Gene Expression Profiling of Renal Medullary Carcinoma

Potential Clinical Relevance

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BACKGROUND. Renal medullary carcinoma is a rare kidney tumor with highly aggressive behavior. This tumor occurs exclusively in young patients with sickle cell trait or disease. To the authors' knowledge, very little is known to date regarding the underlying molecular genetics of this tumor, and no effective therapy has been established.

METHODS. The authors analyzed the gene expression profiles of 2 renal medullary carcinomas from patients with sickle cell trait using microarrays containing 21,632 cyclic DNA (cDNA) clones and compared them with the gene expression profiles of 64 renal tumors.

RESULTS. Based on global gene clustering with 3583 selected cDNAs, the authors found a distinct molecular signature of renal medullary carcinoma, which clustered closely with urothelial (transitional cell) carcinoma of the renal pelvis, rather than renal cell carcinoma (RCC). This finding of a significant difference in the gene expression patterns of renal medullary carcinoma compared with RCC suggests that this tumor should not be treated as a conventional RCC but, rather, as a special malignancy. This study also identified genes/proteins that may serve as biomarkers for renal medullary carcinoma or as potential targets of novel therapies. In addition, comparative genomic microarray analysis allowed the authors to predict the lack of chromosomal imbalances in this tumor.

CONCLUSIONS. To the authors' knowledge, the current study is the first molecular profiling of renal medullary carcinoma, a rare but highly aggressive kidney carcinoma. The genes that are expressed specifically in this tumor may lead to not only a better understanding of its molecular pathways and discoveries of novel diagnostic markers but also, more important, to effective therapeutic interventions. *Cancer* **2004;100:976–85.** © *2004 American Cancer Society.*

KEYWORDS: molecular biology, microarray, renal carcinoma, medullary carcinoma, sickle cell.

Renal neoplasms include a variety of malignant and benign tumors, including several subtypes of renal cell carcinoma (RCC), oncocytoma, urothelial (transitional cell) carcinoma of the renal pelvis, and childhood Wilms tumor.[1] Traditionally, this classification is based on morphologic features defined in the World Health Organization *International Histological Classification of Kidney Tumors.*[2]

Renal medullary carcinoma is a rare tumor of the kidney that develops typically in young patients with sickle cell trait or disease.[3–6] This tumor is highly aggressive and routinely fatal, with nearly all patients dying of the disease within several months after diagnosis. Only very few patient have beenreported to have a response to chemotherapy and prolonged disease remission.[7,8] Metastases usually are present at the time of diagnosis, and the tumor is highly resistant

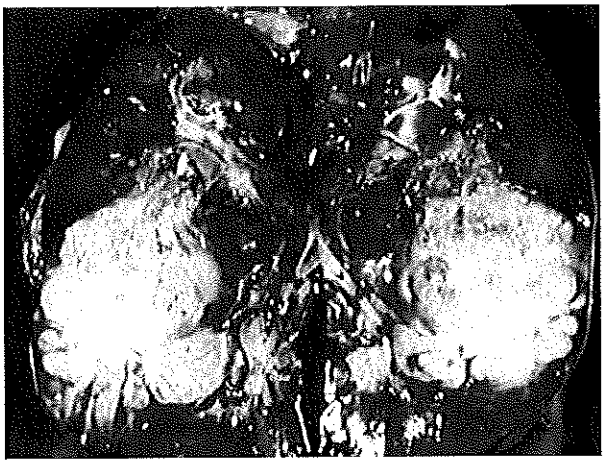


FIGURE 1. Macroscopic pathology. A lobulated tumor measuring 5.3 cm in greatest dimension showed areas of fibrosis, necrosis, and hemorrhage and occupied the renal medulla and cortex. The tumor had replaced the renal papilla adjacent to the calyx.

to conventional chemotherapy; therefore, the development of novel therapeutic agents is warranted.[9] The pathogenesis of renal medullary carcinoma is understood poorly. It is believed that this tumor arises from the epithelium of the calyceal or collecting duct system near the renal papillae. However, there is no biochemical or molecular evidence to substantiate this hypothesis. To our knowledge to date, only very few molecular genetic studies have been conducted on this tumor. Swartz et al. performed comparative genomic hybridization on nine tumors and found that eight tumors showed no changes, whereas one tumor exhibited loss of chromosome 22.[10] Conversely, Stahlschmidt et al. showed the presence of a bcr/abl rearrangement in one tumor.[8] No additional reports have been published that support this finding.

Recent advances in molecular technologies have allowed the genetic characterization of renal carcinoma, and several subtypes have been correlated with distinct genetic abnormalities. In particular, microarray technology has provided comprehensive gene expression profiling that may shed light on the underlying molecular mechanisms of these tumors. In addition to serving as molecular signatures, these gene expression profiles also may correlate with other clinical parameters, such as histologic subtype, prognostic grouping, prediction of survival, and drug response. Recently, we and others identified distinct molecular signatures for several types of renal carcinoma.[9–15]

In view of the lack of understanding of the pathophysiology and genetics of renal medullary carcinoma, we performed a comprehensive molecular profiling in 2 tumors using cyclic DNA (cDNA) gene expression microarrays and comparative genomic microarray analysis (CGMA) and compared the results with the expression profiles of 64 kidney tumors that represented 6 different histologic types. We hypothesized that such an analysis would enhance our general understanding of this disease and would identify potential, novel diagnostic and therapeutic targets for this lethal disease.

