US008361721B2

(12) **United States Patent**
Copland et al.

(10) **Patent No.: US 8,361,721 B2**
(45) **Date of Patent: Jan. 29, 2013**

(54) **METHODS FOR DETECTING, DIAGNOSING AND TREATING HUMAN RENAL CELL CARCINOMA**

(75) Inventors: **John A. Copland**, Ponte Vedra Beach, FL (US); **Bruce A. Luxon**, Galveston, TX (US); **Christopher G. Wood**, Pearland, TX (US)

(73) Assignee: **Board of Regents, The University of Texas System**, Austin, TX (US)

(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 0 days.

(21) Appl. No.: **13/136,588**

(22) Filed: **Aug. 4, 2011**

(65) **Prior Publication Data**

US 2011/0294694 A1 Dec. 1, 2011

Related U.S. Application Data

(63) Continuation of application No. 10/938,973, filed on Sep. 10, 2004, now abandoned.

(60) Provisional application No. 60/539,838, filed on Jan. 28, 2004, provisional application No. 60/502,038, filed on Sep. 10, 2003.

(51) **Int. Cl.**
C12Q 1/68 (2006.01)

(52) **U.S. Cl.** **435/6.1**; 435/6.14

(58) **Field of Classification Search** ..... None
See application file for complete search history.

(56) **References Cited**

U.S. PATENT DOCUMENTS

2003/0224040 A1* 12/2003 Baylin et al. 424/450

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Gumz et al. (Clin. Cancer Res. Aug. 15, 2007, 13(16): 4740-4749).*
P-SCAN (Peak quantification using Statistical Comparative Analysis, http://abs.cit.nih.gov/pscan/, Jan. 23, 2003).*

* cited by examiner

Primary Examiner — Peter J Reddig
(74) *Attorney, Agent, or Firm* — Edwin S. Flores; Chalker Flores, LLP

(57) **ABSTRACT**

Gene expression profiling and hierarchical clustering analysis readily identify differential gene expressions in normal renal epithelial cells and renal cell carcinomas. Genes identified by this analysis would be useful for diagnosis, prognosis and development of targeted therapy for the prevention and treatment of conventional renal cell carcinoma.

