must be accompanied with appropriate bioinformatics tools that have the power to address the specific necessities while accounting for the appropriate controls.

Because DNA microarrays represent a new technology, bioinformatics support is still in its infancy. The connection between the volume of data generated from DNA microarrays and the statistical tools available for researchers to make biological sense out of the data have ample scope to strengthen. Fortunately, biologists and statisticians have joined efforts to bridge the gap. Areas like data normalization techniques, methods for analyzing differential gene expression, statistical distribution of expression data and data clustering have been under development (Pan, 2002; Yang et al., 2002; Xu et al., 2002).

In an effort to establish standards for microarray experiment annotation and data interpretation, the Microarray Gene Expression Data Group (MGED) was established in November 1999 (http://www.mged.org). A set of guidelines (Minimum Information About a Microarray Experiment; MIAME) has been suggested to outline the minimum information required for microarray experiment design and data representation that allows the uniformity for reproduction and verification by other researchers. The MIAME may assist the development of microarray databases and data analysis tools. There are several public gene expression databases available: Gene Expression Omnibus (GEO) by National Center for Biotechnology information at http://www.ncbi.nlm.nih.gov/geo/, RNA Abundance Database (RAD) by University of Pennsylvania at http://www.cbil.upenn.edu/RAD2/about.html, GeneX by National Center for Genome Resources at http://www.ncgr.org/genex/, and ArrayExpress by European Bioinformatics Institute at http://www.ebi.ac.uk/microarray/ArrayExpress/.

3. Genome stability and gene expression regulation

Exposure to exogenous mutagens, carcinogens, and endogenous free radicals have known to result in genome instability and lead to various degenerative diseases such as cancer, cardiovascular disease, and Alzheimer's disease. A high rate of chromosomal aberrations and DNA damage is associated with the initiation of carcinogenic processes and, therefore, increases cancer risk (Bonassi et al., 2000). Dietary components, including macronutrients and micronutrients, play a key role in determining the genomic stability and to prevent genotoxicity caused by exogenous and/or endogenous toxic agents (Fenech, 2001; Tsuda et al., 2000; Chatterjee, 2001). Several micronutrients act as substrates or cofactors in the metabolic pathways that regulate DNA synthesis/repair and the expression of genes (Hageman and Stierum, 2001; Chatterjee, 2001; Friso and Choi, 2002). Deficiency of such nutrients may result in the disruption of genomic integrity and DNA alteration, such as DNA methylation leading to changes in gene expression.

Vitamins C and E are potent antioxidants. Deficiencies of both vitamins are known to cause DNA oxidation and chromosome damage (Halliwell, 2001; Claycombe and Meydani, 2001; Fraga et al., 1991). Vitamin C, for example, inhibits the oxidative modification of guanosine base (Fraga et al., 1991). Vitamin E is a potent lipid peroxyl radical scavenger. It inhibits the DNA single strand breaks caused by reactive oxygen species (ROS) (Tsuda et al., 2000). The protective effects of Vitamin E is linked to its inhibition on the formation of hydroxyl radical ($HO^{\bullet}$) generated from H_2O_2 in the presence of metal ions, such as Fe^{2+}, and the direct effect of H_2O_2 on inducing DNA damage (Machlin, 1991). Vitamin D also exhibits antioxidant property and helps to stabilize chromosome structure and prevent the breakage of DNA double strands by inhibiting iron-dependent lipid peroxidation in liposomes (Chatterjee, 2001; Wiseman, 1993). Vitamin D supplements decrease the incidence of chromosomal aberrations induced by diethylnitrosamine in the rat

liver cells (Chatterjee, 2001). Another nutrient, niacin, plays an important role in DNA repair and cell death as well. Niacin is the precursor of NAD+, which is the substrate of poly(ADP-ribose)-polymerase-1 (PARP). In the presence of DNA strand breaks, PARP signals and initiates cell's response to DNA damage (Hageman and Stierum, 2001). Therefore, niacin status may be critical in maintaining genome integrity and preventing cell death. Folate contributes methyl groups for the conversion of dUMP to dTMP during the de novo deoxynucoleotide triphosphate synthesis and the transformation of methionine from homocysteine in the presence of Vitamin B12. Deficiency of either or both folate and Vitamin B12 results in chromosomal damage and micronucleus formation in humans (Fenech, 2001).