MATERIALS AND METHODS

Clinical History

Patient A, an otherwise healthy black woman age 21 years, presented with painless macroscopic hematuria. A large abdominal mass was found by physical examination, and computed tomography (CT) scanning revealed a large right kidney mass as well as retroperitoneal and mediastinal adenopathy and pulmonary metastases. Preoperative hemoglobin electrophoresis was significant for the presence of 50% sickle cell trait. Radical nephrectomy was performed for control of bleeding and local symptoms. Metastatic disease was treated with investigational bortezomib and, subsequently, with gemcitabine/capecitabine. The patient experienced only a minor response of brief duration to the gemcitabine/capecitabine and died 6 months after diagnosis.

Patient B, a black man age 20 years, was diagnosed with a renal mass, and a CT scan confirmed the presence of a mass lesion in the left kidney. Sickle cell trait was confirmed by hemoglobin electrophoresis. Radical nephrectomy was performed. Despite postoperative chemotherapy, the patient developed extensive pulmonary and bone metastases. He also died of metastatic disease 10 months after nephrectomy. Both patients had TNM Stage IV disease (T4N1M1) at the time they underwent nephrectomy.[16] Previously performed expression profiles for 64 kidney tumors (including 40 clear cell RCCs, 9 papillary RCCs, 5 chromophobe RCCs, 5 Wilms tumors, 3 urothelial carcinomas of the renal pelvis, and 2 renal oncocytomas) were used for comparisons.

Tumor Sample Processing

Institutional Review Board approvals were obtained from The Universities of Chicago, Northwestern University, and the Van Andel Research Institute to study medullary renal carcinoma. The tumor tissues, along with residual nonneoplastic kidney tissues for molecular analysis, were frozen in liquid nitrogen immediately after the kidneys were removed and were stored at − 80 °C. Portions of the tumors and benign kidneys were fixed in buffered formalin and underwent routine tissue processing for conventional light microscopy. A portion of the tumor from Patient A was fixed in phosphate-buffered glutaraldehyde for electron mi-

