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Microarray-Based Expression Profiling of Normal and Malignant Immune Cells

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Recent advances in gene microarray technology have facilitated global analyses of gene expression profiles in normal and malignant immune cells. Great strides have been made in our understanding of molecular differences among various types of immune cells, the process of T and B cell activation, and the genomic changes that convert normal cells to malignant ones. Genomic analysis has become a crucial aspect of cancer classification, diagnosis, therapy, and prognosis. This technology has the potential to reveal the comprehensive transcriptional alterations that dictate fundamental biological processes such as signal transduction in response to specific stimuli, cell growth, differentiation, and apoptosis. While reaping the benefits of genomic analyses, it is important to realize its limitations with respect to accuracy of interpretation, reproducibility, and signal detection. It is crucial to optimize signals for individual probe-target pairs and to develop a uniform set of criteria for data analyses. The development of a public-access database of results from individual laboratories will pave the way for identifying discrepancies and advancing scientific breakthroughs. (*Endocrine Reviews* 23: 393–400, 2002)

I. Introduction

OVER THE PAST decade, the development of microarray technology has opened doors to a treasure of information about patterns of gene expression in diverse biological systems, allowing scientists to channel this technology to address questions in virtually any field of biology. It has the potential to systematically and comprehensively quantify entire genomes in the foreseeable future. DNA arrays enable the simultaneous analyses of expression of thousands of genes, making it possible to evaluate expression profiles in a temporal fashion within a single sample, or to compare profiles among samples. The power of this technology comes from the enormous amount of data it generates, although thus far it has been difficult to utilize the technology to its full potential because data-mining technology has not reached the same level of sophistication. To fully exploit this technology in the future, it will be crucial to construct standardized public databases that can sort and store the enormous quantity of data for reference and comparison for scientists worldwide.

A. Overview of methodology

Various technologies have been developed to produce ordered arrays of several thousand DNA samples on a solid substrate. The DNA samples may consist of either cDNA fragments spotted by robots, or of oligonucleotides directly synthesized on the silicon substrate using photolithography. The former are typically more easily fabricated in individual research laboratories and allow for development of custom-made arrays with the genes of choice spotted on them. The more widely used oligonucleotide arrays are those synthesized and marketed by Affymetrix, Inc. (Santa Clara, CA). Regardless of technology used in their construction, the immobilized DNA serves as a hybridization target of fluorescently tagged cDNA prepared from mRNA isolated from the sample of interest. The fluorescence intensity at each spot is a measure of the abundance of the corresponding gene in the sample. The scanned digital image of the array is processed through sensitive algorithms to obtain quantitative values for the magnitude of expression of each mRNA represented by a complementary DNA on the array. Commercial arrays

Abbreviations: ALCL, Anaplastic large cell lymphoma; ALL, acute lymphoblastic leukemia; ATRA, all-*trans*-retinoic acid; BTG, B cell translocation gene; $[Ca^{2+}]$, intracellular calcium concentration; CLL, chronic lymphoid leukemia; CML, chronic myeloid leukemia; DLBCL, diffuse large B cell lymphoma; GATA2, GATA binding protein 2; LAT, linker for activation of T cells; PIASy, protein inhibitor of activated STAT y; PKC, protein kinase C; SH2, Src homology 2; SLP-76, SH2-containing leukocyte protein 76; STAT, signal transducer and activator of transcription; TCR, T cell receptor; TRIM, TCR-interacting molecule.

containing specialized sets of genes that meet specific research purposes are available from various sources. These include individual arrays representing genes relevant to toxicology, cancer, signal transduction, cytokines, apoptosis, development, or proliferation, and may be more manageable than comprehensive genome arrays. There has been a recent surge in genome-based investigation, and as the technology is refined, it may well become a critical component of clinical diagnosis and disease management (1–3).

