Endocrine, *vol. 28, no. 1, 49–56, October 2005* 0969–711X/05/28:49–56/$30.00

DNA Microarrays

Recent Developments and Applications to the Study of Pituitary Tissues

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Many new techniques are rapidly being developed and applied to the study of normal and neoplastic pituitary. DNA microarrays are a uniquely efficient method for simultaneously assessing the expression levels of thousands of genes, identifying disease subphenotypes, and predicting disease progression. This article reviews the utility of DNA microarray-based tumor profiling including recent developments and applications to pituitary biology in order to demonstrate how these new techniques are providing insights about basic aspects, clinical knowledge, and pharmacologic knowledge of the pituitary gland and about pituitary tumors.

Key Words: Microarray; pituitary; pituitary adenoma.

Introduction

DNA microarrays consist of DNA attached to a surface in an ordered, predetermined fashion at extremely high density. More than 20,000 genes can be arrayed on the surface of a glass microscope slide *(1–3)*. The principle of microarrray analysis is that messenger RNA (mRNA) from a specific tissue or cell line is used to generate a labeled sample or target. This is then hybridized in parallel to a large number of DNA sequences in a specific order immobilized on a solid support *(1,4)*. Thus arrayed, thousands of transcripts can be detected and quantified at the same time. Microarrays are used to study gene expression profiles of normal tissues and tumors *(5–12)*.

Several recent advances in biology have contributed to our ability to generate high-density DNA arrays. One major advance in DNA chip technology is the ability to generate high-density DNA arrays *(4,13)*. Over 30,000 short expressed sequence tags (EST) of DNA sequences can be arrayed onto a thumbnail-sized glass chip at a rate of 800 spots a minute. Alternative methods of creating a DNA array include photolithography, fragment-based DNA printing, and the inkjet-based method *(14)*. Improved fluorescence labeling methods and powerful computing software were developed to analyze data generated from the vast number of hybridizations present on a single chip *(15–25)*.

Received June 2, 2005; Revised June 16, 2005; Accepted July 14, 2005.

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Basic Principles

DNA chip or microarray technology is similar to that underlying Northern and Southern blot analyses *(16)*. DNA microarray relies on the hybridization of a probe to multiple defined cDNAs or ESTs that have designated spots on a solid phase chip. The probe is usually a complex mixture of cDNA fragments generated from mRNA. The fluorescence-labeled probe fragments bind to their appropriate partners and the intensity of emission can be assessed by an argon ion laser *(1–4)*. Using this method, global gene expression can be compared in two populations *(17)*. The whole system necessary for DNA chip technology consists of an arrayer to spot the cDNAs onto a glass slide, a reader with a laser to measure fluorescent emissions, and computer software, both operational and informatic. This integrated system permits the simultaneous analysis of thousands of genes, rather than simply a comparison of only 10–20 genes, as in differential display technology, which involves systematically amplifying mRNAs by PCR and then distributing their termini on a denaturing polyacrylamide gel.

Another method that promises the same order of analysis as DNA chip technology is serial analysis of gene expression (SAGE) *(18,19)*. This method, also based on PCR, involves cutting cDNA with two different restriction enzymes to form ditags with two different primer sites, just nine base pairs from each other. These are ligated to form huge concatemers, which are then sequenced with PCR to form numerous short sequence tags. These tags have been cut at the same place with respect to the restriction enzyme site, so that they can be used to screen the database to identify the gene expressed. The number of tags present for each gene gives a quantitative evaluation of their expression. It is of note that the SAGE method is more technically demanding than chip technology and has been successfully utilized by only a limited number of research groups *(20,21)*.

Array Platforms

The two main forms of microarray are cDNA and oligonucleotide arrays *(20–25)*. cDNA arrays are composed of

PCR-amplified cDNA clones arranged on a non-porous surface. A typical cDNA array is printed onto a 30 mm by 15 mm glass microscope slide by a computer-controlled robotic cantilever arm, each spot being about 50–150 microns in diameter. cDNA clone sets of 15,000–20,000 mouse and 40,000 human genes are available for in-house arrays in addition to ready-made commercial chip sets. About 80% of all human genes can be queried on about two slides printed at high density. Other flexible and porous surfaces such as nylon membranes can be used as alternatives to glass *(1–4)*.

