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Microarray Technology: A Promising Tool in Nutrigenomics

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Microarray technology is a powerful tool for the global evaluation of gene expression profiles in tissues and for understanding many of the factors controlling the regulation of gene transcription. This technique not only provides a considerable amount of information on markers and predictive factors that may potentially characterize a specific clinical picture, but also promises new applications for therapy. One of the most recent applications of microarrays concerns nutritional genomics. Nutritional genomics, known as nutrigenomics, aims to identify and understand mechanisms of molecular interaction between nutrients and/or other dietary bioactive compounds and the genome. Actually, many nutrigenomic studies utilize new approaches such as microarrays, genomics, and bioinformatics to understand how nutrients influence gene expression. The coupling of these new technologies with nutrigenomics promises to lead to improvements in diet and health. In fact, it may provide new information which can be used to ameliorate dietary regimens and to discover novel natural agents for the treatment of important diseases such as diabetes and cancer. This critical review gives an overview of the clinical relevance of a nutritional approach to several important diseases, and proposes the use of microarray for nutrigenomic studies.

Keywords nutritional genomics, gene expression, macronutrients, micronutrients, food

INTRODUCTION

The maintenance of human health requires a well-balanced diet containing a complex mixture of macronutrients (i.e. carbohydrates, lipids, and proteins) and essential micronutrients (i.e. minerals and vitamins). Macronutrients are primarily used as an energy supply, whereas micronutrients, which are organic or inorganic compounds present in small amounts, are not used for energy. Furthermore, a wide range of nonessential micronutrients, such as organic phytochemicals (i.e., quercetin, naringenin, and other bioflavonoids in general) that are not strictly required in the diet, are linked to the promotion of good health, when present in sufficient quantities (Roberfroid, 2000; Graf et al., 2005).

A well-balanced diet represents one of the most urgent worldwide health challenges, for two main reasons—malnutrition, which is the result of a diet with insufficient macro- and micronutrients, and overfeeding, which is characterized by excessive food intake (Cordain et al., 2005). Malnutrition involves over 800 million people worldwide (data provided by FAO's 2006 State of Food Insecurity Report, http://www.fao.org/docrep/009/a0750e/a0750e00.htm) and causes different types of disorders that particularly affect vulnerable individuals, such as children, elderly people, and pregnant women; malnutrition may also lead to death (Muller and Krawinkel, 2005; Webb et al., 2007). Overfeeding is a problem affecting several industrialized nations, where excessive daily caloric intake often leads to disorders such as obesity, diabetes, cardiovascular disease, and cancer. Thus, determining the composition of foods and understanding how food components can modulate health may provide important information for the prevention and management of several human diseases.

Today, the link between diet and health is well established, and thus in the future researchers will focus on how to balance biologically active dietary components and how this may exert beneficial effects; in other words, the concept of "nutritional genomics." To date, many nutritional genomic studies have investigated the relationship between genes and food intake and the biologically active components of food, and how these interactions may have an impact on human health (Ordovas and Mooser, 2004). Nutritional genomic or nutrigenomic strategies could improve health providing molecular biomarkers, preventing diseases, and helping to understand changes in gene expression induced by whole diets or by individual dietary constituents (Kussmann et al., 2006).

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The application of high throughput functional genomic technologies, such as microarrays, in nutritional research may facilitate the definition of a "gold standard" diet for populations and/or individuals, living in different geographical areas. This, in turn, should promote the development of food derived treatments and functionally enhanced foods to improve health.

This review will critically discuss how microarray technology may help to clarify several issues regarding nutritional genomics.

