Evaluation of the Effects of the Dietary Intake of Proteins and Amino Acids by DNA Microarray Technology

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ABSTRACT The DNA microarray technique has been increasingly utilized in various fields of life sciences. It allows us to analyze the expression levels of thousands of genes simultaneously. The high productivity will facilitate the evaluation of changes in amino acid metabolism and their consequences in response to dietary proteins and amino acids. We compared the expression profiles by the GeneChip system in the liver and other tissues among three groups of rats fed with a 12% casein, a 12% gluten or a protein-free diet. Feeding the gluten or the protein-free diet up- or down-regulated a few hundred genes in the liver compared to the casein diet. Although some of the genes were already known to respond to changes in the protein nutritional state, the majority was newly identified responders. This paper also discusses the possibility of a use this technology for safety evaluation of excessive intake of dietary components, especially of amino acids. J. Nutr. 133: 2073S–2077S, 2003.

KEY WORDS: • amino acids • nutrigenomics • DNA microarray • protein nutrition • functional food • nutrigenomics and toxicogenomics

When it became clear that all of the information about any genome could be obtained, it was natural for one to envisage that other aspects of life could be understood in a exhaustive manner. Thus the attempts to understand the full complexity of genes, transcripts, proteins, metabolites and others have evolved to new research areas (Fig. 1). These rapidly growing areas are labeled by the names of the object or field studied, suffixed by “omics”, such as genomics, transcriptomics, proteomics and metabolomics. (1,2). Nutrients or the components of diet can affect the status of an animal’s body by influencing all of the steps. A new field of study referred to as nutrigenomics is the study of both the functions and the pertinent intake levels of food components using the changes of such omics as information sources (3–6).

In this paper, we outline the omics analyses of the effects of dietary amino acids and proteins focusing on the study of the transcriptome. Examples of the transcriptomic analyses of the function of dietary proteins will be described, followed by a look at the application of transcriptomic technology for the study of the toxicity of amino acid excess that is the main theme of this workshop. When it comes to toxicology, the term toxicogenomics has been widely used, and its perspective is not very different from that of the term nutrigenomics (Fig. 1). The subject of toxicogenomics was recently reviewed by Aardema and MacGregor (7).

Use of DNA microarray technology in food and nutrition sciences

The study of transcriptomics has been facilitated by the advance of DNA microarray technology. Because the technology’s principle is well known, we will not describe it in detail here (for details, see (8,9)). In brief, the combination of a highly integrated panel of hybridization probes (microarray) and an apparatus for detecting the hybridization signal enables us to analyze the expression levels of thousands of mRNAs simultaneously. For instance, the GeneChip microarray (Affymetrix, Santa Clara, CA) of the rat, which is described below, carries probe sets of around 7,000 genes.

Transcriptomics represents only one aspect of the multiple omics world shown in Figure 1. It is only natural to argue for the need of a comprehensive analysis of other sets of omics to gain precise insights into the activities and responses of organisms. However, the advantages of DNA microarray analysis, including the prompt output of a huge amount of information and the ease of sharing a common experimental system among researchers, makes this technique the most widely used of the omic technologies (8). In addition, the growth of transcriptome analysis has been promoted by the recognition that the response of each transcript reflects changes in the status of proteins and other metabolites. That is, transcriptome analysis can be used as a global marker of the status and response of cells, organs and bodies, which result from changes of proteomics, metabolomics and other omics. This concept is simply
illustrated in an article by Brazhnik et al. (10), where a global biochemical network is represented by three levels as planes (gene space, protein space and metabolic space) and all interacting networks can be visualized by projecting all interactions on the gene space (transcriptomics).

DNA microarray analysis has been abundantly utilized in many of the life science fields and has provided us with an unprecedentedly immense body of valuable information. Researchers of nutrition and its related areas promptly turned attention to this technology (3–6). Some early DNA array works relating to nutrition include a series of studies on the effects of calorie restriction on aging (11–15). The impact of these studies helped many nutritionists recognize the fruitfulness of transcriptomics analysis. However, there is little published research about the effects of other nutritional factors on gene expression profiles. Some studies on the effects of lipids and vitamins have appeared (16–18), and a huge amount of data is likely to have already been accumulated around the world. It seems likely that most of the data has not yet been published because this field is so new that publication standards have not been developed for this research.