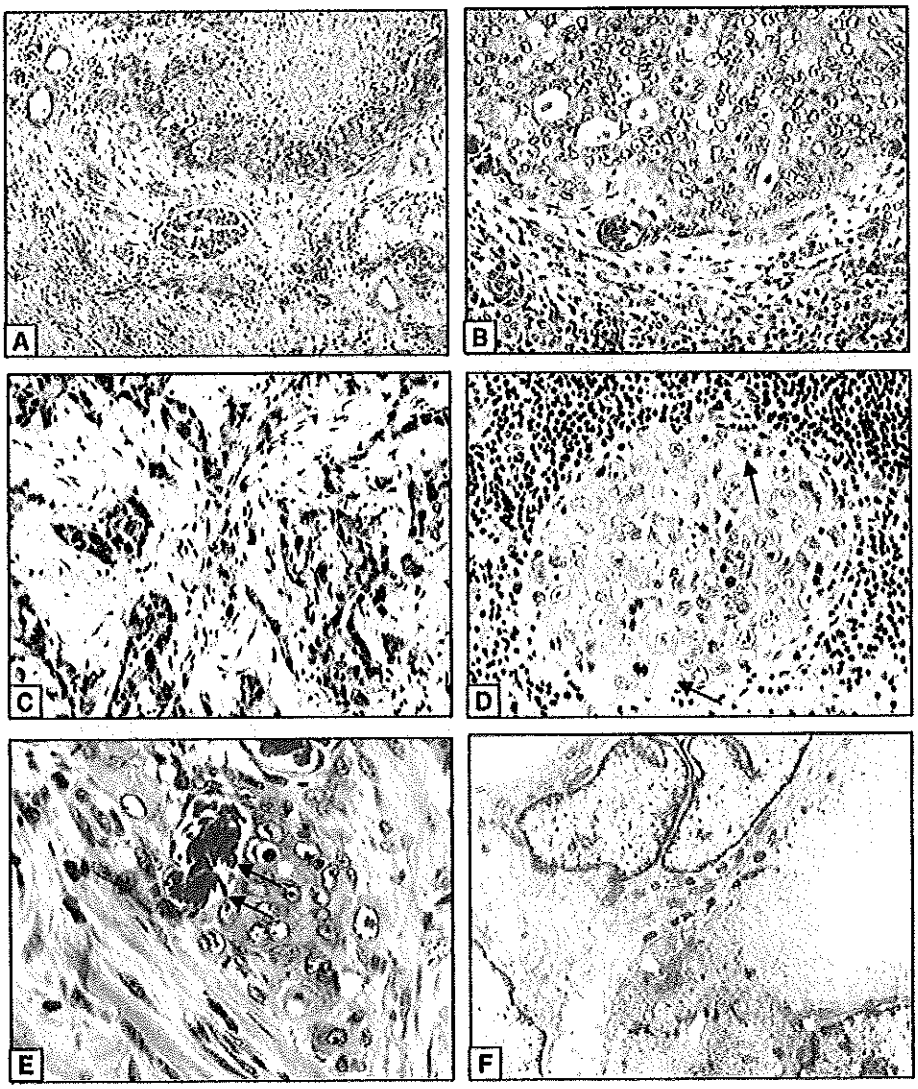


FIGURE 2. Light microscopy and electron microscopy. (A) Tumor cells comprised of nests or cords infiltrating renal parenchyma with areas of necrosis. (B) A cribriform pattern of tumor cells demonstrating intracellular pattern and inflammatory infiltrates. (C) Tumor cells forming infiltrating cords and fibrotic stroma. (D) A cluster of tumor cells with abundant, eosinophilic cytoplasm and rhabdoid features (arrows) metastasizing in lymph node. (E) Sickling red blood cells (arrows) in a capillary in a section of renal medullary carcinoma with intracellular lumen. (F) Ultrastructurally, tumor cells displayed tight junctions and intracellular lumen with microvilli.

croscopy. The tissue was postfixed in osmium tetraoxide. Thin sections were stained with lead citrate and uranyl acetate. Examination under a Philips CM10 electron microscope (Philips Medical Systems, Andover, MA) was performed as described previously.[17]

cDNA Gene Expression Microarray Studies

Microarray production was performed as described previously[18,19] with slight modification. Briefly, 21,632 cDNA clones that were amplified by polymerase chain reaction directly from bacterial stocks (purchased from Research Genetics, Huntsville, AL) were printed onto aminosilane-coated glass slides using a home-built, robotic microarrayer. Fifty micrograms of total RNA from tumors and from normal kidney reference tissues were reverse transcribed with oligo (dT) primer and Superscript II (Invitrogen, Carlsbad, CA) in the presence of indodicarbocyanine-deoxycytidine triphosphate (Cy5-dCTP) and indocarbocyanine (Cy3)-dCTP (Amersham Pharmacia Biotech, Peapack, NJ). The Cy5-labeled and Cy3-labeled cDNA probes were mixed with probe hybridization solution containing formamide. After hybridization, slides were washed and then dried by snap centrifugation, and they were scanned immediately using Scan Array Lite operating at 532-nanometer (nm) and 635-nm wavelengths (GSI Lumonics, Billerica, CA). Each experiment on medullary carcinomas was repeated three times.

Data Analysis

Images were analyzed by using the GENEPIX PRO software package (version 3.0; Axon Instruments, Foster City, CA). The local background was subtracted from the remaining spots, and the ratios of net fluo-

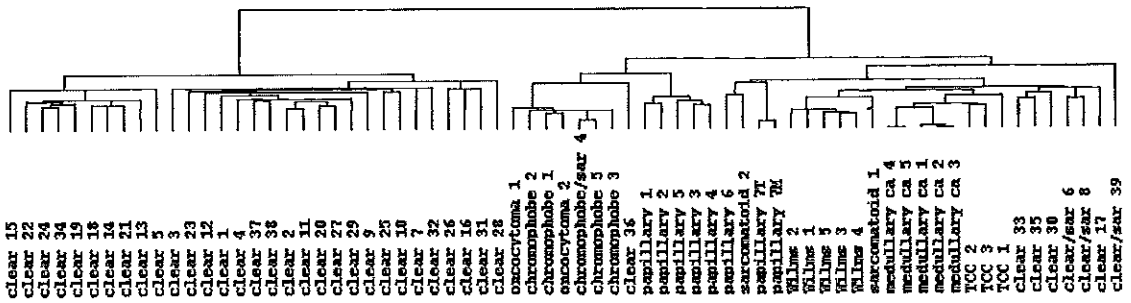


FIGURE 3. Molecular clustering of renal medullary carcinoma and other renal tumors. The clustering of patients (using Pearson correlation) is based on the gene expression profiles comprised of median polished data from 4569 selected spots. Rows represent individual cyclic DNAs (cDNAs), and columns represent individual tumor samples. The color of each square represents the median polished, normalized ratio of gene expression in a tumor relative to reference. Expression levels greater than the median were shaded in red and expression levels below the median were shaded in green (black, equal to the median; gray, inadequate or missing data). The color saturation indicates the degree of divergence from the median. Medullary carcinoma (ca) 1, 2, and 3 represented triplicates of hybridization experiments using tumor RNA from Patient A. Medullary ca 4 and 5 represented duplicates of hybridization experiments using tumor RNA from Patient B. Supervised clustering-based 487 cDNA showed that the tumors closest in resemblance to renal medullary carcinomas are urothelial (transitional cell) carcinomas (TCC).