B. Applications of microarray technology

One can envision several applications of microarray technology in basic research, including functional characterization of genes identified via the genome project based on their spatial and temporal expression patterns, expression clustering to identify genes associated with common metabolic and physiological processes, identification of genes regulating specific cellular pathways or responses to specific stimuli, toxicogenomics, and pharmacogenomics. Several of these applications have the potential to address important clinical issues such as identification of genes related to abnormal biological behavior, characterization and use of gene profiles for prediction of disease progression, prognosis and treatment strategies, and understanding the genetic basis of multigenic diseases such as diabetes, atherosclerosis, and cancer. Gene array analysis has facilitated important breakthroughs in our understanding of the molecular basis of cancer, especially with respect to cancer classification and diagnosis, early detection of metastases and relapses, acquired resistance, and development of novel molecular targets for chemotherapy (4). Future advances in this technology may revolutionize medicine by allowing an analysis of the complete genetic profile of patients, empowering diagnosis and therapy.

Here I briefly review how microarray technology has been instrumental in advancing our understanding of immune cell properties, lymphocyte activation, and lymphoid malignancies.

II. Use of Microarrays in Immune Cell Characterization

Microarray analysis is particularly advantageous in the studies of immune cell function and classification. Distinguishing among various subtypes of T and B cells, and pinpointing their functions, is facilitated by analysis of the genes they express. The pathways of immune cell responses to diverse stimuli are also best understood by evaluating the alteration in gene expression triggered by individual agents. The transition from normal lymphocyte to malignant lymphocyte is also a genetic event, which has been studied in the context of genomic changes. Among all the cancers studied for their genome profiles, leukemias have served as informative models for developing genome-based diagnosis.

A. Characterization of T and B cell lineages

It has been confirmed that T and B cell lineages are derived from a common precursor and share a similar pattern of gene expression (5). A number of studies have used subtractive hybridization techniques to identify genes differentially expressed in T and B cells that could account for their remarkably different functions (6, 7). These studies identified T cell-specific genes including those encoding the cytotoxic T lymphocyte-associated antigen family of cell surface proteins. Microarray technology allows addressing the same issues about the molecular basis of differential T and B cell function in a much more sensitive and efficient format. A global view of a multitude of genes in an array enables one to assess the regulation of individual genes in relation to others, and to understand pathways for lymphocyte activation, growth, and malignancy. A compilation of microarray data from different laboratories has confirmed the validity of the approach with the identification of specific genes, a number of which were previously identified via independent techniques. Among the B cell receptor signaling molecules are the novel tyrosine kinase Lyn, Src homology 2 (SH2)-containing inositol phosphatase, non-receptor tyrosine kinase Btk, and protein kinase C (PKC)δ, whereas T cell receptor (TCR) signaling-specific genes include *Zap-70, SH2-containing leukocyte protein 76* (*SLP-76*), *junB*, and *PKC*ϵ and -τ. An interesting observation was that by 48 h post T cell activation, a majority of genes regulated within 8 h are back to baseline expression, emphasizing the importance for temporal monitoring of molecular changes during lymphocyte activation (8).

Alizadeh and colleagues (9, 10) have developed a "lymphochip," which is a micorarray of more than 10,000 individual human cDNAs, representing genes of known and unknown function expressed in lymphocytes. Several researchers have used the lymphochip to define immune cell lineages and differentiation stages. Alizadeh *et al*. (9) and Shaffer *et al*. (11) have developed the concept of "immune cell signatures," which define individual cell lineages based on the specific regions of the lymphochip that show selective hybridization, analogous to a gene expression map for that cell lineage. T cell signatures, for example, contain genes corresponding to the TCR, specific signaling proteins, and surface markers [TCRα and -β, CD3δ and CD3γ, linker for activation of T cells (LAT), TCR-interacting molecule (TRIM), CD2 and CD5]. A B cell signature is defined by genes for the B cell receptor, other cell surface receptors (CD19, CD20, CD22, CD27, CD40, and CD45), and transcription factors such as Oct-2 and the B cell lineage commitment factor Pax 5. Going a step further, they have demonstrated that the lymphochip can identify cells within a lineage that represent different developmental/differentiation stages. Thus, B cells at the germinal center can be distinguished from plasma cells, and proliferative cells can be identified by their own distinct signature, represented by genes involved in cell cycle regulation, DNA synthesis, and protein translation. Similarly, cellular signaling pathways may be distinguished from one another based on the distinct set of genes they regulate. This is a rather elegant use of microarray technology and has the potential to develop into a powerful tool to classify different cell types, differentiation/proliferation stages, or signaling pathways in the foreseeable future. However, it may be difficult to compare expression data generated using different microarrays or generated in different laboratories unless attempts are made to develop a comprehensive public database and methods are devised to compare signals derived from different laboratories and using different meth-

odologies. Discrepancies between laboratories should be identified and resolved if the conclusions are to be validated.