THE POWER OF MICROARRAY TECHNOLOGY

In recent years, the advances in our knowledge of nucleic acid sequences, nucleic acid hybridization, and cloning techniques have led to a clearer understanding of overall gene expression regulation at the transcriptional level. Moreover, oligonucleotide and cDNA microarrays represent powerful tools that allow for a rapid and high-throughput gene expression evaluation. Essentially, the microarray technique is a quantitative assessment of the relative cell or tissue concentration of the specific messenger RNA (mRNA) that is directly related to the level of expression of that particular gene (Fig. 1). The amount of the mRNA transcript present in the cells or tissues can be measured indirectly after its extraction, and is then used to create a complementary labeled strand of DNA (cDNA). The labeled cDNA (target) can be hybridized with a complementary probe, which has been previously spotted on a solid glass slide or nylon substrate. This array contains a known set of gene sequences (genome). The intensity of the color, quantified by a confocal fluorescent scanner (or a chemiluminescence reader) after the hybridization process, is directly related to the amount of target mRNA and reflects the expression level of that particular gene. In this way, it is possible to determine which gene is up-regulated or down-regulated compared to the control sample, as a result of specific biological manipulations or during normal tissue development, as already observed in muscle (Reecy et al., 2006). Microarray technology is an extremely powerful tool that can be used to study metabolic processes at a very basic level and it provides an appropriate mean of understanding the complex interactions regulating gene functions. Gene transcription is only one step in the regulatory pathways that leads to functional protein formation; thus it is not always possible to correlate the increased presence of mRNA with phenotypic or protein changes in tissues (Muller and Kersten, 2003; Moody, 2001).

This technology, however, has certain drawbacks. In fact, in order to obtain reliable and reproducible results, several parameters of the experimental protocol (array production, RNA extraction, cDNA labelling and hybridization, and data analysis techniques) should be optimized. Adherence to protocols of manufacturers for commercial arrays ensures a high degree of standardization, at least in the labeling, hybridization, and other analytical steps. The Microarray Gene Expression Data Society (MGED) has developed guidelines that specify the minimum standards necessary to ensure that experiments using microarrays can be properly interpreted and independently verified. These guidelines (minimum information about a microarray experiment [MIAME]) are available

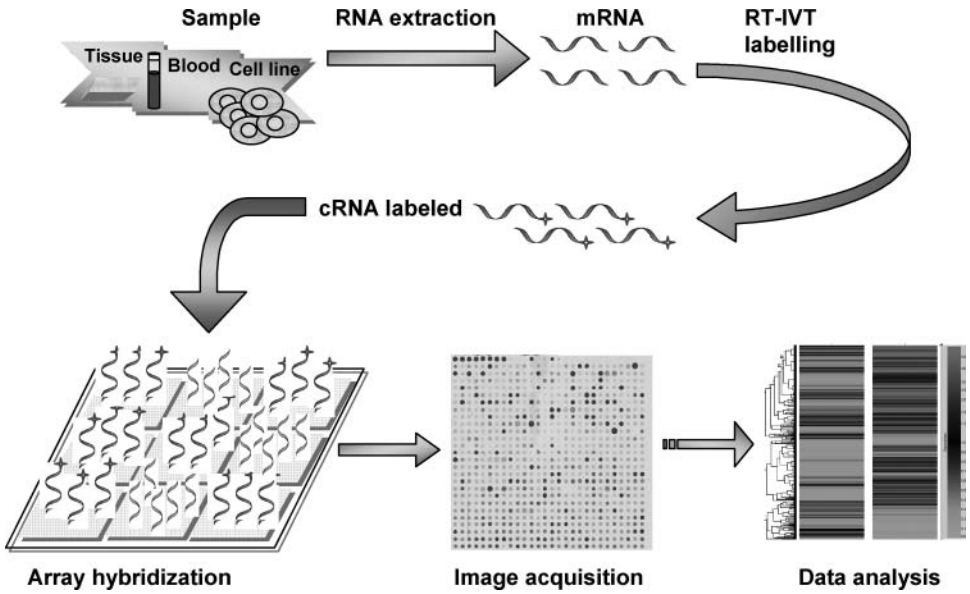

Figure 1 A schematic representation of microarray technology. mRNA transcripts extracted from blood, cells, or tissues are subjected to a Reverse Transcriptase-In Vitro Transcription (RT-IVT) to create labeled cRNA copies. The labeled cRNA is hybridized with a complimentary strand (probe) spotted on a solid glass slide or nylon substrate (array) containing a known set of gene sequences (genome). The intensity of the color is quantified by a confocal fluorescent scanner (or a chemiluminescence reader) and the resulting image is analyzed to extract raw data on gene expression. Then, raw data are analyzed using specific software to determine which genes are up-regulated (red) or down-regulated (green) with respect to the control sample.