Oligonucleotide arrays are usually made from short 20–25 oligonucleotide bases. Presynthesized oligonucleotides can also be printed onto glass slides or by ink-jet technology *(14)*. One advantage of oligonucelotide arrays is that the sequence information is known to be sufficient to generate the DNA to be arrayed. Oligonucleotide arrays use the chemically synthesized oligonucleotide (about 25 base-pairs), which contains only the coding part of the sequence complementary to its mRNA transcript. Oligonucleotide arrays have the advantage of not requiring the laborious process of PCR amplification and clone-insert purification before arraying. It is relatively easy to prepare the array, because it is not necessary to generate thousands of DNAs. Furthermore, the most specific sequences of a gene can be used for the arrays, thus avoiding homologous sequences with other genes and specific variants. The disadvantage of short oligonucleotides is decreased hybridization specificity and reduced sensitivity. The use of longer oligonucleotides (50–100 base pairs) avoids these potential problems *(13)*. Technical issues, such as optimization of the slide surface for oligonucleotide attachment and the design of most specific oligonucleotide probe(s) for each gene, are ongoing challenges *(20–25)*. Cross-platform comparisons often yield significantly non-overlapping results; the source of these discrepancies is unknown *(23–25)*.

Other techniques for the measurement of gene expression that involve large-scale sequencing, such as SAGE *(18, 19)* and massively parallel signature sequencing (MPSS) *(26)*, can be more sensitive. These methods are highly efficient and perhaps more sensitive in that they involve sequencing of short fragments and counting of cDNA clones that represent mRNA expressed in a particular cell or tissue. However, because neither technique is as rapid as a microarray, they are less likely to be useful in the clinical setting.

Microarrays use microscope slides containing hundreds to thousands of immobilized DNA samples hybridized in a manner very similar to the Northern and Southern blot *(16)*. The main function of a microarray is to detect the level of mRNA transcript expression for specific genes of interest. The plates are incubated in a solution containing genetic material under consideration. Upon contact, mRNA transcripts in the solution hybridize to the appropriate cDNAs. Because cDNA on the chip is fluorescently labeled, every spot will emit a light in the ultraviolet environment. The intensity of the fluorescent label depends on the amount of hybridized mRNA present. The differentiation of the cDNAs by ultraviolet dye permits a comparison of gene expression under different experimental conditions. Initial data obtained from DNA microarrays is in the form of scanned images. Coding gene expression by means of different colors can be helpful for building genetic maps and graphical data expression and processing. Expression gene mapping, for example, under multiple experimental conditions or for different patients, can be presented in the form of a table, in which rows correspond to consecutive genes and columns represent different samples.

The sample under study is the source of the extracted mRNA used for microarray analysis. mRNA is converted to a cDNA species on the surface of the array and is then detected by fluorescence scanning or phosphor-imaging *(27–29)*. The oligonucleotide chips are highly reproducible, so accurate comparisons can be made by comparing signals generated by samples from separate arrays. This cannot be done with cDNA arrays. The alternative approach of using two different fluorescent wavelengths, such as Cy5 and Cy3, permits comparison of the intensity of two spots *(15)*. Understandably, a reference RNA must be used when comparing a large number of samples *(21)*.

After generation of microarray data, the greatest remaining challenge is data analysis. Because a large number of spot intensities and intensity ratios are generated, results must be carefully analyzed and then validated *(29)*. Repeat of the experiment is one sure way of reducing potential false positive signals. Many investigators use independent duplicate samples with reciprocal labeling with four microarrays for each experimental point. For genes with low expression levels, variability of microarray results can be considerable. Thus, repeat of the procedures can establish a high degree of confidence in the data. The use of data analysis software is essential for bioinformatic analysis of the data. In order to improve reliability of the data, validation of microarray results by Northern blot hybridization, quantitative or real-time RT-PCR, RNAse protection assays, or RT-PCR should be part of the complete analysis *(16,29)*.