B. Characterization of immune cell activation

A comparison of gene profiles during B and T cell activation has been fundamental to our understanding of their functions, in addition to providing valuable information regarding functional roles of individual genes in the process. Using the microarray approach, Brunet *et al*. (7) and Glynne and colleagues (12, 13) found remarkable similarities in activation-evoked gene regulation profiles in B and T cells. Insights into the mechanisms of B cell tolerance and activation have been obtained by comparing gene profiles in these cells (13). Early response to activation involved regulation of genes that were unchanged in response to tolerance-inducing self-antigen. There was an up-regulation of mitogenic oncogenes c-*myc*, *lymphocyte-specific interferon regulatory factor*, and the bcl-2-like antiapoptotic gene *A1*, and down-regulation of antimitotic genes *lung Kruppel-like factor* and *Id3*. Comparison of responses in resting and tolerant B cells revealed differential regulation of the few genes that may maintain B cell anergy, including the transcriptional repressor NAB2.

Oligonucleotide microarrays were used by Ellisen *et al*. (14) to define sequential changes during concanavalin A-induced human T cell activation. The global patterns of gene expression were sorted into self-organizing gene maps. They have identified eight clusters of genes with similar temporal induction patterns, reflecting broadly related functional roles. The authors have attempted to organize induced genes into functional categories, although they are quite arbitrary. Despite considerable overlaps, it is apparent that the most rapidly induced genes (4–12 h; representing three temporal clusters as defined by the paper) included a disproportionate representation of transcription factors and nuclear cofactors [Rel B, nuclear factor-κB, ATF3, signal transducer and activator of transcription (STAT)-1, STAT5, and cAMP response element modulator), growth factors (several interleukins and their receptors, vascular endothelial growth factor, interferon γ), and intracellular signaling molecules (myristoylated alanine-rich C kinase substrate, Endoglin, Bcl-3, the serine/threonine kinase Pim-1, protein tyrosine phosphatase-1B, tumor necrosis factor receptor-associated factor-1)]. Slower inductions (24–48 h; representing three clusters) were observed for genes encoding metabolic and nucleotide synthesis enzymes (including γ glutaryl transferases, mevalonate-, pyruvate- and phospho glycerate-kinases, fructose 1,6-bisphosphatase, RNA polymerase II, ribonuclease, acid phosphatase, AMP deaminase, thymidylate synthase, DNA polymerase I, and thymidine kinase) and structural proteins (tubulin, porin). Repressed genes (representing two clusters) corresponded to all categories, including transcription factors (Jun B, c-Fos, nuclear factor-E2, YY1 repressor), intracellular signaling molecules (calmodulin kinase II, PKCβ-I and -II), integrins (β7, α4, intracellular cell adhesion molecule-3), and metabolic enzymes (alcohol dehydrogenase, dihydropyrimidine dehydrogenase, transketolase, catalase). These data suggest that targets of early mitogenic regulation (transcription factors) are capable of regulating genes at later time points that may have a more direct role in cellular processes. These data are largely consistent with previously reported induction profiles of genes known to be involved in T cell activation; however, some discrepancies exist in their time of peak induction. This observation emphasizes the importance of monitoring global gene induction profiles at close time intervals, which can only be possible using high-density microarray analysis. This is a valuable preliminary set of data, which will need to be further supported and refined to achieve a better understanding of B and T cell activation and function.

III. Microarray-Aided Cancer Diagnosis and Identification of Prognostic Markers

Cancer is a family of primarily genetic diseases in which altered gene expression is the main molecular characteristic. Therapeutic efficacy and clinical prognosis are highly variable even for cancers that are classified under the same category based on symptomatic and conventional diagnosis. Currently, in an effort to account for their variable clinical behavior, apparently similar cancer classes are being evaluated for subtle differences in gene expression. Microarray technology has facilitated unforeseen advances with this approach; in fact, it has been proposed that genomic profiles can serve as a diagnostic tool, and mircoarray technology has even led to some refinement in tumor classification (15). Here, I focus on the latest developments on the applications of microarray technology in lymphoma and leukemia diagnosis and therapy.

