Minireview

Asthma investigators begin to reap the fruits of genomics

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Abstract

Microarray experiments have identified novel candidate genes in animal models of asthma. In the near future, genomics may have a profound impact on the way we think about this common and complex disease.

People with asthma have recurrent episodes of airway narrowing that can cause wheezing, coughing, and shortness of breath. Episodes of airway narrowing are often triggered by respiratory infections or allergens. Nearly all asthmatics have exaggerated airway narrowing in response to a variety of bronchoconstrictor agents, a phenomenon that can be documented by measuring changes in lung function following inhalation of the pharmacologic agent methacholine in the pulmonary-function laboratory. Several pathological abnormalities, including airway inflammation and airway remodeling (thickening of the smooth muscle layer, increased epithelial cell mucus content, and subepithelial fibrosis), are commonly seen in asthma [1]. Although some of the underlying mechanisms have been elucidated in recent years, much more needs to be done to gain a clearer understanding of the many genetic and environmental factors that contribute to the development of various forms of asthma. Asthma prevalence and morbidity has increased dramatically in recent decades, and there is an urgent need for better approaches to prevention and treatment. Some investigators have begun to use DNA microarrays to advance our understanding of asthma [2-4]; their results demonstrate that microarrays can accelerate the discovery process and suggest that functional genomics may soon help lead to a more complete understanding of the molecular nature of asthma.

Identifying differentially expressed genes in animal models

Microarrays are often used as a high-throughput tool for the identification of differentially expressed genes, and they have been used to study animal models of allergen-induced asthma. Zou *et al.* [2] used arrays containing 40,000 human cDNA probes to analyze lung gene expression in a monkey model. They identified 149 genes whose expression was changed by at least 2.5-fold at 4, 18, or 24 h after inhalation of *Ascaris suum* antigen, or 24 h after treatment with interleukin 4 (IL-4), a cytokine implicated in asthma pathogenesis. These differentially expressed genes included several novel genes, together with many for which a role in asthma pathogenesis had been implicated in previous studies, such as chemokines and other inflammation-associated genes, and matrix proteins and proteases likely to be involved in airway remodeling. RT-PCR analysis of a subset of the genes identified in the microarray experiments verified that most were differentially expressed, often to a greater extent than was estimated from the microarrays [2]. Technical issues, including limited sample size and a dependence on interspecies hybridization, probably account for the relatively small number of differentially expressed genes detected in the microarray experiments; nonetheless, this study demonstrates that microarrays are useful for describing patterns of gene expression in asthma models and for identifying novel candidate genes for further study.

More recently, Zimmermann and colleagues [3] examined gene expression in two widely used murine models of allergic asthma. Mice were challenged with intranasal ovalbumin or *Aspergillus* extract or a control and lungs were harvested 18 h later. Lung gene expression was analyzed for two or three mice per treatment using oligonucleotide microarrays with 12,422 probe sets (Affymetrix U74Av2 GeneChips). Each

allergen caused a two-fold or greater increase in the expression of genes recognized by roughly 500 probe sets ($p < 0.05$); this represents somewhat fewer than 500 distinct genes because of the redundancy of the probe sets. Nearly 300 genes were increased by both allergens and were therefore considered to represent 'asthma signature' genes [3].

Of the hundreds of differentially expressed genes, the investigators focused their attention on three functionally related genes that were not previously known to be involved in asthma pathogenesis but were induced in both asthma models [3]. These genes, the cationic anion transporter CAT2 *(SLC7A2)*, arginase I and arginase II, play a role in the metabolism of L-arginine: CAT2 transports L-arginine, and arginase I and arginase II convert L-arginine to L-ornithine. Increases in expression of these genes might be expected to result in increased production of L-ornithine. This could be relevant to airway remodeling, as L-ornithine is a precursor of polyamines such as putrescine, spermidine and spermine, which help control cell proliferation, and of proline, a major constituent of collagens.