3 Claims, 20 Drawing Sheets

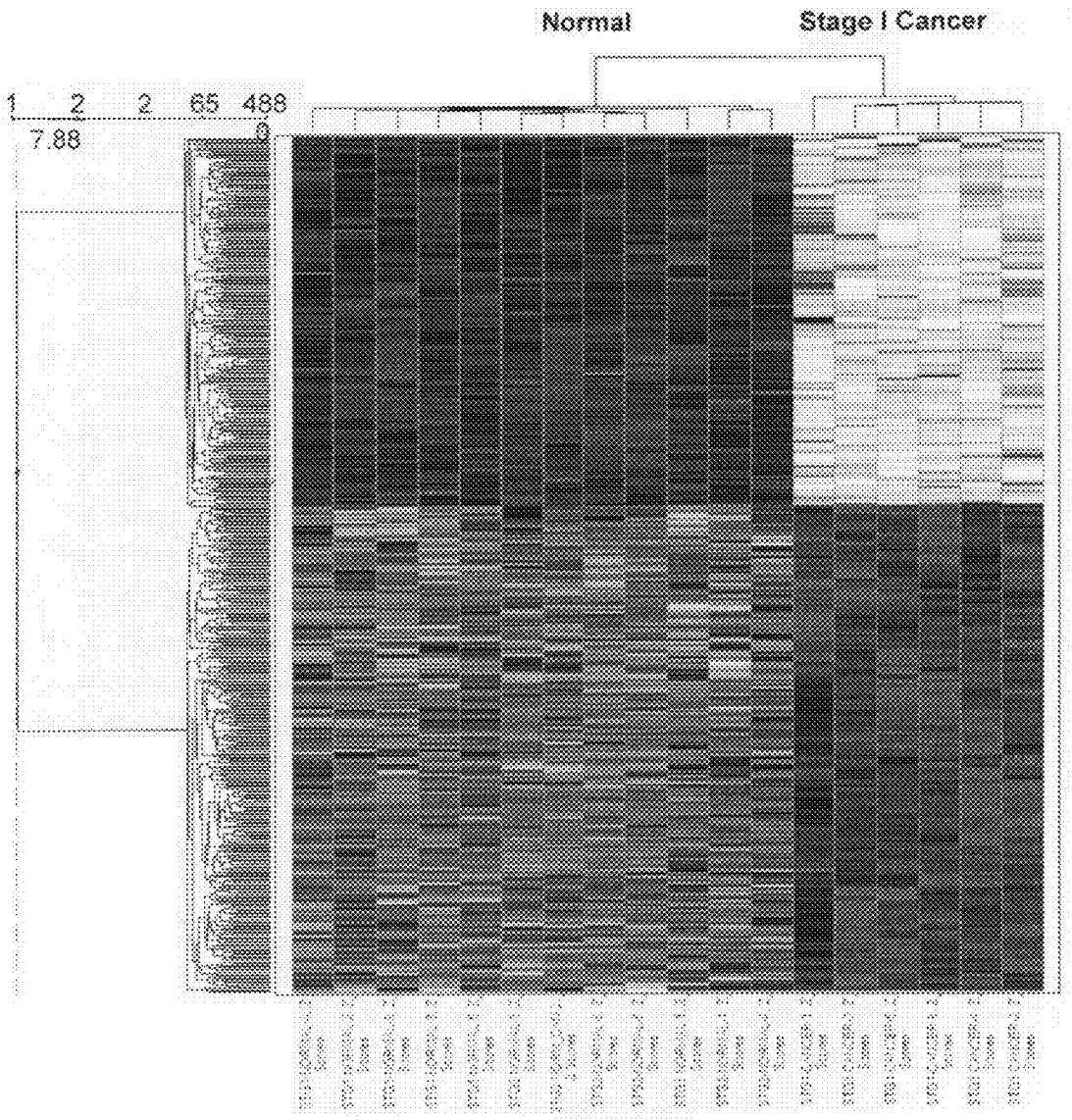

FIG. 1A

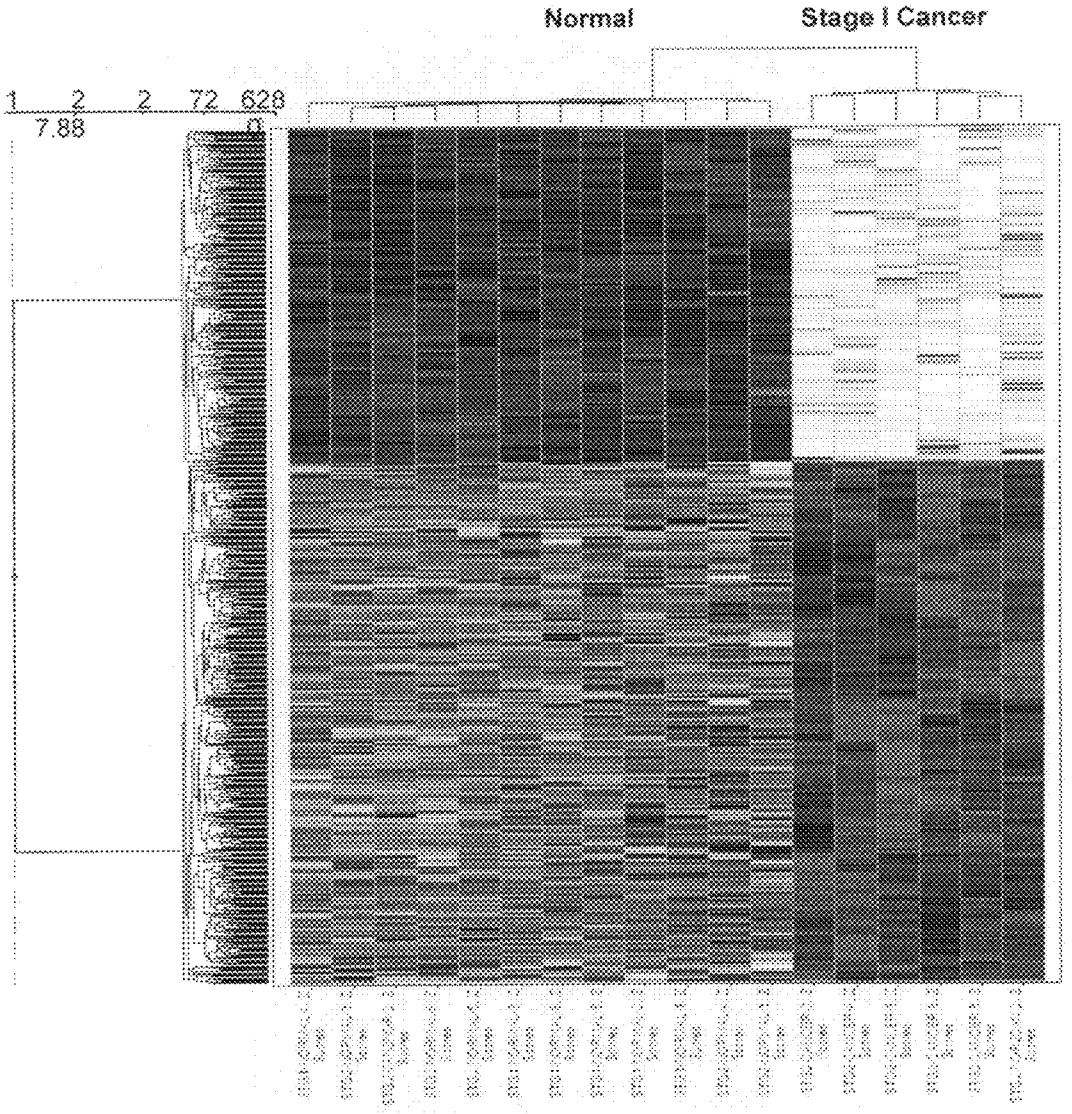

FIG. 1B

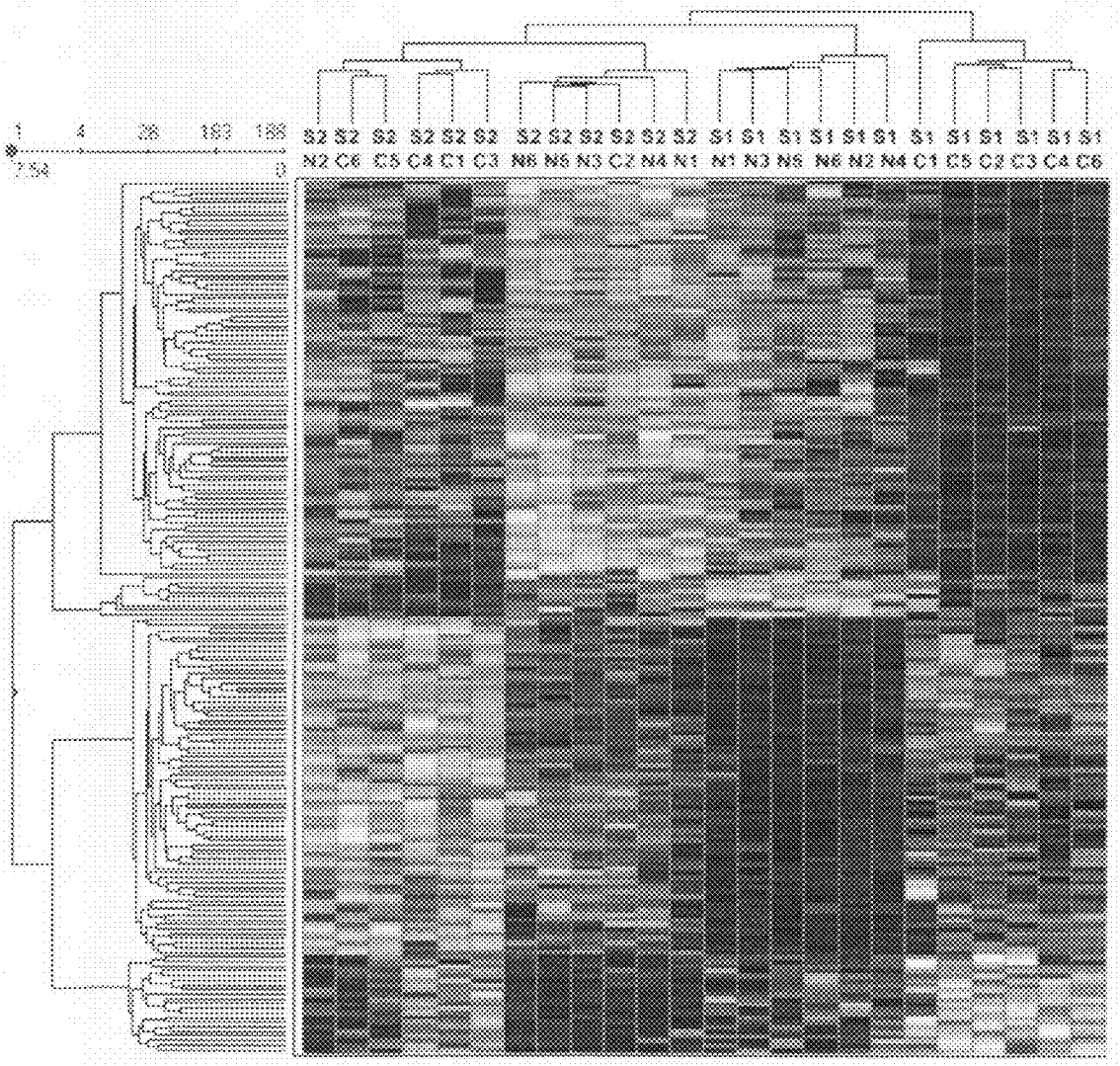
1 4 26 163 186
7.58 0
S2 S2 S2 S2 S2 S2 S2 S2 S2 S2 S2 S2 S1 S1 S1 S1 S1 S1 S1 S1 S1 S1 S1 S1
N2 C6 C5 C4 C1 C3 N6 N5 N3 C2 N4 N1 N7 N3 N5 N6 N2 N4 C1 C5 C2 C3 C4 C6

