Gene Expression in the Normal Adult Human Kidney Assessed by Complementary DNA Microarray

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Submitted June 24, 2003; Revised October 13, 2003; Accepted October 17, 2003
Monitoring Editor: Keith Yamamoto

The kidney is a highly specialized organ with a complex, stereotyped architecture and a great diversity of functions and cell types. Because the microscopic organization of the nephron, the functional unit of the kidney, has a consistent relationship to the macroscopic anatomy of the kidney, knowledge of the characteristic patterns of gene expression in different compartments of the kidney could provide insight into the functions and functional organization of the normal nephron. We studied gene expression in dissected renal lobes of five adult human kidneys using cDNA microarrays representing ~30,000 different human genes. Total RNA was isolated from sections of the inner and outer cortex, inner and outer medulla, papillary tips, and renal pelvis and from glomeruli isolated by sieving. The results revealed unique and highly distinctive patterns of gene expression for glomeruli, cortex, medulla, papillary tips, and pelvic samples. Immunohistochemical staining using selected antisera confirmed differential expression of several cognate proteins and provided histological localization of expression within the nephron. The distinctive patterns of gene expression in discrete portions of the kidney may serve as a resource for further understanding of renal physiology and the molecular and cellular organization of the nephron.

INTRODUCTION

The kidney is a multifunctional organ with critical roles in selective elimination of soluble wastes, acid base, electrolyte, and hormonal homeostasis, and regulation of blood pressure and erythropoiesis. The nephron, the functional unit of the kidney, is a highly ordered structure, consisting of several distinct specialized cell types. An ultrafiltrate of the blood, formed in the glomerulus traverses the nephron, where essential solutes are selectively reabsorbed, whereas wastes are concentrated and excreted. The diverse functions of the kidney are divided in a stereotyped manner among the cells of the nephron and associated structures. The specialization of function along the nephron is reflected in the gross anatomy of the renal lobe. The cortex contains glomeruli, the juxtaglomerular apparatus, and the proximal and distal convoluted tubules, where the plasma ultrafiltrate is formed and the majority of its components are reabsorbed, and the medulla contains the loop of Henle and collecting ducts, where the ultrafiltrate is concentrated.

The characteristic pattern of functional specialization of cells along the length of the normal nephron should be accompanied by corresponding regional variation in gene expression patterns. Gene expression profiles of each segment could provide insight into the proteins and systems involved in the function of each portion of the nephron. A comprehensive view of gene expression would therefore contribute to an understanding of normal renal physiology

and its derangement in renal disease. To further explore the genetic programs that underlie the function of the nephron, we have analyzed the gene expression patterns in isolated normal glomeruli and in different portions of normal kidney distinguished by their gross anatomic location within the renal lobe.

MATERIALS AND METHODS

Tissue Samples

Informed consent was obtained from all patients before tissue harvest. Samples were obtained from five fresh nephrectomy specimens removed for renal neoplasia. The ureter was probed and the kidneys were bivalved through the collecting system. Normal kidney samples were obtained from areas of the nephrectomy specimens uninvolved by neoplasm. Renal pelvic mucosa was removed, individual lobes were isolated, and the lobes were dissected into papillary tip, inner medulla, outer medulla, inner cortex, and outer cortex fractions. Fresh whole cortex was used for isolation of glomeruli using a sieving technique (Misra, 1972), which was successful in four of the five cases. All specimens were immediately frozen on dry ice and stored at –80°C. Paraffin sections from each case were reviewed by a single pathologist (J.P.T.H.) in order to exclude medical renal disease.

Microarray Analysis

The cDNA microarrays used in this study included ~28,000 unique characterized genes or EST's represented by a total of 41,859 unique cDNAs printed on glass slides by the Stanford Functional Genomics Facility (<http://www.microarray.org/sfgf/jsp/home.jsp>). Approximately 10,000 of these genes are characterized, whereas the remainder represent expressed sequence tags. Methods for RNA extraction, hybridization to arrays, and interpretation of data have been described elsewhere (Eisen *et al.*, 1998; Alizadeh *et al.*, 2000; Perou *et al.*, 2000; Ross *et al.*, 2000; Garber *et al.*, 2001) and detailed protocols are available at <http://cmgm.Stanford.EDU/pbrown/>.

Tissue was homogenized in Trizol reagent (Invitrogen, Carlsbad, CA) and total RNA was prepared. Preparation of Cy-3-dUTP (green fluorescent)-labeled cDNA from reference RNA using Universal Human Reference RNA (Stratagene, La Jolla, CA) as an internal reference standard and Cy-5-dUTP (red fluorescent) labeled cDNA from kidney RNA, microarray hybridization

Article published online ahead of print. Mol. Biol. Cell 10.1091/mbc.E03-06-0432. Article and publication date are available at www.molbiolcell.org/cgi/doi/10.1091/mbc.E03-06-0432.

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and subsequent analysis was performed as described (Skena *et al.*, 1995; Eisen *et al.*, 1998; Ross *et al.*, 2000). The Stratagene Universal Human Reference RNA represents total RNA pooled from 10 different cell lines. The methods and protocols are also included in the Supplementary Information, available at https://www.med.stanford.edu/jhiggins/Normal_Kidney/index.shtml. After washing, the microarrays were scanned on a GenePix 4000 microarray scanner (Axon Instruments, Foster City, CA) and, after normalization of fluorescence intensities to control for experimental variation, fluorescence ratios (kidney/reference) were calculated using GenePix software. The primary data tables and the image files are freely available from the Stanford Microarray Database (<http://genome-www.stanford.edu/microarray>; Gollub *et al.*, 2003).