Microarray Experiments

The design and implementation of microarray experiments demands special attention, because small variations in conditions can induce significant changes in gene expression *(2–7)*. For human tissue, these variables include post-operative and postmortem intervals, dissection methods, tissue preservation methods, and, of course, RNA quality. Frozen tissue generally provides the best material, although ethanol fixation can be used. Differences in gene expression between samples may be due to differences in genetic background as well. Other general sources of variability include cDNA amplification methods, probe labeling, hybridization conditions, and washing. Nonetheless, when all these issues are taken into account in the experimental design,

microarray experiments provide reliable data on gene expression at a systems level *(30)*.

Perhaps the most obvious impediment to the use of arrays by developmental biologists is the limiting amount of RNA available in standard embryo dissections. Conventional labeling of RNA for microarray hybridization requires 5–100 μg of total RNA. Embryonic organs, especially at early stages, yield only a fraction of the RNA needed for analysis *(31)*.

Limitations of DNA Microarray

Although DNA microarray has proven useful in the study of human malignancies in recent years *(5–12)*, its limitations and pitfalls must be considered when designing experiments, analyzing results, and generating of hypotheses *(32–40)*. Both cDNA arrays and commercial oligoarrays often underestimate relative expression changes when compared with the results of quantitative PCR assays and Northern hybridizations *(39)*. The bias associated with cDNA arrays can be easily corrected for because it is more predictable than that of commercial oligoarrays *(38,40)*.

A common difficulty encountered in the use of DNA microarray technology is analyzing the large amount of data amassed in the study of multiple samples. Another disturbing feature of these studies is that, using different platforms, different genes are variably overexpressed or underexpressed *(35–39)*. This leads to confusion and, of course, underlies the need for validation techniques. Another issue is reproducibility. Microarray results may significantly vary from one experiment to another. This is especially true when studying low-abundance RNA species due to the effect of background signals. Given the complexity of the procedures and the large number of genes involved, errors and inconsistencies may be introduced at any stage of array fabrication and utilization. These include slide heterogeneity (slide to slide variation), nonuniform surface properties of the slides, spot irregularity, printing fluctuation, uneven hybridization and processing, as well as contamination by dust and fibers. These variations can be reduced or minimized by proper controls and adequate replication of the studies *(38–40)*. Using a set of 185 common genes selected by Tan et al., the results could not cross-validate using Affymetrix, Agilent, and Amersham array systems; only four behaved consistently on all three platforms *(25)*. Using less rigorous criteria, about 30% agreement was reached, but never was there more than 52% concordance between two different systems. Manufacturers were not eager to share information regarding the short DNA sequence probes used in their kits to spot gene activity. Although manufacturers identified which genes the probes targeted, they would not reveal the actual nucleotide sequence of each probe. This made it difficult to know exactly what the probes were detecting. Technical differences may influence the results of transcriptional profiling *(5–8)*, but with high-quality arrays and appropriate normalization, the principal factor determining variance is biological rather than methodologic *(5)*. This is reassuring with respect to comparison of data from different microarray platforms. It lends credence to vigorous efforts aimed at obtaining and collating gene expression data in order to permit laboratories around the world to compare and share results *(20–40)*.

Another limitation involves the preparation of RNA samples used to make probes for microarray hybridization *(31)*. Cellular heterogeneity in normal and diseased tissues from various tumor specimens changes gene expression profiles. At present, the best solution to the obtaining of homogeneous populations of solid tumor cells is to use the laser-capture microdissection technique to separate the tumor cells of interest from other contaminating cells *(33,34)*. However, laser-capture microdissection is both expensive and labor-intensive. Samples obtained by this method are generally insufficient to produce adequate RNA for conventional microarray probe-making methods.