FIG. 1C

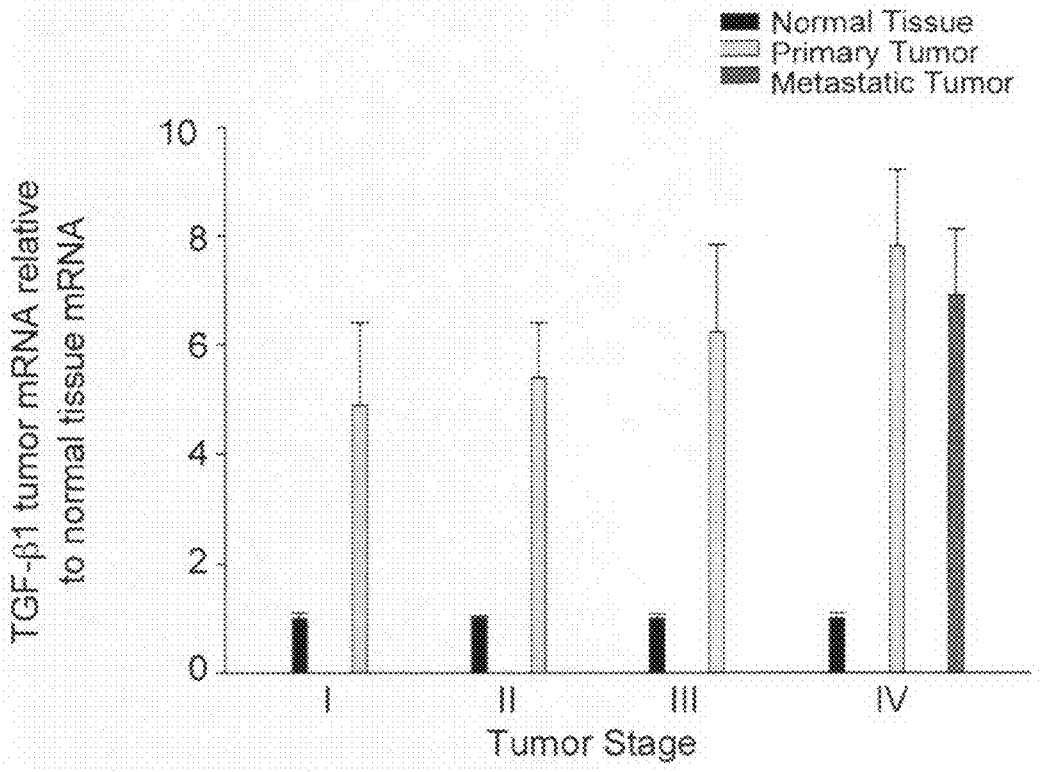
Normal Tissue
Primary Tumor
Metastatic Tumor
TGF-β1 tumor mRNA relative to normal tissue mRNA
10
8
6
4
2
0
I
II
III
IV
Tumor Stage

FIG. 2

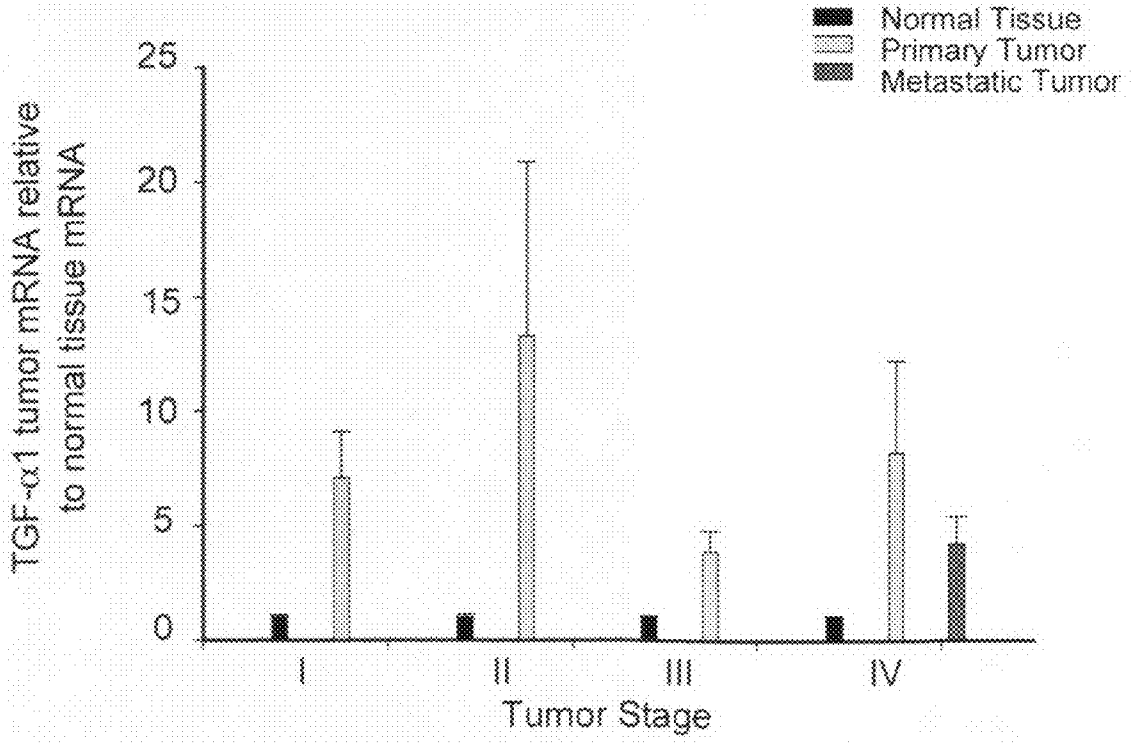
Normal Tissue
Primary Tumor
Metastatic Tumor
TGF-α1 tumor mRNA relative to normal tissue mRNA
25
20
15
10
5
0
I
II
III
IV
Tumor Stage