DNA microarray analysis of the effects of dietary protein on the gene expression profile

A number of genes have been reported to be transcriptionally regulated by amino acids (19). C/EBP homologous protein (CHOP) and asparagine synthase (AS) are the most intensively studied among the genes whose transcription is stimulated by the deficiency of amino acids (20,21). The transcription of the insulin-like growth factor binding protein-1 (IGFBP-1) gene is also up-regulated by amino acid deprivation in cultured cells as well as protein malnutrition in vivo (22,23). The induction of this gene is thought to be one of the mechanisms of growth retardation in protein malnutrition (24). Obviously, plenty of genes are down-regulated by protein malnutrition. Thus, altering the expression of a variety of genes is one of the major strategies of the body to adapt to or resist dietary protein deficiency. Driven by the desire for a more inclusive list of genes that are affected by protein malnutrition, we drew on the power of the DNA microarray technology (25).

Rats were fed a protein-free diet (PF), a 12% gluten diet (12G) or a 12% casein diet (12C) for 1 wk. RNA extracted from the tissues of five rats from each diet group was pooled and then subjected to GeneChip microarray analysis (Rat Genome U34A, Affymetrix). The genes that exhibited up- or down-regulation of twofold or more by PF or 12G as compared with 12C were regarded as responders and were categorized according to their functions. The results are summarized in Table 1, in which the numbers of the responder genes belonging to respective functional groups are shown (25). The expression levels of 281 genes were increased or decreased by twofold or more by PF, whereas 111 genes were prominently affected by 12G. Though some of them were genes already known to respond to protein nutrition, a majority were newly identified as responders to protein nutritional status.

Interesting findings included the drastic changes in the levels of genes for Id (inhibitor of DNA binding) proteins, which bind to bHLH transcription factors and are involved in the regulation of multiple genes (26). Expressions of many other transcription factors were found to respond to protein malnutrition, a finding that was in line with our classic molecular biological finding that hepatocyte nuclear factor-3g (HNF-3g), a forkhead transcription factor, is highly up-regulated by dietary protein deficiency (27). These observations present a notion that protein nutrition affects transcription of a host of genes through regulation of the expression of transcription regulators. Another interesting observation was the up-regulation of the expression of the small heterodimer homolog (SHP) gene by protein malnutrition. SHP has been implicated in feedback regulation of bile acid synthesis (28), and polymorphism of this gene has been shown to be related to mild obesity in the Japanese population (29). Thus, dependence of this gene on protein nutrition might represent one example of the link between protein and cholesterol nutrition and single nucleotide polymorphisms (SNPs).

An additional notable discovery of the relationship between protein and lipid metabolism was the response of a set of...
searchers, which include database and analysis tools. An efficient sharing of information among nutrigenomics researchers is important to understand the implications of individual changes. It is essential to develop an infrastructure for the analysis of information. Global analysis of the relationship of many tissues and will result in an even greater accumulation of knowledge.

We obtained from the rats fed the three diets. Exhaustive enzyme genes in the pathway of cholesterol biosynthesis and disposal (25). The results indicated that genes for many enzyme genes in the pathway of cholesterol biosynthesis and disposal (25). The results indicated that genes for many of the enzyme genes of the cholesterol synthetic pathway and for the rate-limiting enzyme of cholesterol catabolism (CYP7A1) were up-regulated by 12G feeding and down-regulated by PF feeding. Because levels of serum cholesterol in both gluten- and PF-fed rats were reduced (25,30), the mechanism and significance of these changes remain unknown.

We thought it necessary to confirm the expression levels of the genes of interest by other methods, because the experiment was performed using only one chip per dietary group by pooling the RNA of five rats each and also because this technique was new to us. RNase protection assay was carried out for more than ten genes including six of the above mentioned cholesterol-related genes. Comparison of the mean of the expression levels between the simple system of cultured cells and a more complex system of the whole body. Studies on the differential responses of each cell type in a tissue and on responses independent of the influence of neuronal and endocrine factors are needed in the near future.

**Omic approaches in safety evaluation of excessive amino acids**

The adverse effects of excessive amino acids derive from toxicity, antagonism or imbalance (35). Determining the adequate intake of amino acids is challenging because of the scarcity of scientific evidence. There is little solid information about safe intake levels, and a new methodology for determining upper limits is needed.