Applications

Understanding the role of genes in these processes necessitates the use of methods to determine patterns of transcription during development with a high degree of sensitivity and specificity. Conventionally, this is done by *in situ* hybridization. However, some investigators maintain that description of gene expression patterns is of no immediate functional relevance. Although identification of these genes has not yet led to major therapeutic advances, DNA microarray technology has recently been applied in genome-wide expression profiling *(3,6)*. Most successes in the search for genetic links to human diseases have been in rare disorders with mendelian inheritance and have led to major advances in our understanding of several neurological disorders *(12, 41)*, including Alzheimer's disease *(42)*. Now we face the challenge of understanding how genes contribute to normal human development, individual variability, and a variety of other common diseases.

Gene Expression

Each cell of an organism contains a copy of the same genome. The latter is identical apart from changes in individual cells caused by environmental factors. One to two percent of the human genome codes for expressed genes via mRNA which is translated into protein. The rest of the genome is repeat sequences, regulatory regions, or unique non-coding sequences of unknown functional significance. It is estimated that the human genome contains roughly 30,000 genes. Although the bulk of the human genome sequence is known, much remains to be discovered regarding how these genes function. Before introduction of the microarray chip, most research into gene function was done on a per-gene basis. The gene chip and its ability to measure simultaneous expression of the bulk of the human genome provides unique opportunities to discover the "needle in a

haystack," i.e., those few genes that characterize a particular disease *(7,8)*. Owing to the scale of the analysis required, human inspection is no longer a reasonable approach to understanding experimental results. Automated analytic techniques that exist in a variety of disciplines, including machine learning and statistics, have been applied to interpreting microarray data *(43–45)*.

Although cDNA microarray technology allows inexpensive study of all genes expressed in parallel, each method of investigation has its limits and must be interpreted properly. For example, although three genes in a particular metabolic pathway may show changes at the RNA level, the actual function of that pathway may not be affected if none of these genes are rate-limiting in nature. Hence, these high-throughput genome-level technologies must be interpreted in their appropriate biological context *(20–25)*. The aim of typical microarray experiments is to measure the amount of a given mRNA species in the transcribed gene present in a tissue or cell type. To do this, the mRNA is normally transformed by reverse transcription into cDNA, a more stable substance.

Determining the Significance of Identified Genes

Identifying genes that differ between normal and those of a patient group is an important goal of numerous research efforts. These genes may be new therapeutic targets or prognostic indicators, or may simply be related to a particular condition, their function or significance not yet understood. In any case, further biological insights to the disease or condition under study are possible. A variety of methods have been applied to the identification of potentially important genes. The simplest way to identify potentially important genes is to observe a twofold or greater difference in mean gene expressions between normal subjects and those under study. However, small changes in gene expression may not have a large impact in biological systems; therefore, this approach may be misleading. Statistical techniques are the most common methods of identifying potentially important genes in microarray data *(43)*. Tests exist to identify significant changes between group means or correlation with an outcome variable. The Student *t* test is often used to measure the difference between two group means, whereas the ANOVA test is useful for more than two groups *(44)*. Correlation coefficients and regression techniques are of utility when assessing a continuous outcome such as survival time or drug response.

Functional Analysis of Genes

After examining differences in gene expression, the next step is to look at the possible functional significance of these differences *(45)*. To date, functional analyses have almost exclusively fallen into the realm of molecular biologists working with a fully sequenced genome, such as that of *Saccharomyces cerevisae* yeast *(46)*, with its 6000 genes. At present, mutant yeast strains created by gene disruption are used to identify the biological functions of proteins derived from as yet uncharacterized genes *(47)*. The fitness of the mutant strain is monitored under a variety of selective growth conditions, such as exposure to ultraviolet light. This is both time- and labor-intensive. A recent approach used by investigators is to create insertional mutant yeast strains by adding a unique sequence or tag. The molecular tags are amplified from the surviving strains under a particular growth condition and hybridized to a high-density array, thus determining the relative abundance of the strain. The process can be repeated at various time points to more precisely compare the fitness of each deletion strain. This method of using chip technology has been likened to detecting a kind of mutant yeast "molecular bar code." Another functional assay used by researchers for studying transcription factors is proteomics that direct gene expression. Knowledge of the targets of transcription factors will no doubt permit more accurate control of gene expression *(48)*.