We restricted our analysis to genes for which the mean fluorescent hybridization signal intensity divided by the median background intensity was ≥ 1.5 in either the sample or reference channel for at least 80% of the samples analyzed. We selected for further analysis genes that showed highly variable expression between the different samples. A mean level of expression was calculated for each gene by averaging $\text{Log}_2(\text{Cy5}/\text{Cy3})$ net fluorescence intensity ratios. Values for each gene were then adjusted so that the mean would equal zero. Genes with the most variable expression were selected as genes for which at least 2 of the tissue samples had mean-centered $\text{log}_2(\text{Cy5}/\text{Cy3})$ net fluorescence ratios greater than +2 or less than -2 (fourfold variation in expression from the mean). From the initial list of 41,859 cDNAs, 1903 cDNAs (1548 unique Unigene clusters) met these criteria. The complete dataset, representing the measured transcript levels for each of these genes in each of the tissue samples, was organized by average-linkage hierarchical clustering, after first centering the expression measurements relative to the mean for each gene and each array (Eisen *et al.*, 1998). This dataset was the basis for all further analyses in this report.

The 41,859 cDNAs were also filtered using less stringent criteria. When mean fluorescent hybridization signal intensity divided by the median background intensity was greater than or equal to 1.5 in either the sample or reference channel for at least 60% of the samples analyzed and only genes whose expression level differed by a log ratio of 1.25 or greater from their mean level of expression in at least two of the samples were considered, a total of 16,293 cDNAs (12,432 unique Unigene clusters) were available for analysis; this dataset is not shown in this manuscript but is available through the accompanying website: https://www.med.stanford.edu/jhiggins/Normal_Kidney/index.shtml.

As a complementary approach to unsupervised hierarchical clustering, a supervised method, Significance Analysis of Microarrays (SAM; Tusher *et al.*, 2001) was used to identify genes differentially expressed between selected sets of tissue samples (for example, genes expressed in the outer cortex vs. the inner cortex). This program uses normalized log ratios of gene expression levels and ranks genes according to the strength of correlation with a given parameter. The false discovery rate, estimated using randomly permuted data, serves to indicate the statistical significance of the genes on positive significant and negative significant gene lists.

Immunohistochemistry

Immunohistochemistry using antisera to osteonectin (Zymed, South San Francisco, CA) and SFRP1, keratin 19, S100P, aldo-ketoreductase family 1, and solute carrier family 9 (AGI, Sunnyvale, CA) was performed according to previously published protocols (Higgins and Warnke, 1999). To avoid interference from endogenous biotin, a biotin free method, EnVision, was used for amplification of the signal (DAKO, Carpinteria, CA). Antisera to SFRP1, keratin 19, S100P, aldo-ketoreductase family 1, and solute carrier family 9 were raised by injecting peptides predicted from each gene sequence (AGI). The peptides were conjugated to KLH, and injected into two outbred rabbits. The serum was harvested after the rabbits demonstrated significant antipeptide titer. Affinity-purified antiserum was obtained by binding the antiserum to an affinity column conjugated with the three peptides; the bound antibodies were eluted with a pH gradient.

RESULTS

Gene Expression

Distinct anatomical regions of the kidney were distinguished by corresponding differences in global gene expression patterns using unsupervised hierarchical cluster analysis of 1548 genes with highly variable expression (Figure 1). The main branch of the dendrogram separated the samples from the cortex, including the samples of purified glomeruli, from the samples of the medulla and pelvis. Within each of these branches a further subdivision could be seen, with all glomerular samples forming a tight cluster (branch A) distinct from the cortical samples (branch B). Likewise, samples of pelvis (branch E) clustered on a branch distinct from the medullary samples (branch C), and papillary tips (branch

D). The majority of medullary samples clustered on a separate branch with only 2 of 10 medullary samples clustering with the samples obtained from the papillary tips. The distinction between inner and outer cortex samples and inner and outer medulla samples was less significant.

Cortex Cluster

More than 250 genes were predominantly expressed in the renal cortex and distinguished these samples from the other anatomic segments. This distinctive pattern of gene expression is probably due in large part to the characteristic molecular features of the cells of the convoluted tubules and glomeruli. Genes specifically expressed in glomeruli were further highlighted in the samples from purified glomeruli (see below). The proximal convoluted tubule is the predominant cell type in the renal cortex and is not present in any of the other anatomic segments of the kidney. Seventeen transcripts expressed predominantly in the cortex encode members of the solute carrier family, including sodium/chloride, glucose, organic ion, and amino acid transporters. Iodothyronine deiodinase, types I and II are found in this cluster, suggesting that the cortex and cells of the proximal convoluted tubule are responsible for the metabolism of thyroid hormone in the kidney. Many genes from the cortex cluster are also expressed in the small intestine. Several of these are involved in the transport of nutrients such as the facilitated glucose/fructose transporter (SLC2A5) and the intrinsic factor-cobalamin receptor (cubilin). Others appear to play a role in intestinal mucosal defense including fucosyltransferase 6, which appears to be involved in the synthesis of e-selectin ligand (Lewis antigen) and may facilitate leukocyte homing (Koszdin and Bowen, 1992). Others are thought to play a role in digestion such as maltase-glucoamylase (Nichols *et al.*, 2003), aldolase B (Cox *et al.*, 1982), and ketohexokinase (fructokinase; Bonthron *et al.*, 1994). Expression of many of these latter genes in the kidney is unexpected and suggests that these genes may serve a more general function that is common to absorptive epithelia.