DNA methylation involves the addition of a methyl group at the carbon 5′ position of cytosine within the cytosine-quanine $(CpG)_4$ dinucleotide (Razin and Riggs, 1980). DNA methylation typically occurs in the CpG-dinucleotide-rich regions known as "CpG islands", which are highly abundant in the promoter regions or the initial exon of genes (Robertson and Wolffe, 2000). CpG islands usually are not methylated. When CpG islands of certain genes are methylated, the expression of those genes is inhibited. Therefore, the patterns of DNA methylation can affect the genome-wide gene expression profile. Folate has a direct role in DNA methylation by acting as a methyl donor via its main circulating form, 5-methyltetrahydrofolate (Friso and Choi, 2002). Deficiency of folate decreases genomic DNA methylation. By observing the alterations of DNA methylation during hepatocarcinogenesis with chronic dietary folate deficiency, Pogribny et al. (1995) noted that the level of p53 gene expression was increased and associated with hypomethylation of the coding region in pre-neoplastic tissues. However, after the tumor formation, the coding region of p53 gene was re-methylated and its mRNA level was decreased. Folate deficiency may induce liver cancer by affecting the methylation status of the p53 gene coding region and consequently change the expression of p53 gene. Zinc deficiency reduces the utilization of methyl groups from S-adenosylmethionine (SAM) and leads to genomic DNA hypomethylation in rat liver (Wallwork and Duerre, 1985), whereas Vitamin C deficiency is noted to be associated with DNA hypermethylation of lung cancer cells (Piyathilake et al., 2000). Niacin helps to maintain the unmethylated state of CpG dinucleotides by inhibiting DNA methylation (Zardo and Caiafa, 1998). The active form of Vitamin D, 1α, 25-dihydroxyvitamin D3 [$1,25(OH)_2D_3$], also regulate a variety of genes that are involved in intestinal calcium transport, DNA replication and cellular proliferation-differentiation in the skin and immune systems (Minghetti and Norman, 1988). For example, Vitamin D suppresses T-cell proliferation and the expression of cytokines such as interleukin-2 and interferon-γ (Bhalla et al., 1986). This vitamin also exhibits the anti-tumor effects by down-regulating the expression of proto-oncogene c-myc and up-regulating the expression of *c-fos* and *c-jun*, both are differentiation associated oncogenes (Brelvi and Studzinski, 1986). Besides Vitamin D, Vitamin E also plays a protective role against DNA damage caused by H_2O_2 and UV radiation via the up-regulation of *c-fos* and *c-jun* expression as well as the increased binding of activating transcription factor to a common DNA site (AP-1) to regulate transcriptional activities (Claycombe and Meydani, 2001). Vitamin D induces apoptosis in breast, colon, and glioma cell lines via bcl-2 and bax regulated pathways (Mathiasen et al., 1999) without the involvement of caspase, death receptors and p53 (Ashkenazi and Dixit, 1998). $1,25(OH)_2D_3$ increases Bax protein, which promotes cell death, and decreases the expression of bcl-2 gene (Mathiasen et al., 1999). In this way, Vitamin D may promote tumor regression by inducing tumor cell apoptosis.

Many of the aforementioned results were acquired using conventional gene expression analyses, such as Northern blot, reverse transcription PCR, and quantitative real-time PCR. Up until recent years, most studies were pursued based on the hypothesis-driven approach. The scope of genes to be analyzed is limited and may be biased, since the approach has to be based on certain known information such as metabolic functions or signaling pathways that may be influenced by dietary nutrients or components. Use of the DNA microarray technology allows unbiased approach to study genome-wide gene expression patterns in response to dietary intervention. This represents a powerful hypothesis-generating approach. Most nutritional scientists and biologists are trained to perform studies in a hypothesis-driven fashion. Although there is

some initial resistance to depart from the conventional approach, with time more scientists are starting to appreciate the merits of embracing a change in mind-set.