at http://www.mged.org/index.html (Brazma et al. 2001). Other minor drawbacks are the technical variations between array platforms and analytical procedures that may lead to differences in the observed transcriptional responses. The MicroArray Quality Control Consortium (MAQC) recently evaluated the performance and reproducibility of the most common platforms and generated a set of data that justifies their use for gene expression profiling, both in basic and applied research leading to clinical diagnostic tools (D'Ambrosio et al., 2005).

THE GOAL OF NUTRIGENOMICS

There is considerable evidence to indicate that food-intake is a key environmental factor affecting the incidence of many chronic diseases, including obesity, diabetes, and related metabolic syndromes, cardiovascular diseases, and cancer (Ruan and Teng, 2002). Acceptance is growing among the scientific community that the onset, incidence, severity, and progression of the majority of these chronic diseases are dependent on the quantity of food eaten and diet composition. However, the exact number of biologically active components and their effects on our daily diet is unknown. Therefore, determining the composition of foods and understanding how food components can modulate health may help to improve the prevention and subsequent management of the above-mentioned chronic diseases. In addition, human dietary constituents represent a heterogeneous mixture of many biologically active substances, which can directly or indirectly affect the transcription of a large number of genes (Kaput, 2007). The identification and analysis of gene/nutrient interactions are necessary steps in the process of designing and producing foods able to ensure population health and are therefore the main objective of nutrigenomics. Nutrigenomic studies investigate how dietary constituents interact with genes and their products, and conversely how some genes (i.e., genes coding for glucose or insulin receptor) influence the metabolic conversion of dietary constituents into nutrients, anti-nutrients, and bioactive compounds such as vitamins. However, defining the interaction between a particular nutrient and a specific gene is a difficult task due to the chemical heterogeneity of food, the genetic variability of humans, and the complexity of mechanisms whereby nutrient intake influences health and disease (Kaput and Rodriguez, 2004). Nutrigenomics provides the means to find an answer to these many questions.

All current nutritional interventions are based on an individual's diet and genotype, two important elements that can be used to prevent or cure chronic diseases. In fact, nutrigenomic studies are principally focused on the analysis of whether (i) substances contained in the food can directly or indirectly affect the human genome by changing its structure and gene expression; (ii) diet can be an important risk factor for the development of chronic diseases; (iii) some diet-influenced genes play a crucial role in the onset, incidence, progression, and severity of diseases.

COUPLING OF MICROARRAYS AND NUTRIGENOMICS MAY ENSURE GOOD RESULTS

Microarray technology is a powerful tool for the study of gene-diet interactions. This technique has many disadvantages such as the cost of array apparatus and reagents and these, coupled with technical challenges associated with nutritional microarray studies, often mean that compromises have to be made in the number and type of samples analyzed. In the last decade, microarray technology not only provided femtomolar-level sensitivity, allowing for the detection of less than one transcript per cell (i.e., chemiluminescence labeling and reading), but also seems to be well-suited for nutrigenomic expression profiling studies.

The application of microarray technology to nutrigenomic studies is incredibly powerful. In fact, with this approach it is possible to obtain not only clinically important data, for instance, to determine the impact of diet and/or of a single nutrient on a particular human pathology, but also to acquire new information about the effect of a specific food compound on the human gene profile. In the first case, the coupling of microarrays and nutrigenomics permits to identify the therapeutic properties of a natural food compound, whereas in the second case it allows to understand why and how some natural foods may induce different gene responses. In the following paragraphs, we provide some interesting examples of the results obtainable with these two approaches.