A. Cancer classification

Scientists are struggling to determine the fundamental differences that distinguish normal and malignant lymphocytes. Microarray analysis has allowed a systematic analysis of gene expression profiles in normal *vs.* malignant cells in a model of B cell lymphoma, and has identified key deviations from normal differentiation in malignant cells (9). Early experiments with leukemias demonstrated the success of microarray analysis in distinguishing acute lymphoblastic leukemia (ALL) from acute myeloid leukemia (16). A set of 50 genes was sufficient to classify a leukemia into one of these categories. In addition, it was also possible to use self-organizing maps to predict additional classes/groups of leukemias, and in one study this approach was used to sub classify ALLs into T cell and B cell ALL. More importantly, microarray analysis allowed detection of a misdiagnosis, in which a rhabdomyosarcoma was classified as a leukemia based on clinical presentation (17). Using microarrays, Chen *et al*. (18) have identified CD58 as a cell surface antigen that is abundantly overexpressed in most forms of ALL, and they have demonstrated that it can be reliably used for detection of minimal residual disease.

Non-Hodgkin's lymphomas generally classified as diffuse large B cell lymphoma (DLBCL) have a very diverse clinical course, with only 50% of the patients achieving remission. Microarray analysis and hierarchical clustering was used to analyze genes from 9 follicular lymphomas, 11 chronic lymphocytic leukemias, and 42 DLBCLs. The relatively sluggish chronic lymphocytic leukemias and follicular lymphomas

clustered next to resting B cells, whereas DLBCLs could be clustered into two separate categories based on genomic signatures generated using the lymphochip (19, 20). The lymphochip was originally designed to enable classification of Non-Hodgkin's lymphomas; hence, it contains genes from a cDNA library of germinal center B cells (9). One cluster resembled the germinal center B cell signature, whereas the second cluster resembled the signature presented by activated B cells. Clinical data revealed that the classification correlated with survival rates, with germinal center B cell-like DLBCLs having a good 5-yr survival record and the activated B cell-like DLBCLs having an overwhelmingly poor long-term prognosis. These studies demonstrate that genomic analysis is successful not only in classifying leukemias and lymphomas, but may also improve diagnosis and predict clinical progression of disease. Furthermore, efforts are underway to identify key/feature genes that may be used for the same purposes (21), and it may soon be possible to obtain much information about a tumor by molecular analysis of a few genes rather than an entire microarray. This, of course, would be cost effective if it could indeed be reliable.

B. Diagnostic markers for cancer

One long-term goal of microarray analysis should be the development of diagnostic markers of individual subtypes of cancers and their adoption for routine diagnosis in the clinic. Advanced stages of chronic myeloid leukemia (CML) are associated with blast crisis and poor prognosis. Microarray technology was used to evaluate gene expression changes associated with stage progression of CML, and the gene encoding protein inhibitor of activated STAT y (PIASy), an inhibitor of STAT proteins, was found to be consistently down-regulated. Further studies revealed that enforced PIASy expression in CML cell lines induces their apoptotis (22). In the model of chronic lymphocytic leukemia (CLL), a set of genes was identified whose expression levels correlate with clinical staging and patient survival (23). Microarray analyses have defined at least two types of memory B cell-derived CLL, with varying tendency for disease progression (24). Those genes that were abundantly expressed in B-CLL relative to normal human tissues were those previously known to be B cell specific. These include genes encoding proteins involved in B cell antigen receptor signaling [including phospholipase C-γ2, SH2 containing inositol phosphatase, Grb2, ERK1, JunB, ornithine decarboxylase, and B cell translocation gene (BTG)-1]. Parallel analysis of expression levels for each gene and patient survival suggested that reduced expression of genes coding for IL-1β, IL-8 and L-selectin correlate with low survival. Clinical disease stage is an established predictor of patient survival; hence, it was important to determine whether any genes are differentially expressed during different stages of leukemia. Higher clinical stage correlated with decreased expression of IL-1β-, IL-8-, and early growth response 1-encoding genes. This study is a significant step toward identifying a key set of genes that may be sufficient to evaluate cancer progression and survival chances.