Analyses of this sort will sometimes lead to the identification of a gene (or a small group of genes) that directly explains the mechanism of a specific toxicity. In contrast, when the adverse effect of interest cannot be assigned to a specific causative gene(s), finding similarities in the changes of expression profile compared to those found in other conditions might lead to an inferred understanding of the mechanism. An example may be the observation by Nur et al. (18) that the gene expression profile in the intestine of vitamin A–deficient rats has greater similarity to that in rats with chemically induced colitis. To facilitate this type of analysis, it would be highly desirable if public databases encompassing gene expression profiles in a variety of conditions were readily accessible. Researchers have started to develop such databases, and an example is the mini-

### Table 1

**EVALUATION OF THE EFFECTS OF THE DIETARY INTAKE**

<table>
<thead>
<tr>
<th>Functions</th>
<th>PF</th>
<th>12 G</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Up2</td>
<td>Down2</td>
</tr>
<tr>
<td>Growth factors</td>
<td>5</td>
<td>3</td>
</tr>
<tr>
<td>Receptors and signal transduction</td>
<td>25</td>
<td>3</td>
</tr>
<tr>
<td>Energy metabolism</td>
<td>10</td>
<td>3</td>
</tr>
<tr>
<td>Transport and binding proteins</td>
<td>19</td>
<td>2</td>
</tr>
<tr>
<td>Gene expression control</td>
<td>16</td>
<td>6</td>
</tr>
<tr>
<td>Stress responses</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>Cholesterol metabolism</td>
<td>11</td>
<td>15</td>
</tr>
<tr>
<td>Lipid metabolism</td>
<td>8</td>
<td>0</td>
</tr>
<tr>
<td>Metabolism of xenobiotics</td>
<td>6</td>
<td>5</td>
</tr>
<tr>
<td>Amino acid metabolism</td>
<td>13</td>
<td>3</td>
</tr>
<tr>
<td>Biologic oxidation</td>
<td>8</td>
<td>7</td>
</tr>
<tr>
<td>Inflammatory responses</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Cell cycle</td>
<td>0</td>
<td>6</td>
</tr>
<tr>
<td>Cell structure</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>Ribosomal proteins</td>
<td>11</td>
<td>0</td>
</tr>
<tr>
<td>Unassigned</td>
<td>38</td>
<td>51</td>
</tr>
<tr>
<td>Total</td>
<td>97</td>
<td>184</td>
</tr>
</tbody>
</table>

1 The genes were tentatively categorized according to their biochemical functions.

2 The numbers of genes whose mRNA levels were higher or lower by twofold or more in the PF group or the 12G group compared with the 12C group are shown.
Considerations in the application of DNA microarray technology for nutrition science

With respect to the gene-nutrition relationship, most studies including ours described above measure gene expression levels on a tissue-by-tissue basis. However, none of the tissues in the body are merely an agglutination of a homogenous population of cells. Organs are made up of various cell types, and even the cells of the same type have different functions according to their location; an example of the latter is the zonation of liver parenchymal cells (37). The analysis of the changes in the gene expression profile for each tissue has significance when the response as a whole is the matter of interest (e.g., the response of the liver to a gluten diet or a PF diet as in the example described above). In contrast, if a more precise mechanism of response needs to be considered, the heterogeneity of the cell types within a tissue should be taken into account. This issue must be carefully addressed in the application of microarray technology when it is applied to the study of toxic effects.

The strongest feature of DNA microarray analysis, which is its ability to generate huge amounts of data, can also be a source of troubles, especially when tissue samples in vitro are used. First, contamination of the very small amount of a different tissue such as, for example, fat and pancreas in intestinal samples could result in misleading results. In addition, if pieces of tissues used are differently vascularized, misleading results may be obtained. More care thus needs to be taken in planning, performing and interpreting in vivo experiments using DNA microarray technology.

Another problem is the amount of information to be handled. The development and refinement of databases and analytical tools, as well as the fostering of researchers well prepared for working with the information and tools, are challenges that will need to be met in the fields of nutrigenomics and toxicogenomics.

Our studies indicated that DNA microarray analysis is a highly effective way to understand the function of dietary proteins and the global response of the body to protein nutritional status. This feature of the technology can be efficiently utilized to develop new functional foods (38,39). In addition, DNA microarray analysis is a promising tool for the safety evaluation of dietary components, including the determination of the adverse effects of an excess of an amino acid, because it offers an extremely wide range of biomarkers for this purpose. Safety evaluations using microarray analyses will probably greatly reduce the period of animal testing needed, because changes of gene expression usually occur faster than phenotypic changes.

LITERATURE CITED