4. Individual genetic variations

Individuals carrying single nucleotide polymorphism (SNP) of specific nutrient-sensitive genes are likely to have different requirements for certain nutrients. Such individuals may have genetic predispositions that cause them to be more (or less) sensitive to dietary nutrients and be more prone or resistant to the development of a certain disease. For example, methylenetetrahydrofolate reductase (MTHFR) catalyzes the conversion of 5, 10-methylenetetrahydrofolate to methyl-THF. One variant of MTHFR containing a mis-sense mutation, a cytosine-to-thymine transition at base pair 677 (C677T), reduces the activity of the enzyme and consequently decreases the availability of methyl-THF. Only individuals who carry homozygous mutation of MTHFR (TT genotype) and have inadequate folate status are noted to have higher levels of homocysteine (Jacques et al., 1996), which is an independent risk factor for developing cardiovascular disease (Ueland et al., 2000). Individuals with TT genotype may require more folate than those with CC genotype (Bailey and Gregory, 1999). People who carry TT genotype of MTHFR also have a lower risk of developing colorectal cancer. However, this protective effect is observed only in those who have adequate folate status (Ma et al., 1997). Among the persons with low folate status, a higher risk of colorectal cancer is reported (Ulrich et al., 2000).

While nutrigenomics focuses on the influence of nutrients on the genome, the studies of interactions between genetic variations and nutrients and their impact on health and disease are in the field of nutrigenetics. There are commercially available high-density oligonucleotide microarrays that are specifically designed to identify tens of thousands of SNP simultaneously. The use of SNP arrays may help to determine the genetic predisposition of individuals and link the SNP to the altered risk of disease. Eventually, the findings may lead to the adjustment of dietary recommendation based on genotype variations.

5. Toxicology and dietary components

Life and health are heavily influenced by the quality and quantity of the diet. Diet plays an important role on how an individual deals with environmental stressors and toxins to prevent or lessen the impact of diseases. Many components in the diet, either endogenous or exogenous, can modulate the net impact of specific toxicants by acting as inducers, activators, suppressors, inhibitors, or substrates of certain toxifying or detoxifying enzymes.

Cytochrome P450 (P450) enzymes are a group of hemoproteins that are involved in the metabolism of drugs, xenobiotics, carcinogens, steroids, pesticides, hydrocarbons and natural products. Since its discovery in the 1950s, this family of enzymes has been studied and reviewed extensively (Coon et al., 1992; Poulos and Raag, 1992). Dietary constituents influence cytochrome P450 enzymes by altering the expression levels of P450 genes, rates of certain mRNA degradation, translation process, and/or degradation of P450 proteins (Yang et al., 1992). For example, a diet with low protein content or poor quality of protein decreases the metabolism of xenobiotics in humans (Anderson et al., 1985) and animals (Birt et al., 1983), probably due to the decreased protein synthesis of P450 caused by the protein deficiency. Levels of dietary fat, especially polyunsaturated fatty acids, also influence the gene expression of P450 enzymes. Many non-nutrient dietary compounds modulate P450 enzymes as well (Surh and Ferguson, 2003; Yang et al., 1992). Resveratrol, a compound that is present in red wine, potently inhibits P450 1B1 (Guengerich et al., 2003). Because P450 1B1 catalyzes the 4-hydroxylation of estrogens to yield metabolites that eventually produce reactive species, the inhibition of this P450 enzyme by resveratrol may help to prevent breast cancer in humans (Damianaki et al., 2000; Hayes et al., 1996; Li and Li, 1987; Liehr et al., 1986). Dietary compounds such as indole-3-carbinol, indole-3-acetonitrile, and indole-3-carboxyaldehyde found in cruciferous vegetables, like brussel sprouts, cabbage, turnips, cauliflowers and broccoli, induce intestinal and hepatic benzo(a)pyrene (BP) hydroxylase due to increased gene expression of P450s 1A1 and 1A2 (Vang et al., 1990). It has been suggested that those compounds, especially indole-3-carbinol, may protect against colon cancer

in a multiple intestinal neoplasia mouse model (Kim et al., 2003). Other detoxifying enzymes such as glutathione S-transferase, UDP-glucuronosyltransferase and N-acetyltransferase and sulfotransferase function with overlapping specificity and synchrony to prevent the accumulation of toxicants (e.g. pesticides) ingested with food. It is the balance of these and related enzymes in combination with other modulators that confer net protection against dietary toxins (Guengerich, 2000; Kassie et al., 2003).