The investigators took several approaches to validating the microarray data. First, they used northern blot analysis to confirm that the arginases (especially arginase I) were strongly upregulated in both allergic models and in other models of asthma induced by administration of the cytokines IL-4 and IL-13. Second, they performed *in situ* hybridization and found intense arginase I staining in macrophages from the lungs of allergen-challenged mice. Third, they showed that both arginase enzymatic activity and levels of putrescine were increased in the lungs of allergen-challenged mice. Finally, they showed higher expression of arginase I in lung macrophages and airway epithelial cells from humans with asthma than in control subjects. Although further work will be required to establish whether arginase plays a causal role in asthma pathogenesis, this study provides a fine illustration of how microarray data can be used to inspire novel areas of investigation.

Other publicly accessible microarray datasets [5] provide more information about the pattern of arginase I expression in different lung-disease models. Arginase I expression was increased in a model of allergic asthma triggered by a different allergen, ragweed pollen, lending further support to the idea that arginase I induction is a general feature of allergic airway disease. Another publicly available dataset [5] documents gene-expression changes in lungs following treatment with bleomycin, an agent that induces lung fibrosis in some susceptible strains of mice. Arginase I expression was increased following bleomycin treatment of fibrosis-susceptible C57BL/6 mice. Although the overall pattern of gene expression was similar in susceptible C57BL/6 and resistant BALB/c mice, bleomycin had little or no effect on arginase I expression in BALB/c mice [5]. This observation is consistent with the possibility that arginase I is involved in both allergen- and bleomycin-induced fibrosis, and suggests that strain-specific differences in arginase I expression might help to determine susceptibility to bleomycin-induced fibrosis.

Microarrays can identify large numbers of genes that are differentially expressed in asthma models and there is a clear need for creative strategies to allow investigators to identify which of these genes play key roles in asthma pathophysiology. One study, by Karp and colleagues [4], provides an outstanding example of the combined use of microarray and genetic mapping data for asthma-gene discovery. These investigators had previously shown that strain-related differences in allergen-induced airway hyperreactivity to acetylcholine could be mapped to two loci on chromosome 2 [6]. Oligonucleotide arrays were used to measure lung gene expression in susceptible (A/J) and resistant (C3H/HeJ) inbred strains and in genetically heterogeneous mice produced by interbreeding of these two strains. A total of 739 genes were differentially expressed between the inbred susceptible and resistant strains, but only 21 genes had expression patterns that correlated with susceptibility in the genetically heterogeneous mice. One of these genes, *C5*, mapped to one of the previously defined loci on chromosome 2 [4,6]. *C5* encodes complement factor 5, and the A/J mouse strain carries a null mutation in the *C5* gene. Additional experiments found correlations between the *C5* genotype and airway responsiveness in larger groups of mice and also demonstrated the effects of C5 on the production of IL-12, a cytokine that is known to have a role in asthma pathogenesis [7]. This work illustrates the power of combining genomic and genetic data in the search for asthma candidate genes.

Towards the clinic

As microarray technology is more widely applied to asthma models, we can look forward to the identification of more genes involved in pathogenesis. Microarray analyses of the effects of targeted interventions, such as transgenic overexpression of cytokines or gene targeting, could help to elucidate the contributions of specific molecules and pathways. Although whole-lung expression profiling has been shown to be valuable for the investigation of asthma and other lung disease models [2-4,8], there are undoubtedly important cell-type-specific effects that are difficult to detect in complex mixtures of cells. Microarray analysis has shown that airway epithelial and smooth muscle cells and lung fibroblasts all responded to the asthma mediator IL-13 with extensive changes in gene expression, but the specific genes affected in each cell type were almost completely different [9]. Approaches such as laser-capture microdissection and flow-cytometric sorting, in combination with emerging nucleic acid amplification methods and more sensitive detection methods, should be useful for dissecting the contributions of specific cell types to asthma pathogenesis *in vivo*.

We can also expect to see microarrays being deployed for studies of people with asthma. Recent microarray studies of human cancers show the potential utility of microarrays for disease classification and prognosis and suggest that microarray data may be useful in selecting appropriate therapies [10-12]. Asthma microarray studies will be challenging because of the requirement for recruiting large, clinically well-characterized cohorts of subjects and the difficulties inherent in obtaining suitable tissue for study. As these obstacles are overcome, microarray-based approaches may help identify distinct classes of asthma, something that has proved difficult using conventional clinical criteria. Comparing gene expression patterns in classes of asthmatic individuals with those in various animal models may shed new light on asthma mechanisms and suggest focused therapeutic approaches for different forms of asthma.

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