Cytogenetic abnormalities are highly prevalent in CLL, the most common ones being trisomy 12, deletion in 13q, 14q, and 11q. Loss of the 11q region has been reported to occur frequently and defines a new subset of B cell CLL associated with poor survival statistics (25, 26). In a microarray-based study (27) comparing gene expression profiles in 34 cases of CLL and normal B lymphocytes, 78 critical genes with differential expression properties were identified. These included genes for cell surface antigens, transcription factors, oncogenes, and protein kinases. The poor prognosis in some cases of CLL associated with 11q23 deletions has been attributed to disrupted down-regulation of genes including those encoding poly ADP ribose polymerase, the splicing associated factor DEK, nucleoplasmin, and the antiapoptotic protein Mcl-1, and enhanced up-regulation of genes encoding CD25, bcl2, SLP-76, and signal-transducing adaptor molecule. Aalto *et al.* (27) suggest that these gene alterations result in the loss of tumor suppression and apoptotic regulation in cases of CLL associated with 11q23 deletion, contributing to the poor prognosis. The authors have made a correlation between expression of certain genes and clinical staging of CLL. Advanced stages of B-CLL were linked to higher expression of proliferative genes such as those coding for T cell factor and granzyme K and the cell surface antigen CD14. In another study, Wellmann *et al*. (28) demonstrated that the gene *clusterin* is specifically expressed in anaplastic large cell lymphoma (ALCL)-derived cell lines but not in other lymphoma-derived cell lines. Their observation was confirmed by immunohistochemical screening of 198 primary lymphomas for clusterin expression, where all 36 ALCL cases demonstrated clusterin expression and only 2 non-ALCL cases expressed detectable amounts of clusterin. A set of genes was found to be overexpressed in multiple myeloma-derived cell lines when compared with autologous lymphoblastoid cell lines derived from the same patients. Significant differences were found in genes encoding the oncogene Tyr3 tyrosine kinase receptor, the heparin binding epidermal growth factor-like receptor, the G protein-coupled thrombin receptor, and the oncogenic Wnt signaling protein Frz-B. These data were confirmed using purified primary polyclonal plasma cells from various sources (29).

C. Development of a comprehensive leukemia database

A compilation of microarray data from various laboratories is necessary to allow identification of overlapping gene profiles or signatures that may shed light on the genetic basis of specific cancers. The consolidated data would accelerate identification of genetic markers for the diagnosis, staging, and prognosis of cancers. Ideally, such a database would be integrated and dynamic, compiled with information from various laboratory and clinical sources, and continually updated with follow-up data documenting clinical course, response to therapy, survival times, and complications (if any). Such a nationwide effort has begun in Germany under the auspices of the German Leukemia Research Project (30). The parameters taken into account include cytogenetics, immunophenotyping, morphology, molecular genetics, microarray data, and clinical information. This database can serve as an example for setting up and maintaining international databases and for developing universal diagnostic and therapeutic criteria.

D. Experimental limitations

These examples bring our attention to other critical issues pertaining to experimental design during microarray analysis. The most significant decision by far is the choice of a reference control. In most cases, cancer cells are compared with tissue/cell type-matched normal samples, but in the case of leukemias and lymphomas, in view of the diversity of malignant phenotypes and the variability within a subclass, it is crucial to use appropriate control cells for the study. Another highly debated issue is the validity of the use of transformed cell lines as models representing the genomic make of clinical cancers. In support of this approach, there is substantial evidence that cancer cell lines largely maintain the same gene expression pattern that the original cancers from which they were extracted did. A pioneering study in this regard was the typing of 8000 genes in 60 cancer lines (designated NCI60 panel) by the Developmental Program of the National Cancer Institute (31). It was demonstrated that cell lines from many tissue types (*e.g.*, leukemias, melanomas, colon cancer, and ovarian cancer) were more closely related to lines from the same tissue rather than other tissues, thus confirming that the salient features of their original sources were maintained.

A common limitation of the technique is the lack of reproducibility when experiments are conducted in two independent laboratories. In most cases, this has been attributed to the use of different sets of microarrays representing different sets of genes, and when a relevant gene is identified by one group but not by the other, it may be a simple matter of that gene not being represented in the array used by the second group. It may also reflect different time points selected, or different origins of the cancers. It is absolutely imperative that all laboratories cross-check for expression patterns of genes that other laboratories identify, to ensure that such discrepancies are not the result of varying data analysis, sensitivity, or selection of complementary probes. We need a more consistent and reliable pool of data from multiple laboratories if we are to make significant advances in diagnostic applications of the technology. In most cases, individual laboratories test a subset of genes by conventional Northern hybridization or quantitative RT-PCR procedures, but it is not feasible to do these analyses on all genes; that would defeat the purpose of microarray analysis.