Genotyping

Insight into differences between alleles or the mutations present in different individuals can also shed light on the interplay of environment and disease susceptibility. For example, an array of a cell sample of peripheral T cells with an unknown contaminant could be compared with a database of thousands of expression profiles of T cells subjected to different contaminants. This may, for example, detect evidence of prior mercury exposure. The potential of chip technology in clinical research has been demonstrated in several genotyping studies, particularly of hematologic disorders *(3,9)*.

Some companies have produced more than one chip for specific arrays, for example, chips for the detection of protease and reverse transcriptase in HIV-1 *(49)* and for p53 anticodon detection *(50)*. The p53 chip includes over 400 mutations that have been found to be associated with tumors and has been marketed to determine individuals at increased cancer risk *(50,51)*. This carries with it particular ethical dilemmas, particularly if there is no available early therapeutic intervention. There is a risk that stigma will be attached to affected individuals, similar to the present implications inherent in HIV status.

In the future, it is conceivable that data regarding gene expression in a particular tumor in combination with knowledge of the certain aspects of the patient's genotype will permit selection of an ideal treatment modality for each individual. Such "customized therapy" may be applicable to many clinical situations. The long-term implications for pathology and for medicine in general are exciting. It may be that the role of the histopathologist will change, less emphasis being put on histology as compared to tumor genotype. In order to point the clinician down the preferred therapeutic pathway, pathologists may have to assess and

integrate a number of factors, not only tumor genotype genetic risk of the individual under study *(52)*.

Pharmacogenomics

The inherent complexities of human cancer have frustrated attempts to understand its genetic underpinnings and to explain the unpredictable behavior of individual tumors. High-output screening systems and advanced bioinformatics will no doubt identify biomarkers that favor one therapy over another, as well as drug(s) to be avoided in certain individuals to prevent adverse reactions. Thus, it will be feasible to use molecular diagnostics to select drugs safe for the individual patient. This ability to optimize individual therapy for a particular disease differs strategically from the use of pharmacogenomics in cancer therapy, in which microarrays are used to classify tumors of a specific patient *(52–54)*.

Genetic polymorphism of drug metabolizing enzymes and other molecules are no doubt responsible for many observed interindividual differences in efficacy and toxicity of chemotherapeutic agents *(55)*. For example, array technology similar to that used for gene expression can be applied to sequence large portions of an individual's genome to identify single nucleotide polymorphisms underlying disease susceptibility or chemosensitivity. High-throughput, single-nucleotide-polymorphism genotyping technology is changing rapidly, and many new platforms are being developed. Once single nucleotide polymorphisms related to treatment response or adverse effects are identified, individual polymorphisms in genes of interest can be screened to avoid serious adverse drug reactions. In cases wherein therapy is toxic, such as immuno- or chemotherapy, specific polymorphisms that confer risk or predict treatment response could be used to identify patients likely to respond favorably *(54–56)*.

Microarray Analysis of Pituitary Tissues

Major advances in the field can be expected with microarray technology on a genomic scale. This permits simultaneous analysis of the expression levels of thousands of genes relevant to a specific process, e.g., pituitary tumorigenesis. Adenohypophysial tumors, both benign and malignant, are considered monoclonal in origin *(57–65)*. A small number of studies have been published that give us a glimpse of this approach as it relates not only to pituitary tumorigenesis, but also into medical therapy of pituitary tumors and prognosis *(66–70)*.

Tanaka et al. *(69)* constructed a gene expression profile of the normal human pituitary gland. A total of 1,015 randomly collected 31 expressed sequenced tags were grouped into a 527 gene signature species. The relative activities of these genes were unique to the pituitary. Not surprisingly, such pituitary hormone–related genes as prolactin (PRL), growth hormone (GH), luteinizing hormone (LH), follicle-stimulating hormone (FSH), and α-subunit were highly expressed. Interestingly, other genes such as chromogranin B, a secretory granule protein present in all anterior pituitary gland cells, CPE, and 7B2 were expressed at high level as well. Normal pituitary-specific transcripts including pituitary gland specific factors 1a and 1b containing 128 and 91 amino acids, respectively, were also identified, but, the identity and functions of these and other related normal genes is unknown.

