MICROARRAYS

Sugar coating

Studies of the functions of carbohydrates — which are increasingly being implicated in diseases such as cancer — have traditionally lagged behind those for nucleic acids and proteins, primarily owing to the lack of general methods for synthesizing and analysing these structurally complex molecules. Recent advances in methods for synthesizing complex carbohydrates have had a significant impact, and now, with the first report of a chemically defined carbohydrate array, the exciting possibility of chips that present arrays of many different carbohydrates for functional studies seems considerably closer to becoming reality.

Development of a carbohydrate chip for characterizing protein–carbohydrate and carbohydrate–carbohydrate interactions and enzyme activities poses several key challenges. To provide both excellent selectivity and quantitative analysis, the chip must prevent non-specific interactions, and the immobilized carbohydrates must be presented in a regular and homogeneous environment to ensure that they have uniform activity towards soluble proteins and enzymes. Furthermore, because many protein–carbohydrate and carbohydrate–carbohydrate interactions are polyvalent in nature, the density of carbohydrate ligands must be controlled. Finally, to be broadly applicable, the chip should be compatible with several common detection methods, such as fluorescence imaging and surface plasmon resonance (SPR) spectroscopy.

Houseman and Mrksich used the Diels–Alder reaction to link carbohydrate ligands to a gold-based monolayer, prepared from a controlled mixture of two components — one that can be linked to the carbohydrate ligands (1%), and one that prevents non-specific association of proteins (99%). Because the Diels–Alder reaction is rapid, selective and quantitative, it can be used to ensure that the carbohydrates are presented homogeneously at a uniform density.

To test the characteristics of their monolayers, the authors first used SPR, a powerful technique for monitoring biomolecular associations. A monolayer presenting a single carbohydrate ligand was completely inert to non-specific adsorption of fibrinogen, a common "sticky" protein, and the ligand could specifically associate with the lectin concanavalin A, a carbohydrate-binding protein. Next, they prepared an array that presented ten carbohydrate ligands, and showed — this time using fluorescence imaging — that it could be used to selectively identify binding interactions between the ligands and several different lectins. Further experiments showed that such interactions could be analysed quantitatively. Finally, the authors showed that the activity of a carbohydrate-

OBESITY AND DIABETES

Slimming down without DGAT

New research indicates that preventing obesity and diabetes by reducing tissue triglyceride stores might be a good strategy. In the *Journal of Clinical Investigation*, Chen *et al.* show that obese mice that lack a key enzyme in the triglyceride synthesis pathway have increased sensitivity to insulin and to leptin, a hormone that enhances energy expenditure.

The enzymes acyl CoA:diacylglycerol acyltransferase 1 and 2 (DGAT1 and -2) catalyse the final step in mammalian triglyceride synthesis. DGAT1 and -2 expression and activity are widely distributed throughout the body, including adipose tissue. Dgat1-deficient, non-obese mice are resistant to diet-induced obesity because of enhanced energy expenditure, which is partly accounted for by an increase in physical activity. DGAT1 deficiency, under normal circumstances, is not associated with a compensatory increase in DGAT2 expression.

The researchers investigated the effect of DGAT1 deficiency on two mouse models of obesity and insulin resistance: agouti yellow and leptin deficient (ob/ob). Agouti yellow mice suffer from severe insulin and leptin resistance. These genetically manipulated mice have increased levels of tissue triglycerides. In agouti yellow mice that lack Dgat1, the deficiency prevents the onset of insulin resistance and protects against obesity. In fact, a reduction in body weight of ~20% is associated with a dramatic 80% reduction in insulin resistance. Surprisingly, the Dgat1 deficiency in the ob/ob mice did not affect energy expenditure or glucose metabolism. However, this could be due to a compensatory increase of Dgat2 expression that was seen in these mice.

High tissue triglyceride levels are associated with insulin resistance, so the reduced triglyceride content in the Dgat1-deficient agouti yellow mice might enhance insulin sensitivity. However, although obesity is associated with leptin resistance, until now there was little evidence to indicate that decreasing tissue triglyceride content can increase leptin sensitivity. In addition, the decreased adipocyte size in Dgat1-deficient mice might be important, because adipocytes secrete several proteins that modulate glucose metabolism, such as adiponectin and resistin. Interestingly, there is evidence that diacylglycerol antagonizes insulin signalling. Therefore, a reduction in diacylglycerol levels or its precursors, such as fatty acyl CoA, might enhance insulin signalling.