Other genes found in the cortical samples illustrate the role played by the kidney in detoxification. Some of these have been characterized in the liver; such as alanine-glyoxylate aminotransferase, which is involved in glyoxylate detoxification. Defects in this gene are the cause of hyperoxaluria type I, which typically results in renal failure (Danpure and Jennings, 1986). Glutathione S-transferase A2 is a liver enzyme that serves in the detoxification of carcinogens, therapeutic drugs, environmental toxins, and products of oxidative stress, by conjugation with glutathione (Mannervik, 1985). Several members of the UDP glycosyltransferase 2 family are expressed in this cluster and these genes play a role in the elimination of xenobiotic as well as endogenous toxins (Radomska-Pandya *et al.*, 2001). Finally, metallothionein 1G is involved in heavy metal homeostasis and protects against heavy metal toxicity (Nath *et al.*, 1988). Expression of this broad panel of genes involved in detoxification underscores the complex role played by the kidney in the clearance and secretion of toxic compounds.

Glomerulus Cluster

We used a sieving technique (Misra, 1972) to prepare whole glomeruli with ~95% purity when evaluated microscopically (Figure 2). The glomerular samples showed a highly distinctive gene expression pattern, some features of which were also evident in the cortical samples. One-hundred thirty-nine of 1548 differentially expressed genes were predominantly expressed in glomeruli including several known to be expressed in podocytes (osteonectin, Floege *et al.*, 1992;

Figure 1. Unsupervised hierarchical cluster. A representation of the variation in expression of 1548 genes in different compartments of five kidneys. Each row represents a single gene. Each column represents a single sample. Green squares indicate transcript levels below the mean; black squares, transcript levels equal to the mean; red squares, transcript levels greater than the mean; gray squares, technically inadequate or missing data. Gene filtering criteria were for fourfold variation from the normalized mean and at least 80% well-measured spots. Colored bars adjacent to the clustering table indicate the position of the enlarged images. The color of the bar indicates the type of sample in which the cluster of genes is expressed. Red, pelvis cluster; dark blue, papillary tip cluster; brown, tubular cluster; green medullary cluster; turquoise, glomerular cluster; and orange, cortex cluster. Selected gene names are shown. The full dataset can be explored on the accompanying website: https://www.med.stanford.edu/jhiggins/Normal_Kidney/index.shtml. Original magnification: left panel, 5 $\times$; right panel, 10 $\times$.

Figure 2. Isolation of glomeruli from fresh renal cortex shows a high degree of purification. Original magnification: left panel, 5 $\times$; right panel, 10 $\times$.

actinin alpha 4, Kaplan *et al.*, 2000; GLEPP1, Thomas *et al.*, 1994; tight junction protein ZO-1, Kurihara *et al.*, 1992; Schnabel *et al.*, 1990); glomerular endothelium (TEK [tyrosine kinase], Dumont *et al.*, 1994; and EDG1, Liu *et al.*, 2000); and mesangial cells (endoglin, Rodriguez-Barbero *et al.*, 2001; Roy-Chaudhury *et al.*, 1997). However, many of the transcripts in this cluster, including several named genes, were not known previously to be expressed in the glomerulus. For instance, bone morphogenetic protein 7 (BMP7) has been described in renal tubules (Wang *et al.*, 2001) but our data show it is even more highly expressed in the glomeruli. MADH6, which is also found in this cluster, is known to selectively inhibit bone morphogenetic protein signaling by competing with smad4 for receptor-activated smad1 (Hata *et al.*, 1998). Ficolin 3 (hakata antigen) was previously described in the lung and liver (Fukutomi *et al.*, 1996; Akaiwa *et al.*, 1999), and is a target of autoantibodies in some patients with systemic lupus erythematosus (Inaba *et al.*, 1990), suggesting that it may play a role in glomerular injury in lupus patients. Finally, some of the most interesting genes from this cluster may be those about which least is known. Unnamed and uncharacterized genes in this cluster, such as EST Hs.135335, which has only been identified in a nephroblastoma cDNA library (<http://source.stanford.edu>), may be uniquely expressed in podocytes, mesangial cells, or glomerular endothelium.

Renal Tubular Cluster

We identified ~130 genes that were characteristically expressed in the renal cortex and medulla but not in isolated glomeruli or pelvis. We suspect that these transcripts are related to the formation and function of the renal tubular epithelium because the tubule is the component that is characteristically present in both cortex and medulla, but absent from glomeruli and pelvis. Several of the transcripts in this group were already known to be expressed in the kidney, such as prostasin (Yu *et al.*, 1995), NX-17 kidney-specific membrane protein (Zhang *et al.*, 2001), the chloride channel CLCNKA (Matsumura *et al.*, 1999), FXYD2 (Arystarkhova *et al.*, 1999), SLC12A1 (Simon *et al.*, 1996), SCNN1A (Canessa *et al.*, 1994), and carbonic anhydrase XII (Tureci *et al.*, 1998). The latter four genes encode proteins that are targeted by commonly used diuretics and demonstrate the potential utility of this gene set for future gene-directed development of pharmacologic agents.