In a recent study, the pituitary gland was shown to play an important role in the aging process *(71)*. From a total of 1176 genes arrayed on each of the six membranes per group, 542 (46%) were detectable in the anterior pituitaries of both young and old rats. Significance analysis of microarrays (SAM) of these 542 detectable genes revealed 28 that changed significantly in their expression with age. Of these, 24 decreased and 4 increased over the process. Among the five major hormone genes on the membrane, GH and PRL decreased with age, the glycoprotein hormone common alpha subunit gene increased, and the beta subunits of FSH and TSH did not change. Among these genes, the three genes found to undergo change by array analysis were confirmed by Northern blot analysis *(71)*.

DNA microarrays were used to analyze and identify gene expression patterns linked to aging and the associated occurrence of spontaneous pituitary adenomas in a rat animal model *(66)*. RNAs from 3-mo-old rats and tumor-bearing 20- to 28-mo-old rats were analyzed with 588 known gene cDNAs. In the old rats, there were 28 genes that were expressed at higher levels, while in the younger rats, there were 15 genes expressed at higher levels. Galanin, which possess PRL-promoting activity, and glutathione 5-transferase were the genes most differently expressed in old and young rats, respectively. These results were further validated by relative RT-PCR, which was consistent with the microarray data in 14 of the 15 genes tested. Several of these overexpressed genes, including $p27^{kip1}$, have been found to play a role in pituitary tumorigenesis in the rodent pituitary *(72,73)*. cDNA microarray was used to identify estrogen-responsive genes in somatolactotrophic cells of the pituitary gland. GH3 cells respond to estrogen by growth as well as prolactin synthesis. RNAs extracted from GH3 cells treated with 17beta-estradiol (E2) at 10^{-9} *M* for 24 h were compared with those of control samples. The array analysis showed 26 genes to be up-regulated and only seven to be down-regulated by E2. Identification of these estrogen-responsive genes should contribute to our understating of estrogen action upon the pituitary gland *(74)*.

To assess early gene expression changes preceding thyroid hormone–induced involution of a TSH-producing adenoma, Wood et al. *(68)* used an 1176 DNA microarray to detect 7 up-regulated and 40 down-regulated genes by thyroid hormones . The results were validated by Northern blot hybridization. The authors reported that several cell cycle regularity proteins were changed, including up-regulation

of p15 and down-regulation of p57 and CDK2. The latter is known to activate the expression of p27 and c-myc levels to lead to growth arrest in neuroblastoma cell lines *(75)*. Many genes expressed in the pituitary that are targeted by thyroid hormones were also identified, including cell adhesion proteins as cadherin 4 and γ-catenin, tyrosine kinase ryk, inhibin, chromogranin B and C, proconvertase 1, and the apoptosis protein DAD1. Most of these were down-regulated by thyroid hormone treatment *(68)*. Ornithine decarboxylase, a rate-limiting enzyme in the biosynthesis of polyamines, such as putrescine, spermidine, and spermine, was overexpressed only in GH tumors. This enzyme correlates with malignancy in pituitary tumors as well as in gliomas *(76)*. Thus, polyamine metabolism is a potential target for antineoplastic therapy *(77)*. Pituitary adenylate cyclase–activating polypeptide (PACAP) exerts trophic effects on various neuronal, neuroendocrine, and endocrine cells. For example, a recent high-density microarray study showed that the proliferative phenotypes are possible targets of PACAP during differentiation of normal and neoplastic sympathoadrenal cells *(78)*.

