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Minireview

Linking nutrition to genomics

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Abstract

The new scientific field of nutrigenomics utilizes genomic tools, like microarrays, to analyze metabolic adaptations induced by variations in nutritional status. Here we describe how transcriptional regulation patterns caused by nutritional changes can be identified using gene expression profiling. This includes technical remarks on microarray analysis and data processing, as well as giving biological meaning to statistically solid data. We highlight our recent findings of transcriptional regulation of genes representing specific signaling and metabolic pathways in mouse liver under starvation. The results show strong correlations to previously identified responses to caloric restriction, which can be linked to lifespan extension.

Keywords: caloric restriction; hepatic expression profiling; lifespan prolongation; metabolic signaling; microarray analysis; nutrition response.

Introduction

Interaction between nutrition and biochemistry is of fundamental importance in physiology and disease. Many metabolic pathways and signaling cascades are known, based on a large body of biochemical data (Michal, 1999). The best way to analyze and understand the influence of nutrients on physiological processes would be to measure the flux through each pathway and monitor the signals that control it. So far the desired tools for this ideal approach have not been developed and it is doubtful they will be anytime soon. If we look at this challenge in view of the current technical possibilities, microarrays can provide a global assessment of the transcriptionally dependent changes. Although there are several steps and levels in the regulation of gene expression, a change in the level of mRNA is the most basic and points to the direction toward which gene activity shifts. Taking into account biochemical knowledge, genes can be grouped into pathways based on the encoded gene products. If the microarray data is organized using the biochemical matrix, the regulation can be condensed into specific groups (Figure 1). On top of this biochemical outline, signaling components that regulate the metabolic pathways can also be identified. Using this method we have monitored global changes in gene expression in mouse liver in response to fasting and sugar-fed conditions using high density microarrays (Bauer et al., 2004).

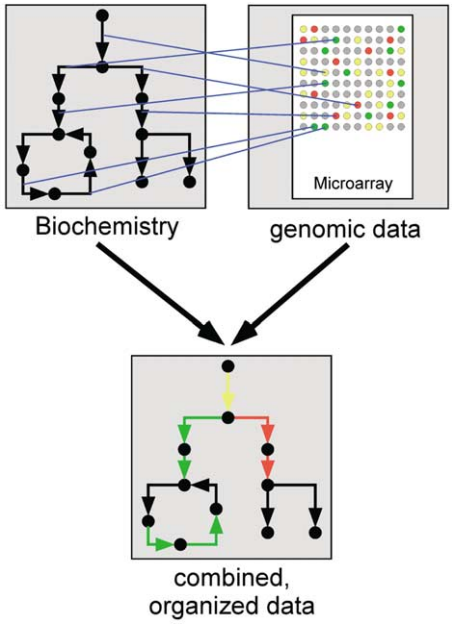

Figure 1 Schematic overview of the approach used to structure microarray data.
Using the biochemical matrix (upper left drawing) and combining it with the microarray results (upper right drawing) it is possible to create a biochemical map of transcriptionally influenced pathways. Yellow arrows in the lower drawing indicate reactions in which the transcription of the corresponding enzyme is not changed, red and green arrows indicate an expression change. The colors correspond to the standard color code used in the visualization of microarray results.

Technical basis

The microarrays contained probes of approximately 20 000 different mouse genes that were generated based on the Lion Bioscience ArrayTag cDNA libraries and spotted in duplicate on each slide. The probes are arranged as two separate subarrays, resulting in a maximum distance between the duplicate spots. The distant spot layout increases the chance that at least one copy can be detected in good quality in cases of local hybridization inconsistencies. This array design was made possible by using 2×12 spotting pins with a Genemachines contact printer Omnigrid 100. This led to a density of 30 by 30 spots per block and 2×24 blocks per array, resulting in a total of 43 200 spots per array.