IV. Microarray Analysis of Gene Expression in Response to Various Stimuli

As the Human Genome Project nears completion, thousands of genes have been identified and sequenced but are in need of functional characterization. Microarrays are the most versatile and high-throughput tools for gene expression analysis and functional genomics. There are unlimited applications of microarray technology for the identification of genes relevant to cell growth, development, differentiation, disease, aging, *etc*. Multivariant gene clustering allows extraction of correlated patterns of expression, which have been shown to represent groups of genes of related function. Deciphering the molecular details of signaling pathways is a crucial step in understanding basic physiological processes and disease pathology. Significant progress has been made in understanding the mechanisms and genes involved in lymphocyte activation in response to diverse stimuli, a summary of which is provided here.

A. Glucocorticoid-mediated changes in gene expression

Glucocorticoids play a crucial role in immune function and T cell selection, primarily by regulating expression of key lymphoid genes, which in turn induce profound biochemical and morphological changes in lymphocytes. Early studies have identified proteins that modulate metabolic processes as being sequentially affected by glucocorticoids via a mechanism that requires new protein synthesis (32). It was demonstrated that glucocorticoids coordinately regulate expression of specific gene networks involving a limited number of target genes, rather than effecting a global transcriptional change (33). Subtractive hybridization approaches were used to identify several genes induced by glucocorticoids in murine thymoma WEHI-7TG cells, including the VL30 retrovirus-like element, calmodulin, mitochondrial phosphate carrier protein, and the chondroitin sulfate proteoglycan core protein (34, 35). Differential display techniques were employed to identify differentially expressed cDNA sequence tags from WEHI 7.2 cells in response to glucocorticoids (36). In a recent study, glucocorticoids were shown to up-regulate IL-7 receptor α, and thus enhance IL-7-mediated signaling, to positively influence T cell survival and function and antagonize TCR signaling (37). Microarray-based gene expression profiles of peripheral blood mononuclear cells showed glucocorticoid-induced expression of genes encoding chemokine, cytokine, and complement family members, and scavenger and Toll-like receptors, as well as a repression of immune-modulatory genes (38). Glucocorticoids also showed opposing regulatory effects on certain genes depending on the activation state of T cells, suggesting a role as immunopermissive agents. Superimposed inhibitory and stimulatory effects were observed on gene clusters involved in inflammatory and apoptosis-related pathways. Several researchers have focused on microarray-based identification of genes involved in GC-evoked thymocyte apoptosis (see *Section E*).

B. Signaling in calcium-mediated lymphocyte activation

Calcium is an essential mediator of lymphocyte activation; however, there is great ambiguity with respect to its molecular pathway. Increase in intracellular calcium concentration ($[Ca^{2+}]$) is an early and essential event during T and B cell activation, and is accompanied by activation of several transcription factors and alterations in gene expression that eventually modulates immune function, cell proliferation, differentiation, and death (39, 40). Comparing normal T cells to calcium influx-deficient T cells in microarray analysis using the lymphochip, Feske *et al*. (41) studied global patterns of phorbol ester-mediated calcium-dependent gene expression. Both Ca^{2+} influx-dependent gene induction and repression were observed and categorized into different expression patterns. The data allowed for segregation of genes whose expression may be influenced by overlapping calcium-independent pathways (*cAMP response element modulator, Fra-2, NOT, STAT-4, granzyme H, IL-2, IL-8, CD30 ligand, or Fas*

ligand), as evidenced by moderate induction by phorbol esters in calcium influx-defective cells, and those that were predominantly unchanged by phorbol esters in calcium influx-defective cells [*protein tyrosine phosphatase-1B, signaling lymphocyte activation molecule, SLP-76-associated protein, p70S6 kinase, cyclin D2, cyclin-dependent kinase (cdk)6, nuclear factor regulated by IL-3-A*]. In addition, genes that were repressed in a Ca^{2+}-dependent manner were also identified. Most of the calcium influx-dependent genes were also calcineurin dependent, indicating that the major Ca^{2+}-dependent pathway for T cell activation required calcineurin as a mediator. This study provides valuable insight into the mechanisms of T cell activation and highlights the potential of microarray technology in delineating complex cellular pathways in other systems.