Several of the as yet uncharacterized genes in this cluster are of potentially great interest. For example, ESTs Hs.144472, Hs.155290, Hs.135787, Hs.126246, Hs.155747, and Hs.128408 are all represented by clones that have only been isolated from cDNA libraries of renal origin and were not found in libraries from other sites (<http://source.stanford.edu>). This suggests that these may be renal specific genes, with as yet unknown functions.

Medulla Cluster

We dissected the medulla into an outer segment rich in proximal straight tubules, thin segments of short loops of Henle, thick ascending limbs, and collecting ducts, an inner segment that includes long Henle's loops and collecting ducts, and the papillary tips, which contain predominantly the distal collecting ducts and the urothelium on their renal pelvic surface. Unsupervised hierarchical cluster analysis was able to distinguish between the papillary tip and medullary samples, but not between the inner and outer medulla samples. There was some overlap in gene expression between the medulla and papillary tip samples, and two samples from the inner medulla clustered with the papillary tips. For instance, the papillary tips share one cluster of transcripts with the medulla and a second, nonoverlapping group with the renal pelvis (Figure 1). This overlap suggests either that the cellular constituents of the papillary tips have features of medullary and pelvic cells or that some components of the papillary tips are also present in the medulla (such as the interstitium and tubules), whereas others are present in the pelvis (such as the urothelium on the outer surface of the papillae). The latter possibility is supported by the observation that several keratins are expressed in both the pelvis and in the papillary tips; these are certain to be expressed by the epithelium. On the other hand, aquaporin 2, a gene expressed in the collecting duct, is expressed in the medullary samples, implying that there may be a continuum of gene expression patterns along the nephron. A similar overlap in gene expression is seen in the cortical and medullary genes. Many of these probably represent widely expressed renal tubule genes (such as Na-K-ATPase), because tubules represent a component present in both cortex and medulla but not in glomeruli or pelvis.

A group of 58 genes was more highly expressed in the medullary samples than in the papillary tips. This group included some expected genes (for example, the antidiuretic hormone responsive water channel aquaporin-2). Two genes in the medulla cluster may play a role in mucosal defense: the Fc fragment of IgG-binding protein and matrix metalloproteinase 7, which is involved in wound healing (Lu *et al.*, 1999) and in the intestine may regulate the activity of mucosal defensins (Wilson *et al.*, 1999). This suggests that mechanisms used in mucosal defense of the intestine may represent general strategies for defense against microbes used by a wide range of epithelia.

Papillary Tip Cluster

The papillary tips share transcript profiles with both the medulla and pelvis, likely reflecting shared cell types with these anatomic regions. Several genes in this 102-gene papillary tip cluster point to unanticipated functions in the papillary tips. SLC21A3 is a sodium-independent transporter that mediates cellular uptake of organic ions in the liver, including bile acids, bromosulphophthalein, and some steroidal compounds (Kullak-Ublick *et al.*, 1995). Aldo-keto reductase family 1, member C1, also found in this cluster, is thought to play a role in the transport of bile acids (Stolz *et al.*, 1993). Several genes involved in alcohol metabolism,

including alcohol dehydrogenase IB and IC, and aldehyde dehydrogenase 1 family, member A3 are also highly expressed in the papillary tips. Whereas expression of alcohol dehydrogenase I has previously been localized to the human renal tubule (Buhler *et al.*, 1983), our finding of several genes involved in alcohol metabolism suggests a significant role for this portion of the renal lobe in this process. Furthermore, the findings of these genes in the medulla suggests that alcohol by-products may be produced in increased amount in the hypoxic conditions of the medulla.

Pelvis Cluster

A rather large cluster of genes was expressed predominantly in the pelvis, attesting to the difference between the pelvic tissues and the other portions of the kidney proper. Many of the transcripts in this cluster, including gamma 2 actin, myosin light polypeptide kinase, caveolin 1, calponin 1, tropomyosin 2, and actin binding protein 280, reflect the smooth muscle in the wall of the renal pelvis. Still other of these genes, including uroplakins 1B and 3 (Lobban *et al.*, 1998) and prostate stem cell antigen (Amara *et al.*, 2001) are known to be expressed by the urothelium that lines the pelvis. A third group of genes may play a role in protecting the urothelium against neoplastic transformation. These include glutathione peroxidase 2, which in the gastrointestinal tract is thought to protect against ingested organic hydroperoxides (Chu *et al.*, 1993) and TP63, which may serve to induce apoptosis and inhibit growth in p53 deficient cells (Suliman *et al.*, 2001).

Inner vs. Outer Cortex and Inner vs. Outer Medulla

The gene expression patterns of samples from inner and outer cortex showed a high degree of similarity and unsupervised hierarchical cluster analysis did not distinguish between these. Instead, three of five outer cortex samples showed a gene expression pattern that most resembled the inner cortex from the same patient. Patient-based grouping of medullary samples was seen in one of five cases. We used SAM (Tusher *et al.*, 2001) to identify genes expressed in the outer and inner cortex and outer and inner medulla. Forty-nine genes were more highly expressed in the inner cortex than in the outer cortex (false significant number: four genes; Web Table I). No genes were more highly expressed in the outer cortex than in the inner. Ten genes were more highly expressed in the inner medulla than in the outer (false significant number: 3; Web Table II). Further studies are required to determine the functional significance of these genes.