Male 129/sv mice (8–15 weeks old) were housed under three different nutrient conditions. One experimental group (n=6) was starved for 24 h and a second group (n=10) for 48 h. The third group (n=10) received in addition to fresh water a 50% sugar solution to which they had free access. The control group (same number as experimental, for a total of 26) received standard laboratory mouse food. To obtain statistically relevant data, a total of 60 microarrays were hybridized comparing the liver transcriptome of experimental versus control mice: 24 microarrays for normal-fed vs. 24 h starved, 24 microarrays for normal-fed vs. 48 h starved, and 12 microarrays for normal-fed vs. sugar. A maximum of 24 and 48 data points per gene was possible in the sugar and in the starvation experiments, respectively. Only those genes that gave rise to at least 16 data points in the sugar, and 36 data points in the starvation, experiments were taken through statistical analysis. The data were evaluated using a Student's *t*-test and only those genes whose expression changes lay in the 99.5% confidence interval were analyzed further. Furthermore, a dye swap, in which the Cy3 and Cy5 dyes are exchanged for labeling of experimental and control mRNAs, was performed. These stringent criteria helped to consolidate the data basis before starting the extensive process of data structuring, which adds biological relevance to statistical significance.

Another technical procedure applied to further improve the data quality was to scan each microarray three times with different amplification ratios of the detection system. In the low intensity scan the detection was adjusted so that none of the spots was in saturation compared to the maximum detection value. From this scan the ratios of expression changes of these genes can be calculated that are expressed at a high level, whereas genes that are expressed on a low level would be missed. In a scan with high detection sensitivity the weakly expressed genes can be detected, but the spots of genes that are expressed at high levels are in saturation; therefore, the two scans complement each other. To supplement the subsequent calculations a third scan is made, which lies in between the high and low intensity scans. The values of saturated spots in the 'medium' and 'high' scans are extrapolated based on the 'low' scan data to gain values that fit to the real intensities. All three data sets are averaged so that the error rate due to the detection system is further reduced.

Extracting biological information

Our next step was to place the regulated genes into known metabolic pathways (Figure 1). Using this approach to organize the data, we were able to draw a picture of the transcriptionally influenced physiological processes upon starvation and sugar feeding (Table 1). In addition to pathways that we expected to be regulated and that helped us to validate the system, we unexpectedly observed changes in several biochemical cascades that have been implicated in regulating the aging process, namely that the starvation response correlated with processes promoting anti-aging and longevity. Interestingly, most of these potentially beneficial changes were suppressed by sugar feeding. We focus below on the starvation response and highlight its relationship to lifespan-prolonging processes.

Table 1 Metabolic processes identified to be transcriptionally regulated in mouse liver upon starvation for 48 h.

Metabolic process	Regulation in starvation
Fatty acid synthesis	down
Fatty acid β-oxidation	up
Xenobiotic and lipid breakdown product detoxification	up
Amino acid breakdown	up
Urea cycle	up
SAM cycle	up
Steroid *de novo* synthesis	down
DHEA anabolic reactions	up
DHEA catabolic reactions	down

Fat and amino acid catabolism

There were alterations in biochemical and physiological pathways already known to be affected upon starvation, and transcriptional regulation of these pathways confirmed the biochemical knowledge. Clear examples are the down-regulation of fatty acid synthesis and the up-regulation of fat breakdown to provide energy. Fat catabolism also entails the activation of the lipid signaling cascade, which provides protection from genotoxic side products (Chawla et al., 2001). This group includes different cytochrome P450 species and lipid-activated nuclear receptors. Many of these components are also part of the xenobiotic response.

There is also an up-regulation of amino acid catabolism and the urea cycle, since endogenous proteins are broken down during starvation to provide fuel and essential metabolites. The urea cycle regulates the nitrogen level and also helps prevent conditions where excess nitrogen and ammonia become toxic. In this regard, the up-regulation of the urea cycle can also be seen as a protective reaction that functions analogously to the antioxidant lipid signaling cascade. Since excess amino acids and proteins cannot be stored (unlike excess fats and carbohydrates), the increase in the urea cycle can be brought about by two opposite physiological conditions, i.e. the absence, as well as an excess, of dietary proteins, as for example in the high protein 'Atkins' diet.