rescence from the Cy5-specific channel to the net fluorescence from the Cy3-specific channel were calculated for each spot, representing tumor RNA expression relative to the normal kidney total RNA. Ratios were log-transformed (base 2) and normalized so that the average log-transformed ratio equaled zero. Genes with good data present in 70% of the experiments and with expression ratios that varied at least 2-fold in at least 2 experiments were selected for the clustering analysis (3583 genes). The gene ratios were "'median polished"' before hierarchical clustering using CLUSTER and visualized using TREEVIEW (available from URL: http: yyrana.lbl.gov). The correlation distances were calculated as $1 - r$, in which r indicates the Pearson correlation coefficient.[20] CIT software was used to find genes that were expressed differentially (using a Student t test) between one histologic subtype and the others.[21] To identify significant discriminating genes, 10,000 t statistics were calculated by dividing patients randomly into 2 groups.[22] A 99.9% significance threshold ($\alpha \leq 0.01$) was used to identify genes that could distinguish significantly between 2 patient groups versus the random patient groupings.

CGMA

Gene expression values were adjusted using within-print tip group normalization followed by scale normalization procedures[23] using the BioConductor software packages (available from URL: www.bioconductor.org) for the R environment.[24] Gene expression values that mapped to each chromosomal arm were collected, and a binomial test was used to determine whether a significant upward or downward bias was present. Sequence comparisons were used to map microarray probe sequences to predicted Ensembl transcripts (Ensembl, version 10.2).[25] Expression values from multiple probes that mapped to the same gene were condensed into a single value by averaging. To apply the binomial test, for n nonzero expression values that mapped to a given chromosomal arm, r gene expression values were scored as "up" if the log2(R/G) value was positive and (n − r) values were scored as "down" if the log-transformed ratio was negative. The test assumed that the frequency of positive values (p) and negative values (q) were equal, such that $p = q = 0.5$. For significant biases ($a = 0.003$), a summary statistic was calculated using the normal approximation to the binomial distribution, such that $z = (2r - n)/\mathrm{sqrt}(n)$. The set of summary statistics was plotted as a heat map to identify predicted cytogenetic features and to predict cytogenetic alterations.[19]

Immunohistochemistry

Immunostaining was performed on 5-μm-thick, formalin fixed paraffin sections using the biotin-avidin system with mouse monoclonal antibodies, respectively, specific for cytokeratin 19 (Dako Corporation, Carpinteria, CA) and DNA topoisomerase II alpha (Topo II α; Vector Laboratories, Burlingame, CA), as described previously.[26]

RESULTS

Pathologic findings

Macroscopically, the kidney from Patient A, including the tumor, weighed 570 g. The tumor occupied 90% of the kidney and measured 9.6 cm in greatest dimension. Sectioning revealed an ill-defined, yellow tumor with necrosis, hemorrhage, and invasion into the main renal vein and perinephritic adipose tissue. The tumor from Patient B weighed 355 g (including the

TABLE 1
Top 40 Genes Overexpressed (Up-Regulated) in Renal Medullary Carcinomas

Accession ID	Gene name	Fold change
AA677534	Laminin, gamma 2 (nicein [100 kD], kalinin [105 kD], BM600 [100 kD])	61.54
R32848	S100 calcium-binding protein P	53.08
N26285	Fibronectin 1	51.65
AA620995	*Homo sapiens* normal mucosa of esophagus specific 1 (NMES1)	43.74
AA845156	Serine protease inhibitor, Kazal type 1	42.11
AA001432	Laminin, alpha 3 (nicein [150 kD], kalinin [165 kD], BM600 [150 kD]), epilegrin)	34.96
N27159	Inhibin, beta A (activin A, activin AB alpha polypeptide)	33.73
N92646	ESTs	22.94
AA194833	Claudin 1	22.65
AA031514	Matrix metalloproteinase 7 (matrilysin, uterine)	22.38
AA172400	Retinoic acid induced 3	21.07
N33590	ESTs	21.04
AA478623	Cathepsin B	20.54
AA663981	Immunoglobulin heavy constant gamma 3 (G3m marker)	20.27
AA452840	Fibulin 2	18.10
T70329	ESTs, weakly similar to ALU7_human ALU subfamily SQ sequence (*H. sapiens*)	17.21
AA448261	High-mobility group (nonhistone chromosomal) protein isoforms I and Y	15.42
T54298	PPAR(gamma) angiopoietin related protein	14.40
AA419015	Annexin A4	14.25
W90740	Collagen, Type III, alpha I (Ehlers–Danlos syndrome Type IV autosomal dominant)	14.09
AA101875	Chondroitin sulfate proteoglycan 2 (versican)	14.07
AA485683	ADP-ribosylation factor-like 7	13.82
N33920	Diubiquitin	13.62
AA425806	Deformed epidermal autoregulatory factor 1 (*Drosophila*)	13.22
AA042990	Sema domain, immunoglobulin domain (Ig), short basic domain, secreted, 3C	13.04
AA488070	Immunoglobulin kappa constant	12.29
AA173454	Macrophage stimulating 1 receptor (c-met-related tyrosine kinase)	12.27
AA156781	ESTs	11.98
H84154	Cyclin D2	11.91
H48533	Baculoviral IAP repeat-containing 3	11.66
H38240	Thrombospondin 2	11.47
T67053	Immunoglobulin lambda locus	11.20
W84711	Fibronectin 1	11.01
AA460152	Serum-inducible kinase	10.98
AA026682	Topoisomerase (DNA) II alpha (170 kD)*	10.79
N50114	Phosphoprotein associated with GEMs	10.77
AA443638	Synuclein, gamma (breast cancer-specific protein 1)	10.68
H44051	ESTs	10.66
H93328	Hypothetical protein	10.11
AA464250	Keratin 19*	9.796