FIG. 3

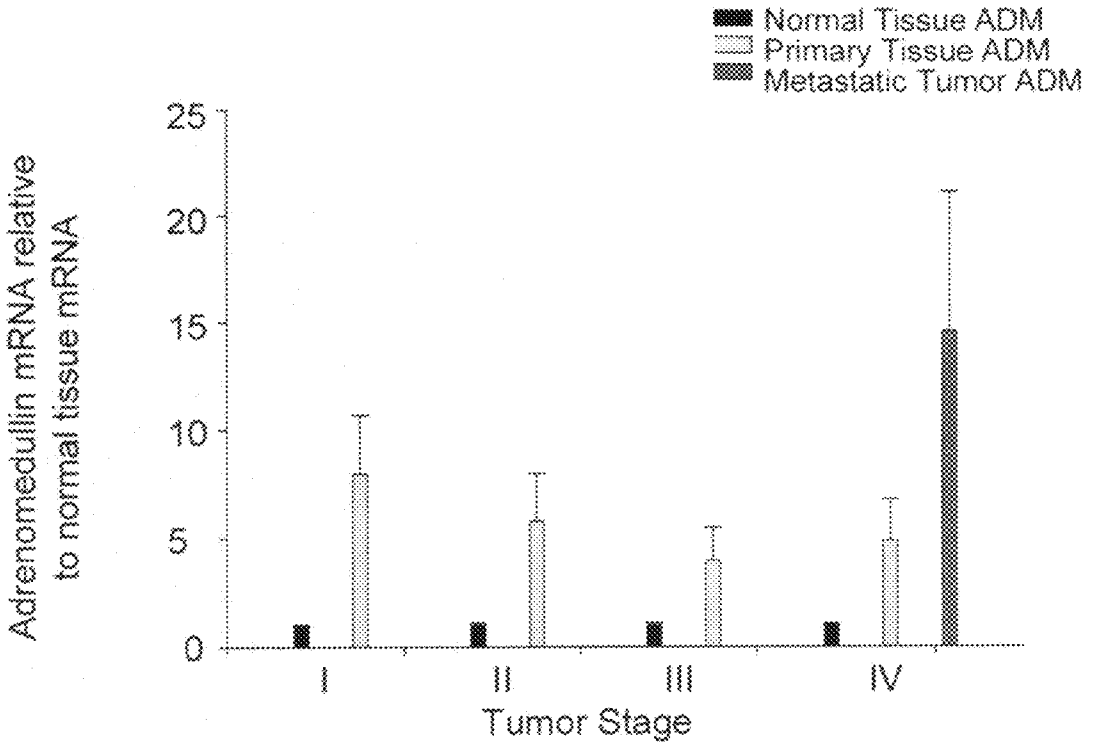
Normal Tissue ADM
Primary Tissue ADM
Metastatic Tumor ADM
Adrenomedullin mRNA relative to normal tissue mRNA
25
20
15
10
5
0
I
II
III
IV
Tumor Stage

FIG. 4

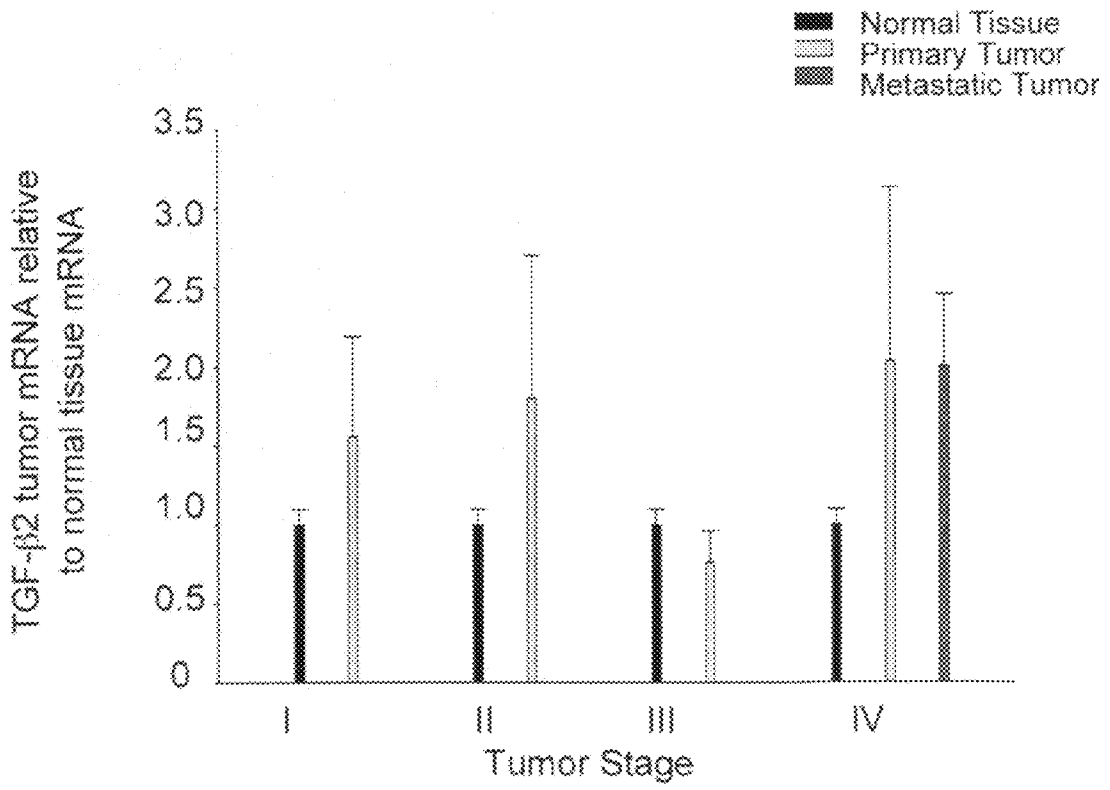
Normal Tissue
Primary Tumor
Metastatic Tumor
TGF-β2 tumor mRNA relative to normal tissue mRNA
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0
I
II
III
IV
Tumor Stage