Results of Relaxed Selection Gene Set

Although our discussion has been largely restricted to the 1548-gene gene set, we also used less stringent criteria for gene filtering (see MATERIALS AND METHODS and supplemental web site). When a 12,432-member gene set was used for unsupervised hierarchical clustering, a highly similar sample dendrogram was obtained (Web Figure 1). The glomeruli and cortex samples were again perfectly segregated from the remaining samples. One papillary tip sample clustered with the pelvis samples, and another was grouped with the inner medullary sample from the same patient. The consistency of the sample-clustering pattern across this wide range of gene sets highlights the robust quality of the individual gene clusters. The large gene set has the advantage that a greater number of genes known to be relevant to renal physiology pass the gene-filtering criteria. For example, podocalyxin, synaptopodin, Collagen IVA3 (Goodpasture antigen), and WT1 all failed to satisfy the stringent criteria of

the small gene set, but all are appropriately present in the glomerular cluster in the larger gene set. Similarly, erythropoietin, HIF-1 α , and ecto 5'-nucleotidase, genes involved in oxygen sensing and erythropoiesis, appear only in the relaxed gene set. Clustering of these genes and arrays with a variety of different stringency criteria can be performed through The Stanford Microarray Database (<http://genome-www.stanford.edu/microarray>).

Immunohistochemistry

We selected five transcripts to evaluate expression of cognate proteins in the nephron by immunohistochemistry. Transcript levels for secreted frizzled-related protein 1 (SFRP1) were highest in the cortex, with lower expression in the medulla as well. Based on immunohistochemical staining, a similar pattern is clearly seen for expression of the cognate protein in the cortex and medulla (Figure 3, left panel). At high magnification (right panel), SFRP1 protein is seen to be strongly expressed in proximal convoluted tubular epithelium, whereas distal tubules stain more weakly. Similarly, transcript levels of solute carrier family 9, isoform 3 regulatory factor 1 (SLC9A3R1), were highest in the proximal tubular epithelium, and we observe similar protein expression by immunohistochemistry (Web Figure 2). This protein was initially characterized in the cerebral cortex but has also been found to be highly expressed in the kidney (Reczek *et al.*, 1997). This protein appears to play a role in the formation of microvilli and maintenance of cell polarity (Bonilha and Rodriguez-Boulant, 2001). Because the cells of the proximal convoluted tubule are replete with microvilli, the high level of expression that we observe is expected. We found the keratin 19 gene to be most strongly expressed in the papillary tip samples. Immunohistochemistry shows expression of the cognate protein to be present in tubules extending through the entire thickness of the renal lobe (Figure 3) but most concentrated in the papillary tips. At high magnification, the antiserum to keratin 19 is seen to stain the principal cells but not the intercalated cells of the collecting ducts as previously reported (Cao *et al.*, 2000). Our gene expression analysis confirms high-level expression of SPARC in the glomeruli (Web Figure 2). Immunohistochemistry demonstrates SPARC protein expression in the visceral epithelial cells (podocytes) as previously described (Floege *et al.*, 1992). We observed high levels of S100P transcripts in the renal pelvis samples, with some expression in the papillary tips and a complete absence of expression in the remainder of the kidney (Figure 3). By immunohistochemistry, this protein is indeed present only in the transitional epithelium of the renal pelvis and that overlying the papillary tips. In agreement with the expression data, none of the tubular epithelium of the kidney expressed this protein. Expression of S100P in urothelium has not been previously reported.

DISCUSSION

Our data show that different macroscopic compartments of the renal lobe show distinct and reproducible patterns of gene expression. Each of the compartments that we dissected showed a highly characteristic pattern of gene expression that was consistent in these samples. Although paired samples from the same patient were more similar in their expression than samples from different patients, the variation in gene expression in each compartment between samples from different individuals was far less than we have observed for renal tumors of a given diagnostic class (Higgins *et al.*, 2003). This consistency of gene expression profiles characteristic of each region or substructure of the kidney is

Figure 3. Correlation of mRNA with protein expression by immunohistochemistry. mRNA expression levels for three genes, SFRP1, keratin 19, and S100P, have been taken from Figure 1 together with the Figure 1 sample dendrogram. Immunohistochemical stains for each of these genes are shown below the mRNA data. Left panel: a full thickness section of a normal renal lobe; right panel: a high-power image taken from the area with the most intense staining. In each case, mRNA expression data correlates with the distribution of the cognate protein by immunohistochemistry. Original magnification: left panel, 5 $\times$; right panel, 10 $\times$.

demonstrated through the tight grouping of similar samples through hierarchical clustering.

By examining the different anatomical subcompartments of the kidney, our study expands upon that of Yano *et al.* (2000), who have previously reported a microarray analysis of gene expression in nine samples of the adult renal cortex. In their analysis of 18,326 genes, they found some results similar to ours, in that genes encoding ion channels/transport proteins were highly expressed in their samples. Furthermore, metallothionein genes were highly expressed in the cortex in both their study and in ours. However, in contrast to the Yano group, we found higher-level expression of ribosomal genes in our medullary samples than in our cortex samples. Our data add a comprehensive profile of genes expressed in the medulla, papillary tips, and renal pelvis and permit a comparison of gene expression in cortex with other compartments of the kidney. Our inclusion of isolated glomeruli may prove particularly useful since genes in this cluster may be associated with genetic and immunologic glomerular diseases unique to the kidney.