* The expression of these two genes was confirmed by immunohistochemistry

kidney) and measured 5.3 cm in greatest dimension. The tumor was lobular, occupying the medulla and cortex of the lower pole, with a yellow-tan cutting surface and focal necrosis (Fig. 1); and it invaded the renal vein macroscopically, although the renal capsule was intact.

Microscopically, tumor cells formed sheets, nests, cords, or glandular structures and extensively invaded the renal parenchyma (Fig. 2A) with areas of necrosis, fibrosis, and inflammatory infiltrates(Fig. 2B,C). Tumor cells were large with moderate or occasionally abundant cytoplasm that had rhabdoid features (Fig. 2D). Mitotic figures were numerous. Red blood cells within the specimen displayed sickle cell morphology (Fig. 2E). In both patients, metastases were identified in the lymph nodes and adrenal glands at the time of surgery.

Electron Microscopy

Ultrastructurally, the tumor cells from Patient A formed irregularly shaped glands with intracellular lumen containing small, finger-like cytoplasmic projects (microvilli) and well formed desmosomes (Fig. 2F).

cDNA Expression Microarray

We compared the expression profiles of renal medullary carcinoma with normal kidney RNA by hybridization to 21,632 cDNA microarrays along with previously

TABLE 2
Top 40 Genes Underexpressed (Down-Regulated) in Renal Medullary Carcinoma

Accession ID	Gene name	Fold change
AA664180	Glutathione peroxidase 3 (plasma)	0.0120
R61229	Glycine amidinotransferase (L-arginine:glycine amidinotransferase)	0.0133
AA863424	Dipeptidase 1 (renal)	0.0133
AA775899	FXYD domain-containing ion transport regulator 2	0.0140
R59722	Hypothetical protein FLJ10851	0.0160
T58896	Betaine-homocysteine methyltransferase	0.0200
W95082	Hydroxysteroid (11-beta) dehydrogenase 2	0.0200
AA872602	Parathyroid hormone receptor 1	0.0200
AA010605	4-Hydroxyphenylpyruvate dioxygenase	0.0200
T98253	ESTs, weakly similar to aldolase B (*H. sapiens*)	0.0220
H63534	Methylmalonate-semialdehyde dehydrogenase	0.0280
N92148	*H. sapiens* HRBPiso mRNA, complete cds	0.0300
AA497001	Hypothetical protein FLJ20920	0.0300
AA863449	Oviductal glycoprotein 1, 120 kD (mucin 9, oviductin)	0.0300
H11346	Aldehyde dehydrogenase 4 family, member A1	0.0320
AA504891	Crystallin, alpha B	0.0340
N62179	ESTs	0.0380
R91950	Cytochrome b-5	0.0400
AA858026	Serine (or cysteine) proteinase inhibitor, clade A, member 5	0.0400
R54416	MAWD-binding protein	0.0433
W37112	Potassium inwardly rectifying channel, subfamily J, member 16	0.0450
R92737	Aquaporin 3	0.0467
AA451904	Epididymis-specific, whey-acidic protein type, four-disulfid; putative ovarian carcinoma marker	0.0480
H50667	Uncharacterized bone marrow protein BM042	0.0500
H62163	Hepsin (transmembrane protease, serine 1)	0.0540
T94781	Potassium inwardly-rectifying channel, subfamily J, member 15	0.0567
AA457718	*H. sapiens* mRNA; cDNA DKFZp564B076 (from clone DKFZp564B076)	0.0567
AA699427	Fructose-1,6-bisphosphatase 1	0.0600
T65736	Selenium-binding protein 1	0.0600
H53340	Metallothionein 1G	0.0620
N72715	Translational inhibitor protein p14.5	0.0640
R42685	Inositol(myo)-1 (or 4)-monophosphatase 2	0.0640
H37827	L-pipecolic acid oxidase	0.0650
N93191	*H. sapiens* cDNA: FLJ22811 fis, clone KAIA2944	0.0650
H24316	Aquaporin 1 (channel-forming integral protein; 28 kD)	0.0660
AA011096	Monoamine oxidase A	0.0667
R44346	ESTs, weakly similar to T26845 hypothetical protein Y43F4B.7 (*Caenorhabditis elegans*)	0.0680

obtained data from 64 renal tumors.[11] The data were analyzed in two ways. First, we compared the gene expression of renal medullary carcinoma with normal kidney RNA to identify gene expression alterations (increase or decrease). Then, we sought to identify particular subsets of genes that defined most strongly the division of renal medullary carcinoma from other kidney tumors.