C. Stress responses

A similar approach has been proposed for studying genomic responses to stress at a molecular level. Different triggers of stress such as starvation, oxidative stress, toxicity, and irradiation may affect a distinct set of genes and may alter their expression. Microarray technology can provide a comprehensive analysis of stress responses and can identify crucial, commonly involved genes that can serve as molecular markers for exposure to stress. Radiation-induced changes in gene expression have been studied by Amundson *et al*. (42) in human myeloid lymphoma cells, and have applications in developing more effective radiotherapy protocols as well as in identification of sensitive biomarkers for accidental exposure.

D. Cell growth and differentiation

Cell growth and differentiation pathways involve a precise balance of opposing growth regulatory signals mediated by altered expression of a multitude of genes. Microarrays have also been used to understand alterations in gene expression profiles during cell cycle progression with rather conflicting results (43, 44), which emphasize the inherent variability in multiple experiments and the importance of a sound experimental system. There have been significant efforts to utilize microarray technology to understand the growth-promoting or differentiation pathways triggered by specific agents. A classic example is the all-*trans*-retinoic acid (ATRA)-induced differentiation of granulocytes and acute promyelocytic leukemia cells. Microarray analysis revealed a coordinated up-regulation of genes suppressing proliferation (the cdk inhibitor $p21^{WAFI}$, *growth arrest and DNA damage-153*, *BTG-1*, and the gene for Src-like adaptor protein) and a parallel down-regulation of cell proliferative genes [c-*myc*, transcription factor encoding GATA-binding protein-2 (*GATA2*), *cyclin A, cyclin B1*] in response to ATRA in acute promyelocytic leukemia cells (45). ATRA treatment also induced antiapoptotic genes (*e.g.*, Bcl2-related gene *A1*), which allowed progression toward differentiation, via parallel induction of differentiating genes such as that coding for the CCAAT/enhancer binding protein ϵ.

The protooncogene product, c-Myc, is a well-characterized growth-promoting transcription factor that has also been implicated in modulating apoptosis in certain cell types (46, 47). To understand c-Myc action, Myc-mediated transcription regulation of growth-regulatory genes has been extensively studied in different systems. In human B cells, microarray analysis revealed c-Myc target genes to include those encoding products involved in amino acid and protein synthesis (such as tRNA synthetases, ribosomal protein L3, and hydrogen carrier protein), transport, RNA splicing and transcription (splicing factor serine kinase, CCAAT-box binding factor, and immunophilin FKBP52), DNA synthesis (carbamoyl phosphate synthetase, phosphoribosylpyrophosphate synthetase subunit II, CTP synthetase, glutamine phosphoribosylpyrophosphate amidotransferase), lipid metabolism (apolipoprotein E receptor 2, fatty acid binding protein homolog, long chain acyl CoA synthetase), and signal transduction (thyroid hormone receptor, protein phosphatase PAC-1, fibroblast growth factor, fibroblast growth factor receptor 3), suggesting that c-Myc serves as a central regulator of growth control (48). Using primary human fibroblasts transfected with cDNA encoding the regulatable c-Myc and estrogen receptor ligand binding domain fusion protein, Coller *et al*. (49) identified genes regulated by c-Myc, which again emphasized the diverse action of c-Myc in cellular physiology, DNA and protein synthesis, metabolism, cell growth and cell cycle regulation, cell adhesion, and apoptosis.