Our attempt to categorize gene expression in the normal nephron via gross dissection of the renal lobe has some limitations. Some functionally distinct boundaries in the nephron are too small to permit gross dissection. The nephron takes a tortuous course through the renal lobe such that the proximal and distal convoluted tubules of the nephron are both located in the cortex. Furthermore, the normal kidney is composed of a variety of cell types other than the epithelium of the nephron. Thus, genes identified as expressed in the cortex may be expressed in the tubular epithelium, in the several different cell types of the glomeruli, in the peritubular capillaries or larger vessels, in the normal resident cells of the interstitium (Lemley and Kriz, 1991), or in infiltrating leukocytes or dendritic cells.

Despite these limitations, the power of DNA microarray technology for discovery of genes relevant to renal physiology and pathology is readily apparent on inspection of the genes that comprise the individual clusters. For example, the glomerular cluster contains 196 genes and includes GLEPP1, ZO-1, actin alpha 4, and osteonectin, all genes known to be expressed in the glomerulus and some documented to be involved in glomerulonephropathies. The "rediscovery" of these important genes using this technique indicates that many of the uncharacterized genes in this cluster will prove relevant to normal glomerular function or to glomerular diseases. One novel candidate gene is ficolin 3 (Hakata antigen) against which lupus patients' sera react, but expression of which has only been described in the liver and lung. Perhaps the most interesting discoveries to come from our microarray experiments are of renal expression of panels of apparently related but unexpected genes. The expression of detoxification genes in the renal cortex, of bile metabolism genes in the papillary tips, of genes involved in intestinal mucosal defense in the medulla suggests that the kidney may play a role in biological processes that were previously thought to be the exclusive domain of other organs. Alternatively, it may be that in the kidney these genes underlie functions other than those that have been previously recognized in other organs.

ACKNOWLEDGMENTS

The authors thank the Stanford Functional Genomics Facility (<http://www.microarray.org/sfgf/jsp/home.jsp>) and the Stanford Microarray Database (<http://genome-www.stanford.edu/microarray/>) for their help with this study. This work was supported by National Institutes of Health Grants CA85129 and CA84967, the Kidney Cancer Association Eugene P. Schonfeld

Award, and the Howard Hughes Medical Institute. P.O.B. is an Investigator of the Howard Hughes Medical Institute.

REFERENCES

- Akaiwa, M., Yae, Y., Sugimoto, R., Suzuki, S.O., Iwaki, T., Izuhara, K., and Hamasaki, N. (1999). Hakata antigen, a new member of the ficolin/opsonin p35 family, is a novel human lectin secreted into bronchus/alveolus and bile. *J. Histochem. Cytochem.* 47, 777-786.
- Alizadeh, A.A. *et al.* (2000). Distinct types of diffuse large B-cell lymphoma identified by gene expression profiling. *Nature* 403, 503-511.
- Amara, N., Palapattu, G.S., Schrage, M., Gu, Z., Thomas, G.V., Dorey, F., Said, J., and Reiter, R.E. (2001). Prostate stem cell antigen is overexpressed in human transitional cell carcinoma. *Cancer Res.* 61, 4660-4665.
- Arystarkhova, E., Wetzel, R.K., Asinowski, N.K., and Sweadner, K.J. (1999). The gamma subunit modulates Na(+) and K(+) affinity of the renal Na, K-ATPase. *J. Biol. Chem.* 274, 33183-33185.
- Bonilha, V.L., and Rodriguez-Boulan, E. (2001). Polarity and developmental regulation of two PDZ proteins in the retinal pigment epithelium. *Invest. Ophthalmol. Vis. Sci.* 42, 3274-3282.
- Bonthron, D.T., Brady, N., Donaldson, I.A., and Steinmann, B. (1994). Molecular basis of essential fructosuria: molecular cloning and mutational analysis of human ketohexokinase (fructokinase). *Hum. Mol. Genet.* 3, 1627-1631.
- Buhler, R., Pestalozzi, D., Hess, M., and Von Wartburg, J.P. (1983). Immunohistochemical localization of alcohol dehydrogenase in human kidney, endocrine organs and brain. *Pharmacol. Biochem. Behav.* 18(Suppl 1), 55-59.
- Canessa, C.M., Schild, L., Buell, G., Thorens, B., Gautschi, I., Horisberger, J.D., and Rossier, B.C. (1994). Amiloride-sensitive epithelial Na⁺ channel is made of three homologous subunits. *Nature* 367, 463-467.
- Cao, Y., Karsten, U., Zerbán, H., and Bannasch, P. (2000). Expression of MUC1, Thomsen-Friedenreich-related antigens, and cytokeratin 19 in human renal cell carcinomas and tubular clear cell lesions. *Virchows Arch.* 436, 119-126.
- Chu, F.F., Doroshov, J.H., and Esworthy, R.S. (1993). Expression, characterization, and tissue distribution of a new cellular selenium-dependent glutathione peroxidase, GSHPx-GI. *J. Biol. Chem.* 268, 2571-2576.
- Cox, T.M., Camilleri, M., O'Donnell, M.W., and Chadwick, V.S. (1982). Pseudodominant transmission of fructose intolerance in an adult and three offspring: Heterozygote detection by intestinal biopsy. *N. Engl. J. Med.* 307, 537-540.
- Danpure, C.J., and Jennings, P.R. (1986). Peroxisomal alanine:glyoxylate aminotransferase deficiency in primary hyperoxaluria type I. *FEBS Lett.* 201, 20-24.
- Dumont, D.J., Anderson, L., Breitman, M.L., and Duncan, A.M. (1994). Assignment of the endothelial-specific protein receptor tyrosine kinase gene (TEK) to human chromosome 9p21. *Genomics* 23, 512-513.
- Eisen, M.B., Spellman, P.T., Brown, P.O., and Botstein, D. (1998). Cluster analysis and display of genome-wide expression patterns. *Proc. Natl. Acad. Sci. USA* 95, 14863-14868.
- Floege, J., Alpers, C.E., Sage, E.H., Pritzl, P., Gordon, K., Johnson, R.J., and Couser, W.G. (1992). Markers of complement-dependent and complement-independent glomerular visceral epithelial cell injury in vivo. Expression of antiadhesive proteins and cytoskeletal changes. *Lab. Invest.* 67, 486-497.
- Fukutomi, T., Ando, B., Sakamoto, S., Sakai, H., and Nawata, H. (1996). Thermolabile beta-2 macroglycoprotein (Hakata antigen) in liver disease: biochemical and immunohistochemical study. *Clin. Chim. Acta* 255, 93-106.
- Garber, M.E. *et al.* (2001). Diversity of gene expression in adenocarcinoma of the lung. *Proc. Natl. Acad. Sci. USA* 98, 13784-13789.
- Gollub, J. *et al.* (2003). The Stanford Microarray Database: data access and quality assessment tools. *Nucleic Acids Res.* 31, 94-96.
- Hata, A., Lagna, G., Massague, J., and Hemmati-Brivanlou, A. (1998). Smad6 inhibits BMP/Smad1 signaling by specifically competing with the Smad4 tumor suppressor. *Genes Dev.* 12, 186-197.
- Higgins, J.P. *et al.* (2003). Gene expression patterns in renal cell carcinoma assessed by complementary DNA microarray. *Am. J. Pathol.* 162, 925-932.
- Higgins, J.P., and Warnke, R.A. (1999). CD30 expression is common in mediastinal large B-cell lymphoma. *Am. J. Clin. Pathol.* 112, 241-247.
- Inaba, S., Okochi, K., Yae, Y., Niklasson, F., and de Verder, C.H. (1990). Serological studies of an SLE-associated antigen-antibody system discovered as a precipitation reaction in agarose gel: the HAKATA antigen-antibody system. *Fukuoka Igaku Zasshi* 81, 284-291.