6. Safety of dietary supplements

There is an increasing trend of dietary supplementation with certain levels of micronutrients, taken in either single or multi-nutrients format. The efficacy, safety and proper dosage of such supplements remain to be addressed for many cases. Several dietary nutrients and components have been used as chemopreventive or anti-carcinogenic agents for lowering the incidence of cancer as well as of other degenerative disease and aging. The use of antioxidant nutrients such as Vitamins A, C, E, and β-carotene to prevent cancer has been well documented (Chang et al., 1979; Olson, 1993; Woutersen et al., 1999). Nonetheless, some promising chemopreventive agents may exert undesired toxicity especially when taken in higher doses. For instance, β-carotene prevents inducible skin carcinogenesis in animals (Santamaria et al., 1988) and β-carotene intake from fruits and vegetables is linked with reduced cancer risk based epidemiological evidence (Olson, 1993). However, a β-carotene supplement taken by cigarette smokers resulted in a higher incidence of lung cancer (Albanes et al., 1996; Mayne et al., 1996; Omenn et al., 1996). Epidemiological studies have shown that Vitamin C has a protective effect against cancer via its antioxidant function (Chan and Reade, 1998). However, the protective effect of Vitamin C supplements against oxidative damage to DNA in vivo is weak for those who are not deficient with Vitamin C. In addition, some claim that this vitamin possesses pro-oxidant properties in vivo (Podmore et al., 1998). Healthy volunteers receiving 500 mg of a Vitamin C supplement for 6 weeks had increased 8-oxoadenine and decreased 8-oxoguanine. Both are biomarkers of oxidative DNA damage (Podmore et al., 1998). Claycombe and Meydani (2001) caution the potential genotoxic effect on DNA damage when both Vitamin C and Vitamin E are supplemented together based on the observation that combined treatment with the two vitamin supplements increased radiation-induced DNA damage in human lymphoblastoid cells (Sweetman et al., 1997) These examples warrant a careful examination of all dietary supplements, especially novel nutraceuticals, for potential risks.

The use of DNA microarray technology offers the opportunity for the simultaneous study of toxin-responsive genes including those responsible for xenobiotic metabolism, DNA repair, cell growth and stress-response. While tissues such as the liver may not be easily accessible in humans, circulating immune cells and muscle biopsies offer reasonable opportunities. The DNA microarray technology should be productive in generating a combination of genes as biomarkers for known dietary toxins or to help establish the proper doses of dietary supplements. Once such distinct sets of genes are identified, efforts to develop custom diagnostic arrays should prove to be useful.

7. Genetically modified food

The safety of foods derived from genetically modified organisms (GMO) has been a great concern since the first introduction of genetically modified tobacco in 1998. The International Food Biotechnology Council (IFBC) published the first report on this issue over a decade ago (IFBC, 1990). Since then, several organizations, including the Food and Agriculture Organization of the United Nations (FAO), the World Health Organization (WHO), the Organisation for Economic Cooperation and Development (OECD) and the International Life Sciences Institute (ILSI), have provided specific guidelines for the safety assessment of genetically modified foods. The concept of substantial equivalence has been used as the comparative approach to assess the safety of genetically modified foods. The substantial equivalence is based on the notion that traditional foods, which are considered safe due to a long history of use, can serve as a reference to compare their genetically modified counterpart. This concept is not a tool for safety assessments. It helps to identify similarities and differences between the

traditional foods and the genetically modified products, but it does not characterize the potential beneficial or adversary effects of the genetically modified foods. The concept of substantial equivalence is a starting point of the assessment, the outcome of this comparative analysis may provide a guide to allow further safety evaluation using toxicological and biochemical analyses. Certain factors may limit the application of the concept of substantial equivalence. For example, the information on natural variations of certain crop constituents may be limited. Current methods may not be sensitive enough to evaluate the differences of genetically modified foods and traditional foods if the parental lines are near isogenic. And the availability of analytical methods to detect unintended or intended effects of genetically modified foods is still relatively limited (Kuiper et al., 2002).

