Analyzing Gene Expression in Depression

The use of DNA microarray technologies has come a long way in the decade and a half since their introduction by Schena and colleagues in 1995 (1). In this issue, Sibille and colleagues (2) triangulate several techniques based on DNA microarrays to study transcriptional variation in major depressive disorder compared to normal controls. DNA microarrays assay a "snapshot" of the transcriptome (the composition of mRNAs being expressed) by measuring the concentration of mRNA molecules after conversion to DNA by a bacterial enzyme called reverse transcriptase. The technique can detect which genes vary their expression across different experimental conditions or disease states. By simultaneously measuring the expression of many genes in a tissue sample, the strategy takes an unbiased "discovery science" approach to identifying genes with altered expression profiles when compared to normal or control conditions. In psychiatry, microarray experiments have identified hundreds of molecules as novel candidates for further research and have seeded new directions to better understand the molecular basis of psychiatric illness. Below we describe the maturation of microarray-based science in brain tissue and place the Sibille et al. article in that context.

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Early arrays were fabricated by printing DNA fragments representing dozens of genes onto the surface of a glass slide and then hybridizing them with cDNA molecules derived from the mRNA in a sample of interest. Although the concept of hybridizing samples to surface-fixed DNAs has remained consistent, technological advances based on microelectronic circuits that detect the DNA hybridization allowed the simultaneous assay of tens of thousands of mRNA species at vastly improved sensitivities and accuracies. However, with massive improvements in assay density technology came analytical challenges in weeding through the large number of false positives and false negatives that are guaranteed to occur with the sheer number of transcripts assayed in any given experiment. Therefore, analytical techniques utilizing bioinformatics and complementary analysis methods have been continuously improving.

A seminal article by Subramanian et al. (3) added significant value to the array analysis field by formulizing a method (gene set enrichment analysis) to increase the value of results for functionally related genes that behave synchronously in the array data. This and other, similar approaches allowed us to layer our knowledge of biological systems—in other words, our knowledge of genes that work together in the same biological function—onto the mathematical assessments of changes in gene expression to further refine the reduction in false positive and false negative error rates. This approach works well if transcripts from the biological system of interest are contained within the sample one is analyzing, as is the case for yeast and bacterial cultures or other self-contained cell culture systems. However, in complex tissues like those from the brain, this approach is not always enough. In neuroscience microarray experiments, one may be analyzing data from a brain region that provides major afferent projections (for example, the serotonergic system in the raphe nucleus), but without simultaneous analysis of target regions that provide receptor and signal transduction systems, a large portion of the picture is missing. Some laboratories, including our own, have begun to use a broader

approach through complementary analysis of brain regions in an anatomical circuit. In the Sibille et al. article, the authors focus on analysis of the amygdala and anterior cingulate in postmortem tissue from subjects with major depression. Although this certainly adds value to understanding the molecular nature of interaction between anatomically distinct but connected brain regions, a large number of false positive is still guaranteed to occur because of the large number of assayed transcripts.

A second, even more powerful, approach to validating DNA microarray data is the use of independent techniques, which were also thoughtfully employed by Sibille et al. In today's microarray literature it is expected that some level of independent confirmation of findings will be employed. Often real-time polymerase chain reaction, in situ hybridization, or other independent assessments of gene expression are employed to confirm differential expression observed in array data sets. A third level of complementary strategies utilizes animal models to confirm *behavioral change* correlates with the observed expression changes. This approach can place transcripts found differentially expressed in human psychiatric illness into a context that tests their role in animal behavior. Our own laboratories have successfully used this approach to validate a role for fibroblast growth factors in mood regulation (4). Sibille et al. employed such an approach by crossing microarray analysis of amydgala and anterior cingulate tissue (implicated in major depressive pathology) obtained from human depressed subjects with analysis of orthologous tissue from a rodent model of depression (unpredictable mild chronic stress) in order to identify shared biological systems altered in both models. The authors further refined their observations by identifying which of the responsive systems were successfully normalized by antidepressant treatment of the animals. Interestingly, this left them with transcriptional variations that also correlated with depression severity in the human subjects. Such an approach allows biologically important results to emerge that would normally be buried in a sea of false positive findings or, alternatively, disregarded through the highly stringent statistical analysis required in "standalone" microarray experiments. Finally, a fourth level of studies involves the use not only of specific pharmacology aimed at the molecules of interest but also of direct genetic alterations allowing more causative inferences about the gene and behaviors in question.

The systems that were identified in the Sibille et al. study both confirm observations from prior studies and add a deeper understanding of the molecular signature of depression on the brain. The authors describe correlated expression profiles across brain regions (amygdala and cingulate cortex) in both human and rodent species, but they found correlated expression profiles across species only in the amygdala. The lack of correlation between species for the cingulate cortices may, as the authors discuss, be a result of significant evolutionary separation between species for this brain region, compared to a closer phylogenetic relationship for the amygdala. The specific systems implicated in the overall analysis were decreased oligodendrocyte gene expression in the amygdala and glial-neuronal communications. The Sibille et al. study establishes a new path for investigating the molecular nature of cell-level communication systems that appear to be dysregulated in depression.

References

1. Schena M, Shalon D, Davis RW, Brown PO: Quantitative monitoring of gene expression patterns with a complementary DNA microarray. Science 1995; 270:467–470
2. Sibille E, Wang Y, Joeyen-Waldorf J, Gaiteri C, Surget A, Oh S, Belzung C, Tseng GC, Lewis DA: A molecular signature of depression in the amygdala. Am J Psychiatry 2009; 166:1011–1024
3. Subramanian A, Tamayo P, Mootha VK, Mukherjee S, Ebert BL, Gillette MA, Paulovich A, Pomeroy SL, Golub TR, Lander ES, Mesirov JP: Gene set enrichment analysis: a knowledge-based approach for interpreting genome-wide expression profiles. Proc Natl Acad Sci USA 2005; 102:15545–15550

4. Akil H, Evans SJ, Turner CA, Perez J, Myers RM, Bunney WE, Jones EG, Watson SJ; Pritzker Consortium: The fibroblast growth factor family and mood disorders. Novartis Found Symp 2008; 289:94–96

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All authors report no competing interests.