Hierarchic clustering[20] was used to classify renal medullary carcinoma and was based on gene expression profiles using the expression ratios of a selected set of 3583 cDNAs . The clustering algorithm grouped both genes and tumors by similarity in expression pattern. Clustering based on the total gene expression profile is shown in Figure 3. A patient dendrogram is shown based on the entire set of 3583 cDNAs, and the gene expression pattern was based on 487 genes that were expressed differentially in renal medullary carcinoma compared with all other types of renal tumors. The renal medullary carcinoma clustered most closely with urothelial carcinoma and with Wilms tumors to a certain degree (Fig. 3).

The top 40 genes that were overexpressed in renal medullary carcinoma are listed in Table 1. The increased levels ranged from 9.79-fold to 61.54-fold compared with normal kidney tissue. The top 40 genes with significantly decreased expression in renal medullary carcinoma, ranging from 83.33-fold (fold change = 0.012) to 14.70-fold (fold change = 0.068) compared with normal kidney tissues, are listed in Table 2. We also identified a set of genes in renal medullary carcinoma that were expressed at a significantly different levels compared with other renal tumor subtypes (Table 3) and thus have potential as specific distinguishing markers.

TABLE 3
Differentially Expressed Genes in Renal Medullary Carcinoma[a]

Accession ID	Gene name	Fold change	*P* value
H11346	Aldehyde dehydrogenase 4	−11.3	0.034
N26658	Transforming growth factor, beta receptor III	−5.3	0.034
AA875888	Neuron-specific protein	−4.9	0.033
AA432152	Hypothetical protein MGC11034	−3.2	0.034
H93086	ESTs	−2.8	0.033
N23174	Solute carrier family 7	−2.7	0.034
W67200	RNA-binding protein gene with multiple splicing	−2.4	0.033
AA482119	Inhibitor of DNA binding 3, dominant negative	2.7	0.034
AA401693	CD163 antigen	2.8	0.033
AA449678	ESTs	2.9	0.033
AA194833	Claudin 1	3.2	0.034
H23235	Platelet-derived growth factor receptor, alpha	3.3	0.034
H18630	KIAA0523 protein	3.5	0.034
N54338	B7 homolog 3	3.6	0.034
T67053	Immunoglobulin lambda-like polypeptide 1	6.7	0.034
AA455925	Four and one-half LIM domains 1	8.8	0.034
AA284669	Plasminogen activator	11.8	0.034
R66139	Small inducible cytokine subfamily D	12.4	0.034

[a] These genes were significantly overexpressed or under-expressed in renal medullary carcinoma compared with other types of renal tumors studied by 10,000 times of permutation test.

CGMA

CGMA profiles were generated using the expression profiles of the two renal medullary carcinomas (duplicates from each tumor) and a set of clear cell RCCs (seven tumors) and papillary RCCs (four tumors) and were displayed as a heat map. The results of CGMA in renal medullary carcinomas and in other RCCs are shown in Figure 4 using two-color microarray gene expression profiling data. In each tumor, the underlying gene expression profile was generated such that each tumor transcript expression value was compared with the expression value from the pooled normal kidney tissues. Chromosomal regions with a significant number of down-regulated genes (indicating a genomic loss) were shown in blue, and genomic regions with a significant number of up-regulated genes (indicating a genomic gain) were shown in red (Fig. 4). A sign test for a sample mean was used to determine whether a significant expression bias was present in each chromosomal region. The lowest intensity color indicates that the sign test z-statistic for that region was at least 1.96 ($P = 0.05$), and the highest intensity color indicates a z-statistic of at least 3.26 ($P = 0.001$). The clear cell RCCs showed the characteristic loss of chromosome 3p, whereas the papillary RCCs revealed the expected gain of chromosomes 7, 16, and 17.[27,28] No evident chromosomal gains or losses were observed in the two medullary carcinomas.