- Kaplan, J.M. *et al.* (2000). Mutations in ACTN4, encoding alpha-actinin-4, cause familial focal segmental glomerulosclerosis. *Nat. Genet.* 24, 251–256.
- Koszdin, K.L., and Bowen, B.R. (1992). The cloning and expression of a human alpha-1, 3 fucosyltransferase capable of forming the E-selectin ligand. *Biochem. Biophys. Res. Commun.* 187, 152–157.
- Kullak-Ublick, G.A., Hagenbuch, B., Stieger, B., Schteingart, C.D., Hofmann, A.F., Wolkoff, A.W., and Meier, P.J. (1995). Molecular and functional characterization of an organic anion transporting polypeptide cloned from human liver. *Gastroenterology* 109, 1274–1282.
- Kurihara, H., Anderson, J.M., Kerjaszki, D., and Farquhar, M.G. (1992). The altered glomerular filtration slits seen in puromycin aminonucleoside nephrosis and protamine sulfate-treated rats contain the tight junction protein ZO-1. *Am. J. Pathol.* 141, 805–816.
- Lemley, K.V., and Kriz, W. (1991). Anatomy of the renal interstitium. *Kidney Int.* 39, 370–381.
- Liu, Y. *et al.* (2000). Edg-1, the G protein-coupled receptor for sphingosine-1-phosphate, is essential for vascular maturation. *J. Clin. Invest.* 106, 951–961.
- Lobban, E.D., Smith, B.A., Hall, G.D., Harnden, P., Roberts, P., Selby, P.J., Trejdosiewicz, L.K., and Southgate, J. (1998). Uroplakin gene expression by normal and neoplastic human urothelium. *Am. J. Pathol.* 153, 1957–1967.
- Lu, P.C., Ye, H., Maeda, M., and Azar, D.T. (1999). Immunolocalization and gene expression of matrilysin during corneal wound healing. *Invest. Ophthalmol. Vis. Sci.* 40, 20–27.
- Mannervik, B. (1985). The isoenzymes of glutathione transferase. *Adv. Enzymol. Relat. Areas Mol. Biol.* 57, 357–417.
- Matsumura, Y. *et al.* (1999). Overt nephrogenic diabetes insipidus in mice lacking the CLC-K1 chloride channel. *Nat. Genet.* 21, 95–98.
- Misra, R.P. (1972). Isolation of glomeruli from mammalian kidneys by graded sieving. *Am. J. Clin. Pathol.* 58, 135–139.
- Nath, R., Kambadur, R., Gulati, S., Paliwal, V.K., and Sharma, M. (1988). Molecular aspects, physiological function, and clinical significance of metallothioneins. *Crit. Rev. Food Sci. Nutr.* 27, 41–85.
- Nichols, B.L., Avery, S., Sen, P., Swallow, D.M., Hahn, D., and Sterchi, E. (2003). The maltase-glucoamylase gene: common ancestry to sucrase-isomaltase with complementary starch digestion activities. *Proc. Natl. Acad. Sci. USA* 100, 1432–1437.
- Perou, C.M. *et al.* (2000). Molecular portraits of human breast tumours. *Nature* 406, 747–752.
- Radomska-Pandya, A., Little, J.M., and Czernik, P.J. (2001). Human UDP-glucuronosyltransferase 2B7. *Curr. Drug Metab.* 2, 283–298.
- Reczek, D., Berryman, M., and Bretscher, A. (1997). Identification of EBP 50, a PDZ-containing phosphoprotein that associates with members of the ezrin-radixin-moesin family. *J. Cell Biol.* 139, 169–179.
- Rodriguez-Barbero, A., Obreo, J., Eleno, N., Rodriguez-Pena, A., Duwel, A., Jerkic, M., Sanchez-Rodriguez, A., Bernabeu, C., and Lopez-Novoa, J.M. (2001). Endoglin expression in human and rat mesangial cells and its upregulation by TGF-beta1. *Biochem. Biophys. Res. Commun.* 282, 142–147.