The "Whys" and "Hows" of Nutrigenomics using Microarrays

In the field of nutrition, we expect that many bioactive dietary compounds, at nutritionally relevant concentrations, will elicit subtle changes in gene transcription that may be critically important in biological terms. Unfortunately, their reliable detection is difficult. To help understand the potential of microarray technology with respect to nutrigenomic research, some examples will be briefly described below.

Royal jelly, one of the most popular and traditional food, has diverse physiological and pharmacological properties and has been studied in a mouse model for its (or one of its components) ability to stimulate the production of type I collagen and other functions affecting bone formation through action on osteoblasts (Narita et al., 2006).

Vitamin E compounds, especially tocotrienol, have recently been investigated to elucidate the bioactive mechanism underlying their inhibition of angiogenesis by specifically down-regulating the expression of VEGF receptor (VEGFR) in endothelial cells (Nakagawa et al., 2004). Tocotrienol was also reported to reduce the telomerase activity of HUVEC cells cultured in vitro, by down-regulating the expression of protein kinase C (PKC).

Polyunsaturated fatty acids (PUFA) are essential structural components of the central nervous system due to their role in

controlling learning and memory. A nutrigenomic approach with high-density microarrays revealed brain-specific gene expression changes in response to different PUFA-enriched diets in rats (Kitajka et al., 2004). PUFA-enriched diets led to significant changes in the expression of several central nervous tissue genes such as transthyretin, α-synuclein, and calmodulins, which play important roles in synaptic plasticity and learning. The direct effects of PUFA on transcriptional modulators, the downstream developmental and tissue-specific activated elements, might be key elements for understanding the beneficial effects of PUFA on nervous system functioning.

High-density microarray experiments have also been successfully used to determine the gene expression patterns in kidney glomerular cells of diabetic mice and to investigate the beneficial effects of astaxanthin (a naturally occurring carotenoid pigment) that alters the expression of certain genes in diabetes (Hussein et al., 2006). The affected genes were associated with complexes I, III, and IV located on the mitochondrial inner membrane and their expression levels were decreased in mice treated with astaxanthin compared to control mice. In addition, the expression of many genes associated with oxidative stress, collagen synthesis, and transforming growth factor-beta signaling was enhanced in the diabetic mice. This enhancement was slightly inhibited in the astaxanthin-treated diabetic mice (Naito et al., 2006). Thus, astaxanthin was reported to reduce glomerular oxidative stress as well as to inhibit the increase in urinary albumin in diabetic mice. Microarray technology provides insight into gene functions and putative proteomic pathways thought to be affected by stimulation with high-glucose concentrations and also helps to gain a better understanding of the anti-diabetic action of astaxanthin.

Interestingly, soy proteins have been extensively studied not only for their intrinsic nutritive value, but also for their biological functions such as cholesterol-lowering and anti-obesity effects. Some reports have suggested that soy proteins alter the expression profiles of genes related to lipid metabolism in liver and in adipose tissue. In fact, statistical analysis reported significant differences in gene clusters which are involved in lipid and energy metabolism, transcription factors, and anti-oxidation enzymes (Takamatsu et al., 2004). Furthermore, soy products, the almost exclusive dietary source of isoflavones, exert a number of cardioprotective effects. In fact, soybean-derived isoflavones promote the reduction of LDL cholesterol and inhibition of pro-inflammatory cytokines, cell adhesion proteins, and inducible nitric oxide production, thus preventing the occurrence of cardiovascular diseases (Rimbach et al., 2008).

Another important application of microarrays in the field of nutrition is that it may provide the means to modify a natural food compound so as to induce a particular gene expression profile. This would have several different implications, such as to protect the human organism from developing diseases, to ameliorate responses to conventional therapies (e.g., chemotherapeutic drugs), and to improve the quality of a particular food. For a fuller comprehension of the importance of the use of microarray technology in this aspect of nutrigenomics, we provide the following explicative examples.