FIG. 5

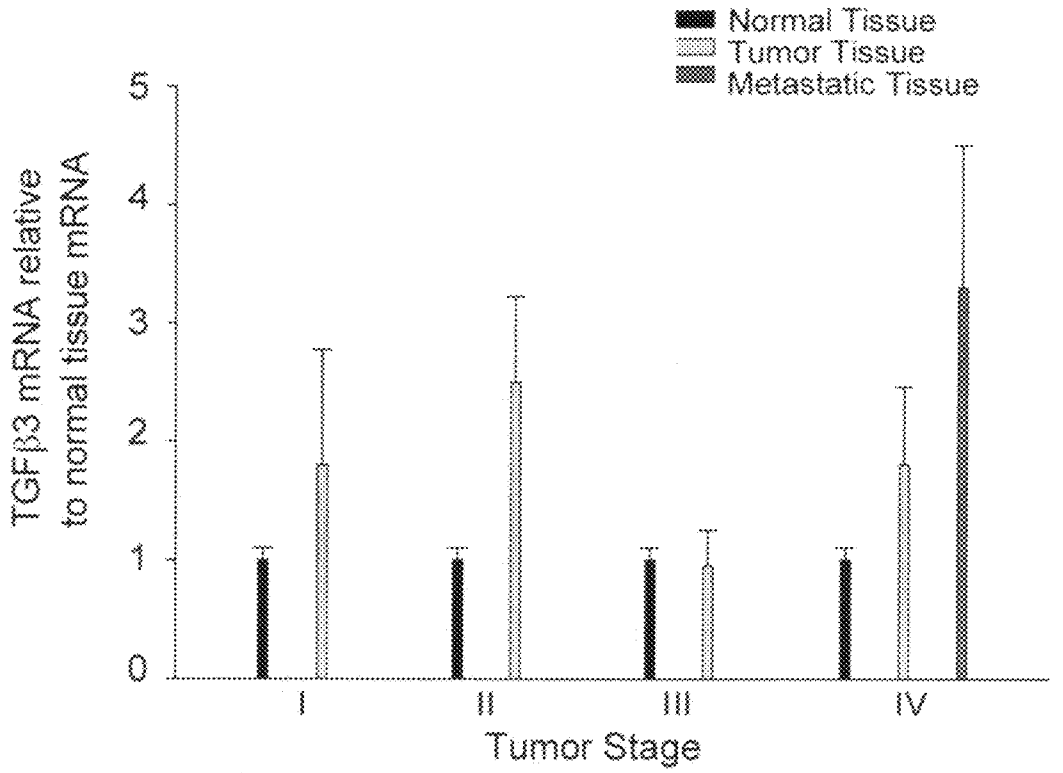
Normal Tissue
Tumor Tissue
Metastatic Tissue
TGFβ3 mRNA relative to normal tissue mRNA
0
1
2
3
4
5
I
II
III
IV
Tumor Stage

FIG. 6

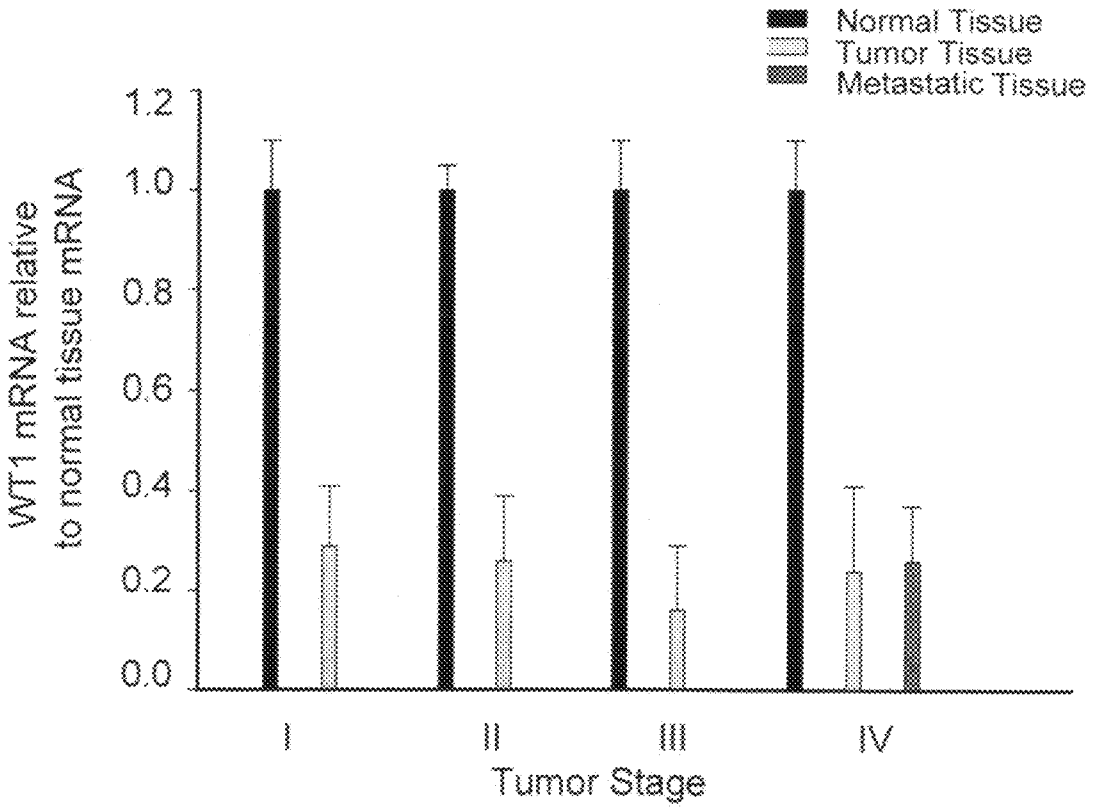
Normal Tissue
Tumor Tissue
Metastatic Tissue
WT1 mRNA relative to normal tissue mRNA
0.0
0.2
0.4
0.6
0.8
1.0
1.2
I
II
III
IV
Tumor Stage

FIG. 7

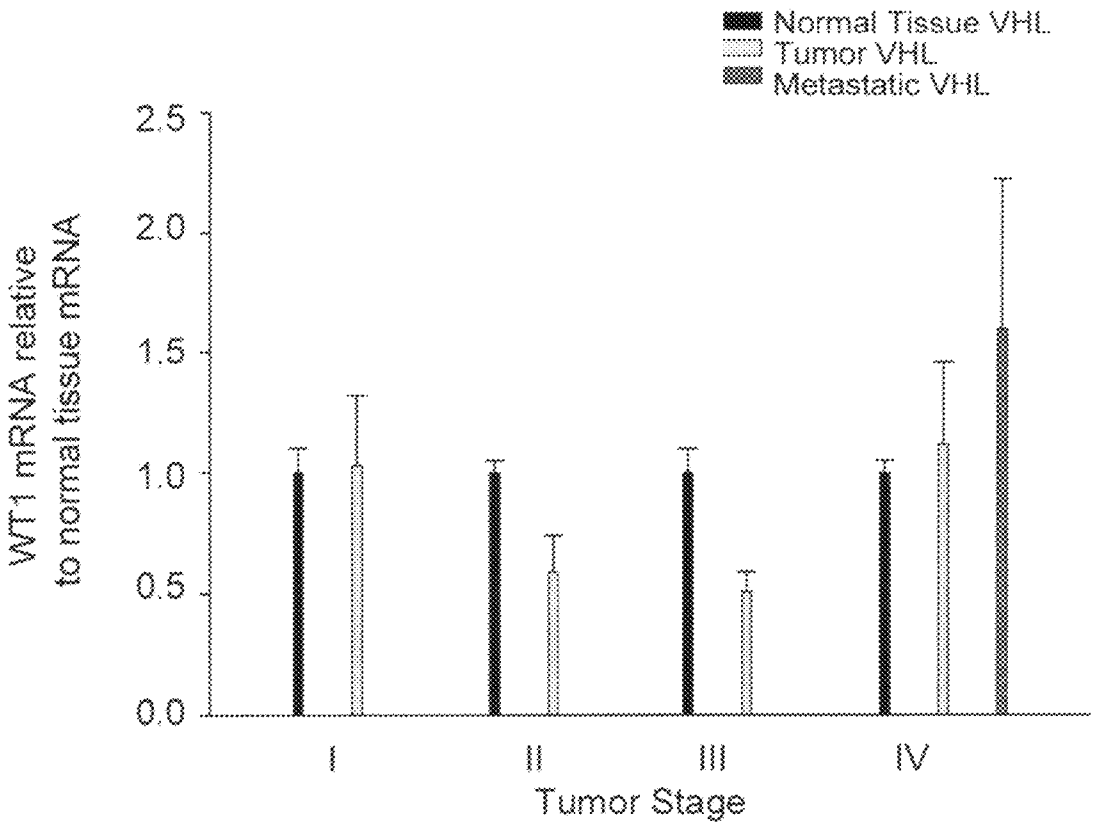
Normal Tissue VHL
Tumor VHL
Metastatic VHL
WT1 mRNA relative to normal tissue mRNA
2.5
2.0
1.5
1.0
0.5
0.0
I
II
III
IV
Tumor Stage