Several safety issues of genetically modified foods should be considered. The process of genetic modification, the safety of new proteins synthesized, the potential occurrence of unintended effects, the possible gene transfer to human intestinal flora, the potential allergenic and/or toxic effects of new protein, and the role of new foods in the diet and the influence on food processing (Kuiper et al., 2001). The same gene located in different types of cells/tissues may produce the same protein manifesting a contrasting function. Therefore, introduction of a gene to two different types of organisms may produce different functional outcomes presenting the risk of adverse effects. Due to gene–gene interactions, the insertion of a new gene may also change the gene expression profile, enzymatic and metabolic pathways, and consequently impact the phenotype of the recipient cells. For instance, the over-expression of a polygalacturonase gene that was involved in the pectin synthesis had no apparent effect on tobacco (Osteryoung et al., 1990). However, the same gene changed the phenotype, including silvery colored leaves and premature leaf shedding, in apple trees (Atkinson et al., 2002). Introduction of a new gene may trigger production of new molecules that are potentially toxic, allergenic, or carcinogenic unless proven otherwise. For example, L-tryptophan produced by transgenic Showa Denko KK bacteria caused the 1989 epidemic of eosinophilia–myalgia syndrome (EMS) in the United States due to the trace contaminants produced by the transgenic bacteria (Kilbourne et al., 1996). The unintended effects of certain genetically modified foods have been well documented (Murrey et al., 1999; Shewmaker et al., 1999; Ye et al., 2000). For example, rice that was genetically modified to promote provitamin A biosynthetic pathway produced unexpected carotenoid derivatives such as β-carotene, lutein, and zeaxanthin (Ye et al., 2000). Wheat that was genetically modified to increase the expression of glucose oxidase exhibited phytotoxicity (Murrey et al., 1999).

The concerns outlined above warrant non-targeted studies using profiling methods to compare genetically modified foods and their traditional counterparts. The introduced gene(s) may disrupt, modify, or silence some active genes or activate certain silent genes due to potential gene–gene interaction or gene regulation and result in changes of downstream proteins and metabolite production. It would thus be prudent to study the gene expression profiles, proteomic profiles, and metabolic profiles of genetically modified foods as well as of tissues from experimental humans and animals who have ingested such food with the corresponding traditional food as a reference for a complete safety assessment. Preliminary study using spotted DNA microarray to evaluate the gene expression pattern of genetically modified tomato has shown reproducible alterations of gene expression patterns outside the ranges of natural variation, due to different stages of ripening (Kuiper et al., 2001). Fortunately, so far no evidence has been found that genes from genetically modified plants are transferred to human or animals that consume the foods (Kuiper et al., 2001; Einspanier et al., 2001; Chambers et al., 2002). A global approach to gene expression analysis will provide critical additional information especially because it is almost impossible to predict the genetic response in such a complex experiment. Under these conditions, not only changes in gene expression but no-change responses would be equally meaningful because one would then be assured of the specific sets of genes that are not sensitive to the specific genetic modification of the food in question.

8. Food-borne pathogens

External contamination of food supplies is another major concern in food safety. Food contamination can occur during food harvesting, processing, storage

and preparation. Although infections of food-borne pathogens have been controlled, the prevalence of food-borne diseases is substantial. In the United Stated, it has been estimated that there are 76 million cases of food-borne illness, 325,000 hospitalizations, and 5000 deaths every year (Mead et al., 1999). Thus, one out of four people in the United States is affected with food-borne illness each year, and more than one in a thousand is hospitalized as a result. The economic burden on the society has been estimated to be US$ 9–32 billion. The spectrum of food-borne pathogens is very broad. It includes, but is not limited to, enteric bacteria, aerobes and anaerobes, viral pathogens, parasites, and bacteria that produce toxins in fish and shellfish. For example, *Escherichia coli* O157:H7, one of the most significant food-borne pathogens, is a leading cause of hemorrhagic colitis and has caused serious public threat world-wide (Allerberger et al., 1996; Altekruse et al., 1997; Bell et al., 1994). Despite the identification of many food-borne pathogens, the pathogens that are responsible for 82% of food-borne illnesses remain elusive (Maslanka et al., 2001; Robert, 2002). Of 1500 deaths caused by known pathogens each year, 75 percent are caused by *Salmonella*, *Listeria monocytogenes*, and *Toxoplasma* (Maslanka et al., 2001). Meanwhile, many new strains of food-borne pathogens continue to be discovered. For instance, the new strains of *Salmonella Enteritidis* infected eggs and broilers were found in most countries of the world during the 1980s (Rodriguez et al., 1990). In the 1990s, the highly resistant strains of *Salmonella Typhimurium* infected cattle and other animals in Europe and North America (Tauxe, 1999).