It has been reported that berries such as bilberry (BB), cloudberry (CB), and lingonberry (LB) contain a great number of different phenolic compounds with potentially anti-carcinogenic properties (Misikangas et al., 2007). The major phenolic compounds found in these berries are anthocyanins, flavonols, and ellagitannins, which are known to inhibit carcinogenesis at different stages both in vitro and in vivo, and to prevent the formation of adenomas. BB contain high levels of anthocyanins, CB are prevalently rich in ellagitannins, and LB contain moderate levels of anthocyanins and flavonols but almost no ellagitannins. Feeding Min/1 mice with BB, LB, or CB, which possess different phenolic profiles, resulted in a significant reduction of intestinal tumors. However, an overall anti-tumorigenic activity is achieved by giving the mice a mixture of these compounds, since they act better synergistically than individually. It seems that CB and LB inhibit the tumor growth, by preventing the nuclear accumulation of β-catenin, ultimately decreasing the expression of cyclin D1, one of the many proteins involved in cell cycle control. However, to utilize fully the anti-carcinogenic properties of berries, further studies should focus on the responsible compound and its mechanism of action, and determine the lowest dose needed to obtain the highest possible effect.

Citrus fruits constitute the main fruit tree crop in the world. They possess an unusual combination of biological characteristics, such as low genetic diversity and a long-term history of tree breeding. We know that the transcriptome is a key element in understanding the developmental biology of plants and how they respond to environmental stresses, which should lead to improvements in fruit quality, higher yields and making them tolerant to environmental stresses (e.g., salinity) (Terol et al., 2007). The difference in behavior of these cultivars during normal fruit growth or when facing environmental adverse conditions were found to be more likely associated with differences in gene regulation rather than with gene polymorphisms. A knowledge and understanding of genomics would provide new tools to produce more efficient varieties and rootstocks, to identify new gene expression profiling, their alleles or genotypes, which would ultimately have a relevant impact on agronomic development.

Another application which uses gene-expression array technology in nutrigenomic studies is the monitoring of cacao beans harvested from the tropical cacao tree (*Theobroma cacao L.*) (Jones et al., 2002). Cacao beans harvested are subject to high losses, mainly as a consequence of pests and diseases. Clearly, the discovery of putative natural resistance mechanisms, which would stabilize the crop for growth, is essential for chocolate production. Microarray platforms are therefore critical for investigators involved in the study of cacao bean stability, pathotype resistance, and for the consequent nutrigenomic effect on humans.

Alfa-chaconine and alfa-solanine are naturally occurring toxins, which account for 95% of the total glycoalkaloids contained in potatoes (*Solanum tuberosum L.*). When present at high

levels, these glycoalkaloids can be toxic to humans, mainly because they can disrupt cell membranes of the gastrointestinal tract. Experiments on gene profiling were performed on Caco-2 cells exposed to pure glycoalkaloid or to mixtures thereof for 6 h to fully elucidate their effects, to determine their transcriptional effects, and to investigate the utility of DNA microarrays used in conjunction with conventional toxicological screening (Mandimika et al, 2007). The differences in response to various glycoalkaloid treatments were mainly due to their different degrees of efficacy, and the DNA microarray technique was able to discriminate the severity of effect/potency amongst the different glycoalkaloid treatments.

It is noteworthy that microarray technology may also have an impact on socioeconomic aspects linked to the agro-food system, which is subject to the influence of markets and globalization. In fact, both food security and productivity may be monitored with this technology. Coffee is a striking example of this concept. During its storage, the high levels of humidity favor the growth of mycotoxin Ochratoxin A (OTA). Roasting at 200°C for 10 or 20 minutes reduces the levels of this toxin by only 10–12% in the dried whole beans (Tsubouchi et al., 1987). Therefore, almost all of the OTA is infused into the coffee, when the powder is extracted with boiling water. For these reasons, the European Food Safety Authority (EFSA; http://www.efsa.europa.eu) issued guidelines for coffee quality control, to avoid contaminated coffee being supplied to consumers. Therefore, microarray technology may be used to identify toxic substances which contaminate coffee and which cause adverse effects if ingested.