FIG. 8

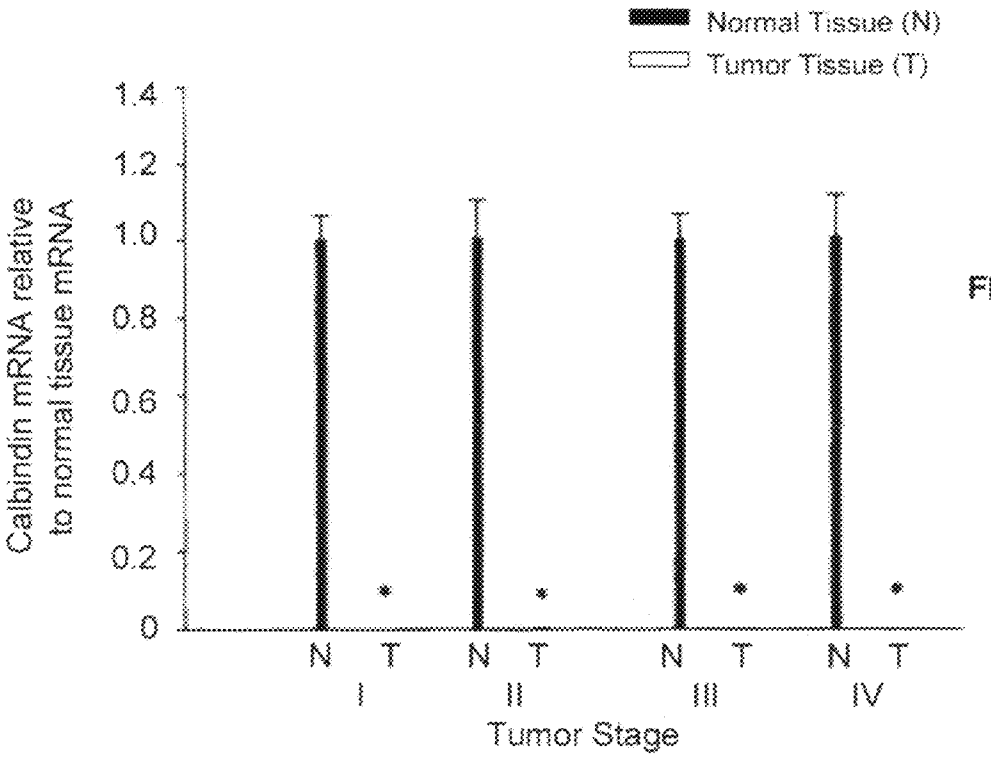
Normal Tissue (N)
Tumor Tissue (T)
Calbindin mRNA relative to normal tissue mRNA
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0
N T N T N T N T
I II III IV
Tumor Stage

FIG. 9

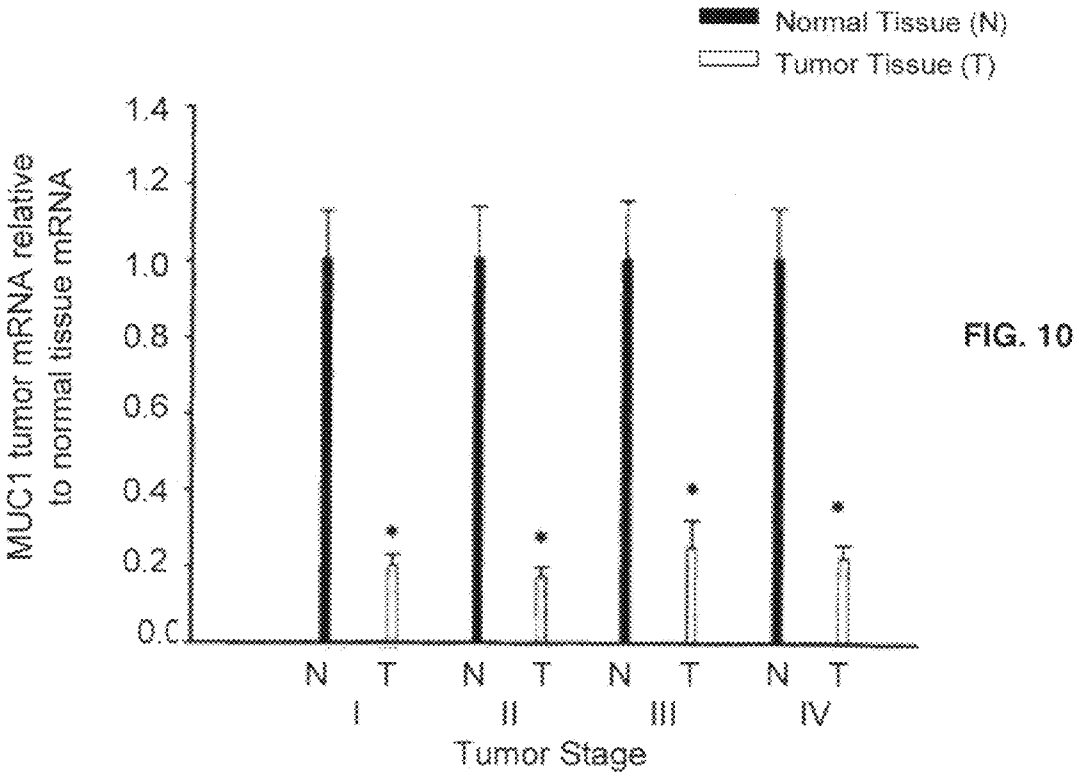
Normal Tissue (N)
Tumor Tissue (T)
MUC1 tumor mRNA relative to normal tissue mRNA
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
N T N T N T N T
I II III IV
Tumor Stage