Traditionally, food-borne pathogens are identified by microbiological culture, followed by immunological methods to detect antigens (March and Ratnam, 1986; Sanderson et al., 1995). Alternative methods have been also employed for the detection of food-borne pathogens (Johnson et al., 1995; Oberst et al., 1998). One major challenge to analyze food-borne pathogens in food and/or environmental samples is the need to detect very small numbers of pathogens in a large volume of samples that may contain a large proportion of interfering substances. Traditional methods require a few days for pre-enrichment, enrichment and post-enrichment to recover pathogens before the conventional detection assays can be conducted. Most of the detection assays are very pathogen-specific and can only be used to detect one pathogen at one time. Assumptions have to be made about the possible pathogens before tests can be performed. Therefore, the variability in pathogen-specific protocols may delay the detection of the agent in the event of an unexpected outbreak. DNA microarray technology offers the opportunity to establish molecular signatures specific to certain forms of contamination. Because formulation of such signatures would be based on hundreds or thousands of genes, a partial homology to the signature would reflect the nature of the pathogen. Custom microarrays formulated based on such signature responses could serve as a rapid and sensitive tool to be used in a field setting. Simplified fluorescence scanners that are designed to provide a yes/no response as opposed to sophisticated laboratory scanners designed to collect detailed information could support such field analysis. While the study of tissue from the infected is of key significance, DNA microarray could also be used to study the pathogen itself. Studies have been done using DNA microarrays spotted with genetics markers in bacteria genomes that are associated with various pathogens and virulence factors (Call et al., 2001; Chizhikov et al., 2001). In one such study, six genes (*slt*-I, *slt*-II, *eae*A, *rfb*E, *fli*C, and *ipa*H) were chosen to identify 15 strains of *Salmonella*, *Shigella*, and *E. coli* (Chizhikov et al., 2001). Both *slt*-I and *slt*-II are toxin structural genes (Nagano et al., 1998). The *eae*A gene encodes the outer membrane protein intimin (Yu and Kaper, 1992), whose product mediates the adherence of the organism to the host cells. The *rfb*E is a more specific target gene that encodes an enzyme involved in biosynthesis of the O157 antigen (Desmarchelier et al., 1998). Another O157:H7-specific gene is *fli*C, which encodes the H7 antigenic flagellin (Prager et al., 2003). Invasion plasmid antigen, *ipa*H, is associated with the invasive phenotype of *Shigella* and enteroinvasive *E. coli*. By combining the multiplex PCR and DNA microarray, Chizhikov and colleagues were able to successfully identify 15 strains of pathogens (Chizhikov et al., 2001). Conventional methods have a detection limit of one cell per 25 g sample (Maslanka et al., 2001). By using spotted DNA microarrays labeled with oligonucleotide probes that were complementary to four virulence loci (intimin, Shiga-like toxins I and II, and hemolyxin A), *E. coli* O157:H7 was detected

from less than one cell equivalent of genomic DNA (1fg) (Call et al., 2001). With the appropriate design of cDNA or oligonucleotide probes, DNA microarrays can be used to characterize the gene expression profiles of hosts induced by various pathogens to better understand the etiology and mechanisms of food-borne infections. This technology can also be used as a universal diagnostic tool for the simultaneous screening of a large number of specimens for a wide range of pathogens.

9. Conclusions

The quality and quantity of diet is a key determinant of human health and disease. Many dietary nutrients and components can act as signals and change the patterns of gene expression, protein expression and switch metabolic pathways to form distinctive dietary signatures. They can also enhance or subdue the toxicity of any given environmental or food-borne toxin. The DNA microarray technology offers substantial versatility to develop powerful diagnostic tools to address issues related to food safety. Since its development a few years ago, this technology has been used for the applications of toxicogenomics, pharmacogenomics, cell biology, and clinical investigations addressing the prevention and intervention of diseases. Optimization of this technology to specifically address nutrigenomics and food safety is a vast resource that remains to be mined. Efforts to develop diagnostic custom arrays and simplified bioinformatics tools for field use are warranted.

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