Insulin and DHEA signaling

In addition to the changes in central metabolic pathways, we found significant regulation of hormonal pathways. We could reproduce the transcriptional regulation of IGFs (insulin-like growth factors) and IGFBPs (IGF binding proteins). IGF1 is a major growth signaling molecule that is transcriptionally activated by insulin and growth hormone (GH) under good nutrient conditions, thereby allowing cell growth and proliferation (Kelley et al., 1996). Under starvation, these signals are absent so that IGF1 expres-

sion is strongly reduced, while its deactivating binding proteins IGFBP1 and IGFBP2 are up-regulated.

We also found an unexpected response to starvation in steroid metabolism. Because of the shortage of acetyl-coA, expression of enzymes that catalyze *de novo* synthesis of cholesterol is strongly reduced. However, due to the breakdown of fat and cell membranes (mouse liver loses approx. 30% of weight during 48 h starvation; Ferraris et al., 2001), cholesterol is still available as a starting metabolite for steroid synthesis. Strikingly, most of the enzymes catalyzing the synthesis of DHEA (dehydroepiandrosterone) are up-regulated, whereas those catabolizing DHEA or converting it to other hormones are down-regulated. This led us to the conclusion that the body tries to maintain as high a level of DHEA as possible during starvation.

Starvation response, stress resistance and longevity

A notable feature of the metabolic and signaling pathways outlined above is that they have been implicated to varying degrees in extending lifespan in different organisms. Oxidative damage is a major factor in the aging process (Sohal and Weindruch, 1996) and the activation of pathways that increase stress resistance, such as up-regulation of cytochrome P450 complexes during fat catabolism, would be beneficial. Although there is to date no direct link between amino acid metabolism and aging, 4-phenylbutyrate (PBA), which is a drug for treating urea cycle disorders, can extend the lifespan in *Drosophila* (Kang et al., 2002), perhaps also by increasing protection against oxidative stress. The role of DHEA in human aging has been a matter of long debate (Allolio and Arlt, 2002). DHEA levels peak in the 20 s and drop dramatically afterward, and recently DHEA has been reported to be involved in neuronal survival and maintenance of human neural stem cells (Suzuki et al., 2004). Our gene expression profile of enzymes involved in DHEA metabolism provides a further connection between the starvation response and the anti-aging process. For insulin and IGF, the opposite appears to be the case, where decreased insulin/IGF signaling is associated with lifespan extension in worms, flies and mice (Longo and Finch, 2003). During starvation, the huge increase in IGFBP would also block insulin signaling, thus also correlating with increased lifespan. As a final example, we have seen a significant up-regulation of GADD45 upon starvation, a gene that is activated during growth arrest and in response to DNA damage. GADD45 has been identified as a target of FOXO3 (Tran et al., 2002), a forkhead transcription factor that mediates insulin signaling and whose *C. elegans* homolog DAF16 plays a key role in lifespan regulation (Finch and Ruvkun, 2001; Kenyon 2001). In a very recent report, FOXO3 has in turn been shown to be regulated by SIRT1 (Brunet et al., 2004), the mammalian homolog of yeast Sir2, which also regulates lifespan in different organisms.

In summary, we postulate that due to metabolic changes and the subsequent increase in stress response, physiological processes evoked by starvation show strong correlation with anti-aging processes (Table 2). These observations suggest that starvation may evoke the same stress response reaction as caloric restriction, which is the only treatment known to prolong lifespan in all organisms tested to date. The difference is that starvation results in a much stronger or prolonged induction. This accentuated response during starvation may facilitate the experimental identification of basic molecular mechanisms linking nutrition and health using a nutrigenomic approach.

Table 2 List of lifespan-prolonging reactions identified at the transcriptional level in mouse liver upon starvation.

Lifespan-prolonging responses to starvation	Underlying metabolic process
Anti-oxidative and anti-xenobiotic reactions	fat breakdown
Elevated urea cycle	breakdown of endogenous amino acids
High DHEA level	steroid metabolism
IGF1 repression	insulin and GH signaling
Up-regulation of genome stabilizing factors	stress response

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