E. Apoptosis

Apoptosis is a well-orchestrated process of cellular suicide involving precise transcriptional alterations of a multitude of genes. The complexity and cross-talk among various apoptotic pathways has eluded a clear understanding of the mechanism. Microarray analysis can now facilitate a comprehensive evaluation of the role of individual signaling pathways in mediating apoptosis and can also provide insights into how apparently distinct pathways mesh with each other to coordinate a balanced response. This is especially relevant when one considers the apparent paradox observed in different systems concerning the role of some genes in the process, *e.g.*, up-regulation of c-*myc* evokes apoptosis in growth factor-dependent cells, whereas c-*myc* down-regulation contributes to apoptosis in growth factor-independent lymphoblastic T cells (47, 50). A comparison of gene profiles in mouse B cells triggered to undergo apoptosis by two different stimuli, growth factor deprivation and PKC inhibition, allowed for the identification of common apoptosis regulatory genes that probably have an essential fundamental function in the process (51). Genes coregulated by both pathways included several expressed sequence tags of unknown function and other genes encoding transcription factors (induction of GATA2; repression of the helix loop helix transcriptional repressor STRA-13, and melanocyte-specific gene-related gene-1 and -3), growth regulatory proteins (induction of BTG-2 and Galectin-9), and proteases (induction of the metallo-protease CD156, and repression of the serine protease inhibitor SPI2).

In a similar approach, microarrays were used to compare alterations of gene expression in response to p53 in two parallel systems: a mouse myeloid cell line LTR6, which undergoes p53-induced apoptosis; and a human lung cancer cell line H1299, which undergoes p53-mediated growth arrest, but not apoptosis. These studies helped identify two distinct programs of p53 signaling, one leading to apoptosis and the other to growth arrest. Apoptosis-triggering genes

were identified, including those for apoptosis protease activating factor-1, small ubiquitin like modifier-1, and gelsolin (52), all previously known to be key regulators of apoptosis. Although these studies provided valuable insight into p53-dependent pathways for growth arrest and apoptosis, it is critical to realize that the comparison was made across species and organ systems – mouse myeloid cells *vs.* human lung cancer cells – thus limiting interpretative potential.

Tonko *et al*. (53) compared glucocorticoid-regulated gene expression profiles in proliferating *vs.* G1/G0 arrested human leukemic cells in an effort to identify those genes that mediate the apoptotic process. This was a reasonable approach because glucocorticoids have overlapping effects on growth arrest and apoptosis and it is difficult to distinguish between genes contributing to the two processes. The authors identified an apoptosis-evoking negative regulatory process that suppressed metabolic pathways critical for cell survival. This study also has some limitations, given that cell-cycle arrest was accomplished by conditional expression of the cdk inhibitor p16/INK4a, which itself may trigger a glucocorticoid-independent gene regulatory response and may not necessarily block upstream/parallel growth arrest pathways. The authors claim that the genes commonly regulated in both proliferating and growth-arrested cells reflect proapoptotic genes, which may not necessarily be true. In another study, genes regulated by glucocorticoids at early time points were compared with two different apoptosis-susceptible cell lines. The data reveal a role for glucocorticoid-mediated gene repression in apoptosis. Repressed genes predominantly included those with established functions in transcription, mRNA splicing, and protein synthesis (54). Our own unpublished studies using parallel sets of glucocorticoid-responsive and -nonresponsive cells (R. Medh and E. B. Thompson, in preparation) in oligonucleotide microarray analysis suggest that both glucocorticoid-mediated up-regulation and down-regulation modulate apoptosis of T lymphoblastic leukemia cells.

Microarrays provide a good tool to understand the relevance of apoptosis in physiology and disease. Disruption of normal physiological apoptosis has been implicated in the pathogenesis of cancer. In the model of mantle cell lymphoma, alterations in apoptotic pathways were detected by oligonucleotide arrays in lymph nodes of lymphoma patients when compared with nonmalignant hyperplastic lymph nodes, strengthening this hypothesis (55). Clearly, a lot remains to be learned about the genomic changes associated with fundamental cellular processes, and microarray technology promises to accelerate our understanding of these phenomena.

V. Conclusions

This review summarizes the recent advances in microarray technology and highlights some of the major insights it has provided into immune-cell characterization and function. One of the areas in which microarrays have pioneered rapid advances is in our ability to classify, diagnose, and predict clinical progression of lymphomas and leukemias. There is great potential for future identification of key molecular markers that will facilitate the design of tailor-made treatment strategies for optimal quality of life and survival. Rapid advances in basic research on the mechanisms of essential cellular processes and signaling pathways have become feasible, thanks to the ability to comprehensively and easily analyze gene alterations in a temporal fashion. There are several shortcomings of this technology, which must be dealt with promptly and openly to maximize the benefits and minimize costly setbacks.

Acknowledgments

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