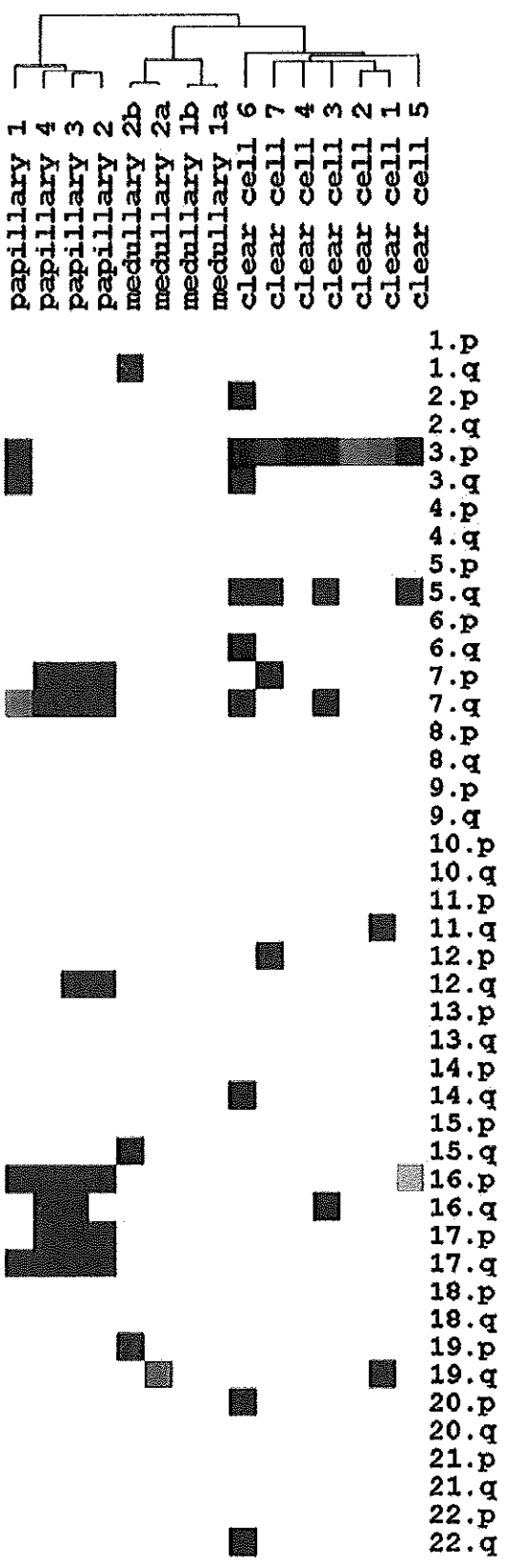
Immunohistochemistry

Immunohistochemistry showed tumor cells that were strongly positive for keratin 19 (Fig. 5A) and Topo II (Fig. 5B), confirming the overexpression identified by cDNA microarrays.

DISCUSSION

Renal medullary carcinoma is essentially a lethal disease with a median survival of 15 weeks. At the time of diagnosis, most patients already have developed widespread, metastatic disease; and to our knowledge, no effective systemic therapy has been described to date. Our molecular profiling of renal medullary carcinoma does not cluster with clear cell or papillary RCC, indicating that it has a unique biology and supporting the clinical observation that its behavior is distinct from classic RCC. Therefore, it is logical that the tumor should be treated differently from RCC, and novel therapeutic agents should be considered based on its expression profiles.

Several genes that were overexpressed in renal medullary carcinoma and are listed in Table 1 may have clinical relevance. For example, RNA expression of DNA Topo II α, an enzyme that controls the topologic state of DNA, is increased significantly (10.79-fold), and its protein overexpression was confirmed by immunohistochemistry. Recently, we reported the finding that the most overexpressed gene in Wilms tumors was Topo II α (80.8-fold),[15,37] whereas Wilms tumor is very sensitive to chemotherapy, including a number of Topo II α inhibitors (such as actinomycin D, doxorubicin, and etoposide). Therefore, these Topo II α inhibitors may be effective as a good first-line chemotherapy. Macrophage-stimulating 1 receptor, a tyrosine kinase, also is increased 12.27-fold. This find-



ing suggests the potential utility of tyrosine kinase inhibitors, such as imatinib mesylate, PKC412, and STI571, in the treatment of this disease. In agreement with recent studies of clear cell RCC,[11,29] we also found high expression levels of angiogenesis-related genes, including peroxisome proliferator-activated receptor gamma angiopoeitin-related gene.[29] Therefore, anti-angiogenesis agents also may be helpful in treating this disease.

In addition, our studies suggested several genes with highly elevated expression levels that may serve as potential biomarkers for the disease. If some of these overexpressed genes can be detected in the serum or urine from patients with renal medullary carcinoma, then they potentially may be used for the detection of disease and recurrence. One of the highly overexpressed genes in renal medullary carcinoma is the activin A gene (33.73-fold). Activin A (also known as inhibin beta A), a member of the transforming growth factor beta superfamily, regulates cell growth and apoptosis, although to our knowledge its functions are not understood fully. In the kidney, activin A appears to be related to renal tubular regeneration after ischemic injury. It is possible that activin A may be generated from the necrosis of renal papillary tubular epithelium or renal medullary carcinoma cells. It is noteworthy that activin A levels can be measured in serum or urine.[30] Whether this protein may be used as a diagnostic marker of renal medullary carcinoma needs to be established.