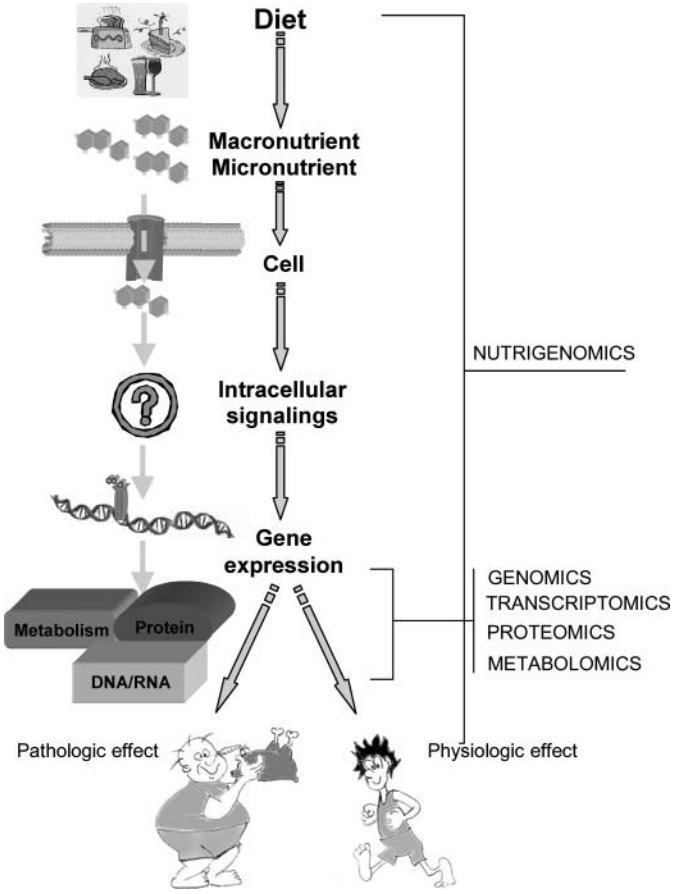

Figure 2 Flow chart representing the different steps involved in gene expression, the stages at which diet can modulate these processes, the functional genomics techniques used to analyze each stage, and their effects on human health.

CONCLUSIONS AND FUTURE PERSPECTIVES

The potential for immense socioeconomic benefit resulting from the successful characterization and exploitation of health promoting factors in foods is a topic which is currently being widely discussed and debated. This area of research can be considered as falling within the scope of the emerging science of nutritional genomics. The science of nutrigenomics uses genome-wide information to evaluate the effects of diet and nutrient management schemes on gene expression. Microarray technologies provide powerful tools for nutrigenomic studies, and other emerging functional genomics techniques should be considered. Nutrigenomics analyzes, as fully as possible, the physical and chemical characterization of food components, as well as target structures and polymorphisms within the genome. One challenging goal of nutrigenomics is to use nutritional systems biology to identify a pool of biomarkers that can predict the beneficial or adverse effects of dietary nutrients or components. These biomarkers could be used as predictive or screening tools to promote health and prevent disease. Furthermore, preliminary studies have suggested that it will be possible to use specific gene expression patterns to evaluate the effects of nutrition on key metabolic processes. However, it is only by combining information from nutrigenomics (Fig. 2), genomics, proteomics, metabolomics, and appropriate bioinformatics, that it will be possible to understand all aspects and implications of nutrition-modulated beneficial homeostasis and its adverse toxicological effects.

We are now combining new technologies like high throughput microRNAs screening with genomic and proteomic analysis to evaluate the effect of dietary and natural compounds (e.g., aloe-emodin) on the development and progression of nonalcoholic fatty liver disease (NAFLD) in animal models and in humans (Alisi et al., 2009).

Unfortunately, these new technologies produce considerable amounts of data requiring the integration of several resources for data interpretation. In this scenario, several different databases will be necessary in the future to identify the physiological and functional significance of the nutrigenomics-derived results (Burgoon and Zacharewski, 2008).

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