FIG. 10

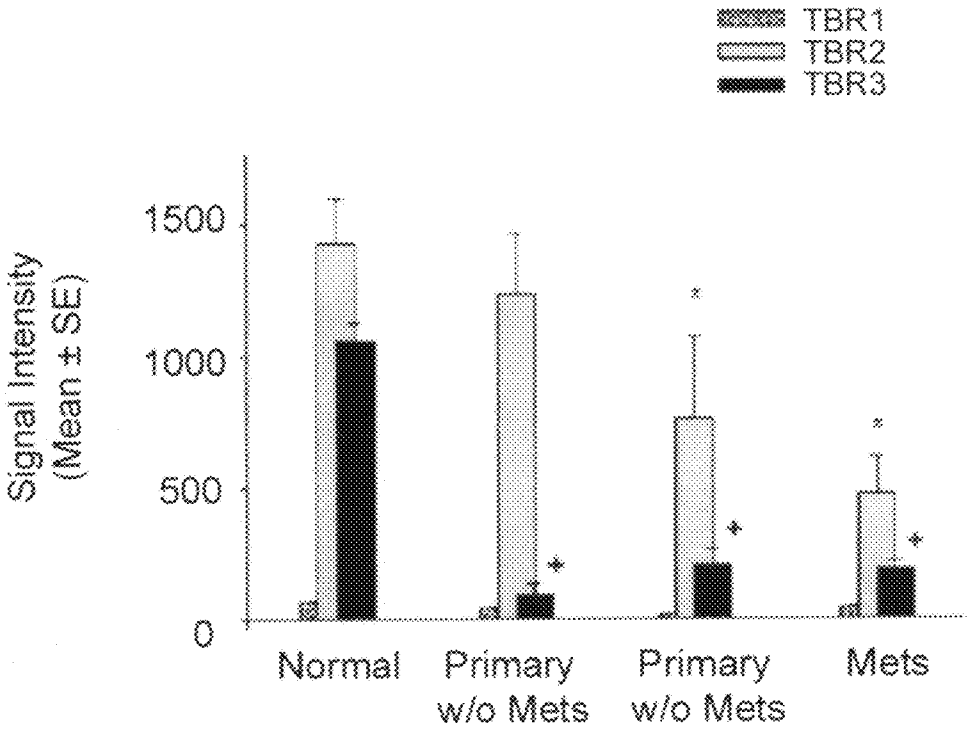

FIG. 11A

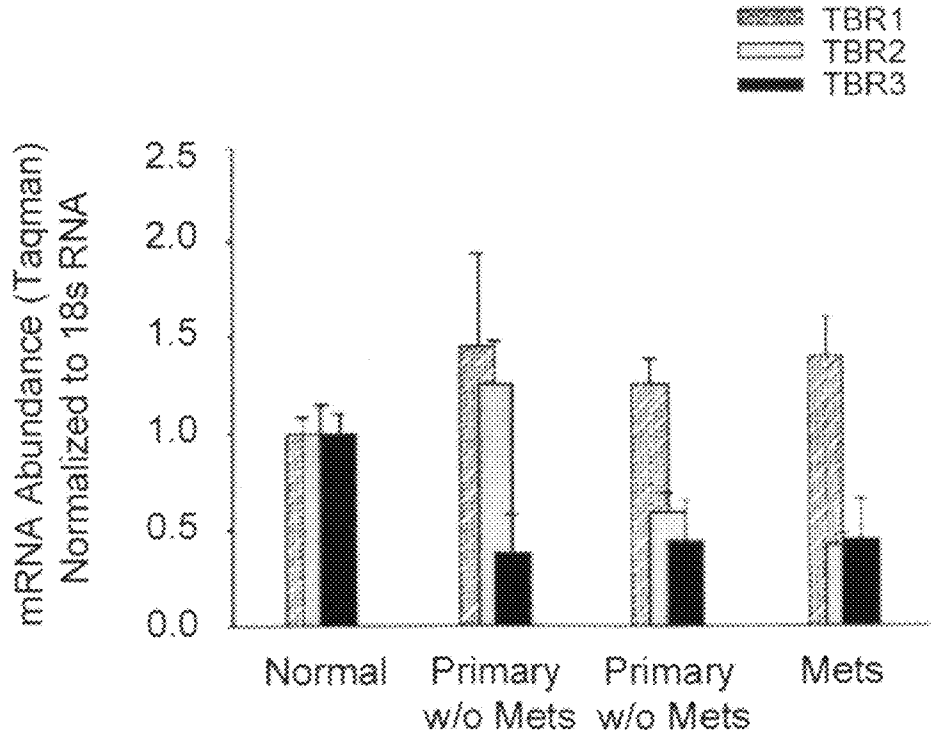

FIG. 11B

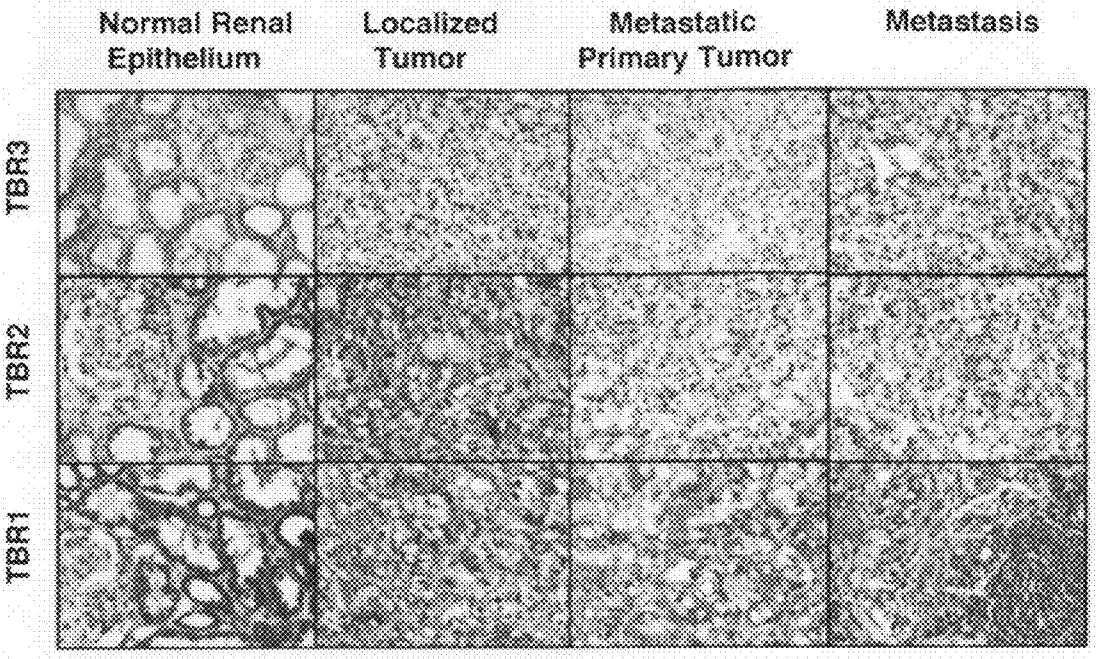
Normal Renal Epithelium
Localized Tumor
Metastatic Primary Tumor
Metastasis
TBR3
TBR2
TBR1