ACTH tumors overexpress CMP-tk, a transmembrane receptor with tyrosine kinase activity. Tyrosine kinase receptors are involved in the control of cellular growth and differentiation *(79)*, thus this gene may play an important role in ACTH tumor initiation and progression. Although PRL adenomas, the most common anterior pituitary tumors in humans, appeared to overexpress trichohyalin, TGF-β receptor III genes, and the protease inhibitor 12 gene; overexpression of these genes was not validated by RT quantitative PCR *(67)*. These results highlight the potential inherent in the discovery of pituitary tumor genes that could serve as targets for radiopharmaceutical therapy.

Among nonfunctioning adenomas folate receptor (FR)-α was overexpressed 2.5-fold as compared to the normal pituitary. This receptor, a glycosyl-phosphatidylinositol-linked protein, is thought to confer growth advantage to cells with limited concentrations of cells with a limited concentration of 5-methyltetrahydrofolic acid with increased uptake of folate *(80)*, so the potential therapeutic approach of using FR by targeted drug delivery has been suggested for recurrent nonfunctioning tumors *(81)* because such studies have been successful in some patients with ovarian tumor and FR-α is overexpressed in ovarian as well as other cancers *(82)*. Evans et al. *(67)* used cDNA arrays to identify gene expression profiles in hormone-secreting and nonfunctioning human pituitary tumors and then compared them to normal pituitary with its 7075 genes. They observed differential expression of 128 genes and then analyzed three genes in 37 pituitaries by RT real-time quantitative PCR.

A cDNA membrane array displaying 1183 probes was used to detect hypothalamic and pituitary changes in gene expression accompanying aging and age-associated pituitary macroadenoma occurrence. Somatostatin and growth hormone–releasing hormone was not altered, while neuropeptide transcripts involved in feeding behavior [orexin/hypocretin, MCH, proopiomelanocortin (POMC), cocaine- and amphetamine-regulated transcript (CART)] were significantly altered. Modifications in hypothalamic orexigenic (orexin, MCH) and anorexigenic (POMC, CART) gene expression are in keeping with an age-associated decrease in energy consumption but a higher one in the presence of macroprolactinomas *(83)*.

A recent preliminary study using the Affymetrix U133A gene chip comparing ACTH pituitary carcinomas to five pituitary adenomas showed increased expression of various genes, including the FGF family (FGF2 and FGF9), the LGALS gene family (galectin-3, -8 and -9), IL6R, and FOS in the pituitary carcinomas as compared to the adenomas *(84)*. It is of note that functional studies have shown galectin-3 to play an important role in pituitary tumor growth *(85)*.

In summary, DNA microarray studies have provided new insights into genes unequally expressed in pituitary tumors. However, several problems and disadvantages have emerged with respect to the technical methods employed. For example, starting materials have usually been contaminated with extraneous cells such as fibroblasts and endothelial cells in studies of the normal pituitary, and with these as well as normal secretory cells in studies of adenomas. The use of sophisticated methods for sampling only the cells of interest, such as laser capture microdissection and combined immunophenotyping and LCM, should aid in increasing the specificity of the array and facilitating the detection of genes expressed at low level *(33,34)*. Determining levels of mRNA regulation is only one approach to understanding dysregulation of various cellular pathways. Methods focused upon protein regulation and post-translational modifications are also important. Proteomics should also complement DNA microarray analysis. Novel approaches combining genomics and proteomics techniques should forward our understanding of normal and abnormal cell functions *(86–88)* and lead to the discovery of yet more important regulatory pathways in normal pituitary cells and tumors.

Conclusion

Microarray technology, by providing novel data on a genome-wide scale, has provided new insight regarding mechanisms underlying human diseases, most notably cancer.

The use of microarrays to explore gene expression on a global level as well with respect to specific clinical disorders is rapidly progressing. When properly applied, microarrays are powerful tools, but given their sophistication and complexity, they are prone to a number of technical and interpretive errors. A combination of approaches such as LCM and immunophenotyping LCM and microarrays should improve sampling specificity. Meticulous attention to methodology and data analysis, as well as the use of ancillary

verification methods, is crucial to drawing reliable conclusions. The studies on human pituitary tumors reviewed herein highlight the potential of DNA microarray analysis, both to the discovery of genes underlying their pathogenesis and to developing improvements in medical treatment.

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