The origin and pathogenesis of renal medullary carcinoma is unknown. It shares some similarities with high-grade urothelial carcinoma with regard to its location, infiltrating pattern, and tumor cell morphology. It was believed previously that it originated from collecting ducts or the calycle epithelium[5] and has been proposed as a special variant of collecting duct carcinoma of kidney.[31] Indeed, the close clustering of renal medullary carcinoma and urothelial (transitional cell) carcinoma (Fig. 3) appears to support the

FIGURE 4. Comparative genomic microarray analysis (CGMA). Using two-color microarray gene expression profiling data, CGMA profiles of renal medullar carcinoma (two samples, each with duplicate experiments) were generated, compared with a set of clear cell (seven samples) and papillary renal cell carcinoma (four samples) expression profiles, and displayed as a heat map. Red bars: chromosomal regions with significant numbers of up-regulated genes (indicating a genomic gain); blue bars: chromosomal regions with significant numbers of down-regulated genes (indicating a genomic loss). A sign test for a one-sample mean was used to determine whether significant expression bias was present in each region. The lowest intensity color indicates that the sign test z-statistic for that region was ≥ 1.96 ($P = 0.05$), and the highest intensity color indicates a z-statistic of ≥ 3.26 ($P = 0.001$).

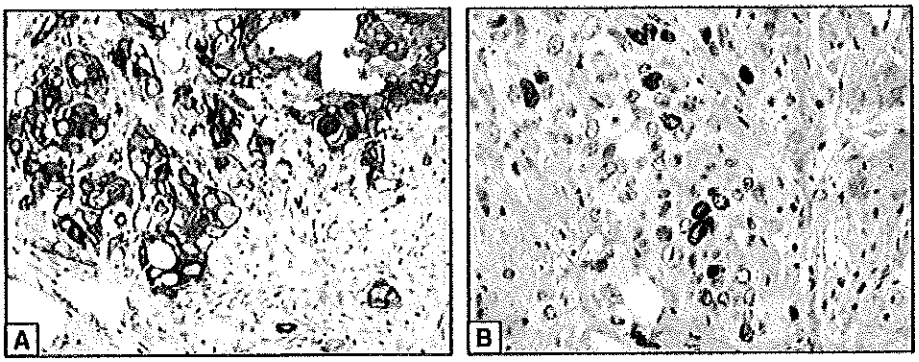


FIGURE 5. Immunohistochemistry. (A) Tumor cells showing immunoreactivity to keratin 19. (B) Tumor cells demonstrating positive nuclear staining for topoisomerase II α.

notion that this malignancy closely resembles urothelium of the renal pelvis. It is noteworthy that, despite its highly malignant behavior, no macroscopic chromosomal abnormalities could be detected, an observation supported by previous studies.[10] The tumors most likely harbor small mutation(s) of critical cancer-related genes that are beyond detection by comparative genomic hybridization, CGMA, or conventional cytogenetic methods. This finding further indicates the significance of studying specific gene expression patterns in this tumor by cDNA microarrays and other molecular and biochemical methods.

The molecular profiles of renal medullary carcinoma and urothelial carcinoma both are characterized by markedly elevated extracellular matrix genes, such as laminin alpha 3 and gamma 2, fibronectin 1, collagen Type III, and fibulin 2. To date, numerous studies have reported elevated expression and altered processing of these genes in several types of carcinoma,[32–34] including carcinomas of the kidney.[35] These genes may act alone or may interact with metalloproteinases in tumor progression, invasion, and metastasis.[36] Their overexpression also may explain the aggressive behavior of this tumor.

The unique expression profile of renal medullary carcinoma also may be used to identify putative diagnostic markers.[37] Genes that can distinguish one renal carcinoma subtype from others can be selected; then, appropriate antibodies can be obtained or produced for more comprehensive analysis by immunohistochemistry. For example, it was found that keratin 19, a smaller keratin in the keratin family of epithelial markers, was overexpressed in both renal medullary carcinoma and urothelial carcinoma, but not in RCC. We also confirmed keratin 19 overexpression in renal medullary carcinoma cells by immunohistochemistry. The overexpression of keratin 19 has been reported in urothelial carcinomas, but not in conventional RCCs.[38] Therefore, keratin 19 immunostaining can be used to distinguish renal medullary carcinoma from conventional RCC. Overexpression of the IGFII gene can differentiate between renal medullary carcinoma and closely related urothelial carcinoma. We recently reported the overexpression of IGFII gene in Wilms tumors (22.1-fold) by microarray analysis.[15] However, IGFII is not elevated typically in other adult renal tumors, including urothelial carcinoma.[11] The significant elevation of IGFII in renal medullary carcinoma may correlate with its accelerated proliferation. These findings suggest molecular similarity between renal medullary carcinoma and Wilms tumors of childhood; although, morphologically, these two tumor types barely demonstrate any resemblance.

In the current study, we report what to our knowledge is the first gene profiling of a rare but highly malignant renal medullary carcinoma. These findings identified distinct genes that were expressed in this tumor compared with the genes expressed in other types of kidney tumors. These differentially expressed genes in renal medullary carcinoma may provide better insights into the pathogenesis of this disease and may lead to potential diagnostic and therapeutic innovation.

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