- Ross, D.T. *et al.* (2000). Systematic variation in gene expression patterns in human cancer cell lines. *Nat. Genet.* 24, 227–235.
- Roy-Chaudhury, P., Simpson, J.G., and Power, D.A. (1997). Endoglin, a transforming growth factor-beta-binding protein, is upregulated in chronic progressive renal disease. *Exp. Nephrol.* 5, 55–60.
- Schena, M., Shalon, D., Davis, R.W., and Brown, P.O. (1995). Quantitative monitoring of gene expression patterns with a complementary DNA microarray. *Science* 270, 467–470.
- Schnabel, E., Anderson, J.M., and Farquhar, M.G. (1990). The tight junction protein ZO-1 is concentrated along slit diaphragms of the glomerular epithelium. *J. Cell Biol.* 111, 1255–1263.
- Simon, D.B., Karet, F.E., Hamdan, J.M., DiPietro, A., Sanjad, S.A., and Lifton, R.P. (1996). Bartter's syndrome, hypokalaemic alkalosis with hypercalciuria, is caused by mutations in the Na-K-2Cl cotransporter NKCC2. *Nat. Genet.* 13, 183–188.
- Stolz, A., Hammond, L., Lou, H., Takikawa, H., Ronk, M., and Shively, J.E. (1993). cDNA cloning and expression of the human hepatic bile acid-binding protein. A member of the monomeric reductase gene family. *J. Biol. Chem.* 268, 10448–10457.
- Suliman, Y., Opitz, O.G., Avadhani, A., Burns, T.C., El-Deiry, W., Wong, D.T., and Rustgi, A.K. (2001). p63 expression is associated with p53 loss in oral-esophageal epithelia of p53-deficient mice. *Cancer Res.* 61, 6467–6473.
- Thomas, P.E., Wharram, B.L., Goyal, M., Wiggins, J.E., Holzman, L.B., and Wiggins, R.C. (1994). GLEPP1, a renal glomerular epithelial cell (podocyte) membrane protein-tyrosine phosphatase. Identification, molecular cloning, and characterization in rabbit. *J. Biol. Chem.* 269, 19953–19962.
- Tureci, O. *et al.* (1998). Human carbonic anhydrase XII: cDNA cloning, expression, and chromosomal localization of a carbonic anhydrase gene that is overexpressed in some renal cell cancers. *Proc. Natl. Acad. Sci. USA* 95, 7608–7613.
- Tusher, V.G., Tibshirani, R., and Chu, G. (2001). Significance analysis of microarrays applied to the ionizing radiation response. *Proc. Natl. Acad. Sci. USA* 98, 5116–5121.
- Wang, S.N., Lapage, J., and Hirschberg, R. (2001). Loss of tubular bone morphogenetic protein-7 in diabetic nephropathy. *J. Am. Soc. Nephrol.* 12, 2392–2399.
- Wilson, C.L., Ouellette, A.J., Satchell, D.P., Ayabe, T., Lopez-Boado, Y.S., Stratman, J.L., Hultgren, S.J., Matrisian, L.M., and Parks, W.C. (1999). Regulation of intestinal alpha-defensin activation by the metalloproteinase matrilysin in innate host defense. *Science* 286, 113–117.
- Yano, N., Endoh, M., Fadden, K., Yamashita, H., Kane, A., Sakai, H., and Rifai, A. (2000). Comprehensive gene expression profile of the adult human renal cortex: analysis by cDNA array hybridization. *Kidney Int.* 57, 1452–1459.
- Yu, J.X., Chao, L., and Chao, J. (1995). Molecular cloning, tissue-specific expression, and cellular localization of human prostasin mRNA. *J. Biol. Chem.* 270, 13483–13489.
- Zhang, H. *et al.* (2001). Collectrin, a collecting duct-specific transmembrane glycoprotein, is a novel homolog of ACE2 and is developmentally regulated in embryonic kidneys. *J. Biol. Chem.* 276, 17132–17139.