Pharmacological inhibition of DGAT1 could be an effective therapy for diabetes and obesity. Further support for the strategy of targeting fat synthesis can be seen in mice deficient for the fatty-acid-synthesis enzyme acetyl CoA carboxylase 2 (ACC2), which have similar phenotypes to Dgat1-deficient mice. However, in the long term, will enhancing energy expenditure cause shorter lifespans?

Melanie Brazil

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WEB SITES

Farese's laboratory: http://gweb1.ucsf.edu/labs/farese/farese.html

Encyclopedia of Life Sciences: http://www.els.net obesity

modifying enzyme towards its natural substrate bound to an array could be characterized.

So, such arrays fulfil the key requirements for the development of a carbohydrate chip, and importantly, are compatible with recently reported methods for automatic synthesis of complex carbohydrates, suggesting that chips constructed by this chemical approach could find broad application in glycobiology, from research to drug discovery and diagnostics. Moreover, the surface chemistry involved could also prove valuable in the preparation of peptide and protein chips.

Peter Kirkpatrick

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MICROARRAYS

Validation on target

Microarray analysis of human multiple sclerosis (MS) lesions has revealed pronounced differences in gene expression in autopsy tissue from MS patients compared with similar tissue from individuals without MS. In a study published in *Nature Medicine*, Lock and colleagues establish the power of the microarray approach by validating two genes as potential therapeutic targets in a mouse model of MS.

MS is an autoimmune disease of the central nervous system (CNS) that is caused by immune-cell infiltration of the CNS white matter. Inflammation and subsequent destruction of myelin cause progressive paralysis. Environmental factors and a genetically determined susceptibility are both implicated in the aberrant immune response to myelin. Patients can have chronic symptoms or suffer acute attacks. Histologically, two types of lesion are seen: acute lesions, which are characterized by inflammation, and chronic regions that show scarring and demyelination.

Comparing transcripts from different lesions uncovered several genes that were previously unassociated with MS, and which showed differential gene expression in the two lesion types. Genes with at least a twofold change in expression were visualized using cluster analysis, which determines the correlation coefficients between pairs of genes and organizes them according to similarities in expression patterns. In each of the MS samples, the expression of 39 genes was increased, whereas the expression of 49 was decreased.

Genes with increased expression included those encoding immune-response proteins, adhesion molecules, complement molecules, B-cell and macrophage-specific proteins and a number of pro-inflammatory cytokines. By contrast, the expression of neuron-associated genes and those associated with myelin production decreased. The reduced expression of myelin-associated enzymes suggests that there is a reduced capacity for repair. For the first time, comparing these two types of lesion supports a molecular difference between the acute and chronic lesions, with selective upregulation of 22 and 32 genes, respectively.

The authors tested the value of the microarray approach using the experimental autoimmune encephalomyelitis (EAE) mouse model of MS. The immunoglobulin G (IgG) Fc receptor was upregulated in chronic lesions. EAE mice that were deficient for the IgG Fc receptor had less severe acute disease and no chronic disease compared with wild-type mice. Granulocyte colony-stimulating factor (G-CSF) was upregulated in acute lesions. The capacity of G-CSF to alter the course of EAE was tested by treating the mice with G-CSF before and during onset of the disease. Early treatment decreased the severity of the disease but had no effect later, which indicates that G-CSF might be a regulatory molecule that naturally suppresses acute attacks.

Although EAE is a useful model for MS, clinical trials have shown that many efficacious therapies in the mouse have not translated into humans. So, caution must be exercised in spite of animal validation of two MS targets. However, this study provides proof of principle for the power of microarray technology to identify new therapeutic targets. An enormous amount of data are produced in these types of microarray experiment. Databanks that contain such raw gene-array data should allow more meaningful comparisons across datasets, enabling the increasing numbers of these analyses to be exploited to their full advantage.

Melanie Brazil

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