FIG. 12

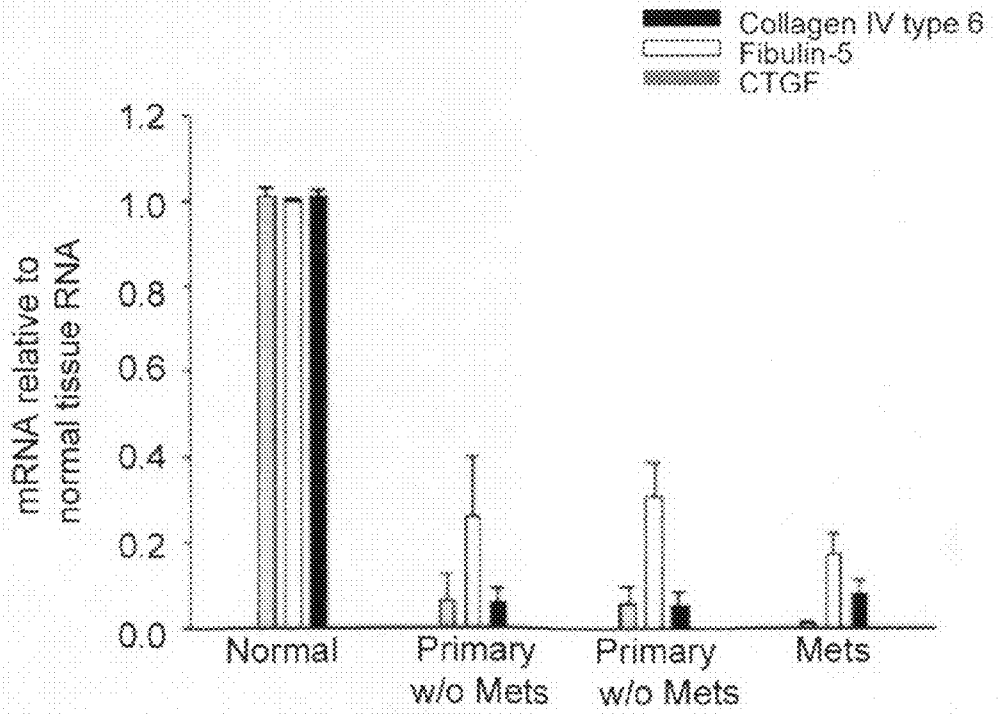
Collagen IV type 6
Fibulin-5
CTGF
mRNA relative to normal tissue RNA
1.2
1.0
0.8
0.6
0.4
0.2
0.0
Normal
Primary w/o Mets
Primary w/o Mets
Mets

FIG. 13

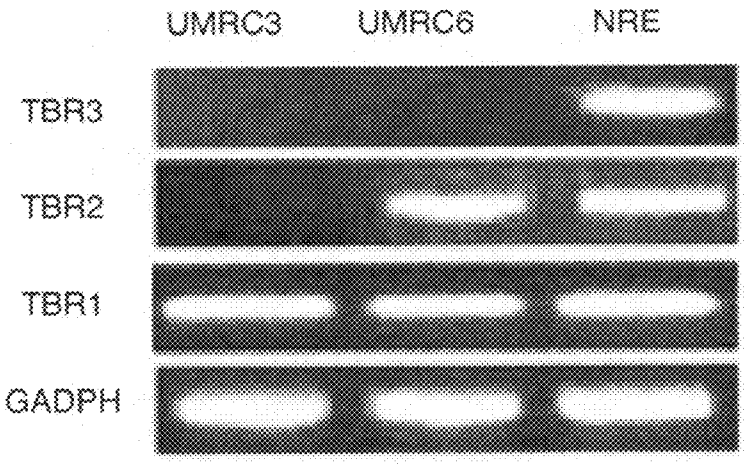

FIG. 14A

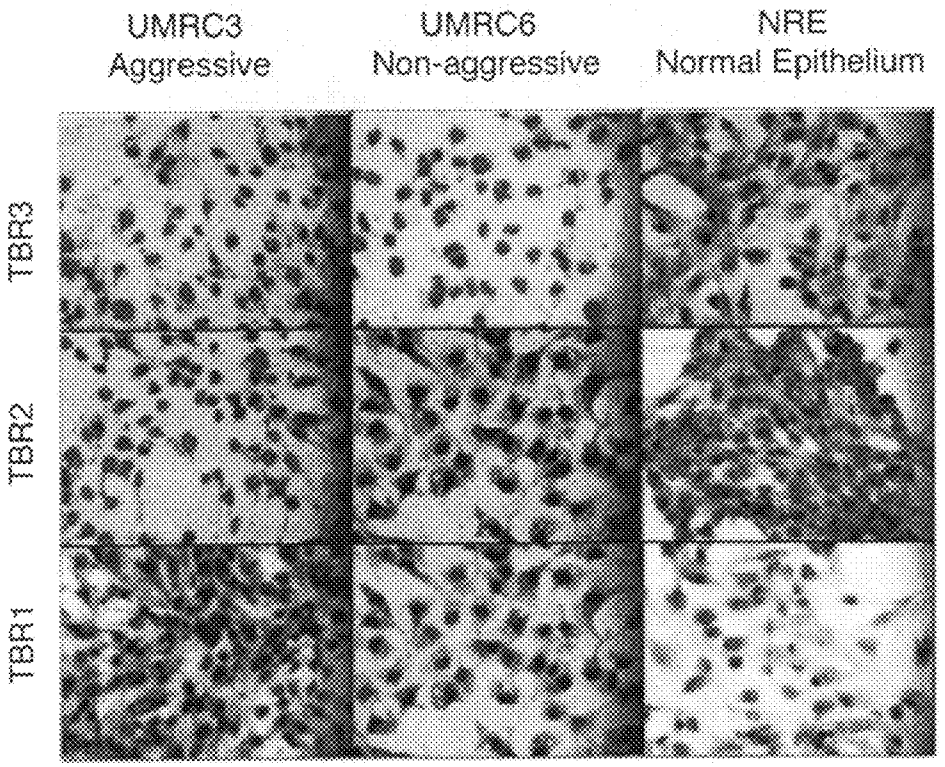

FIG. 14B

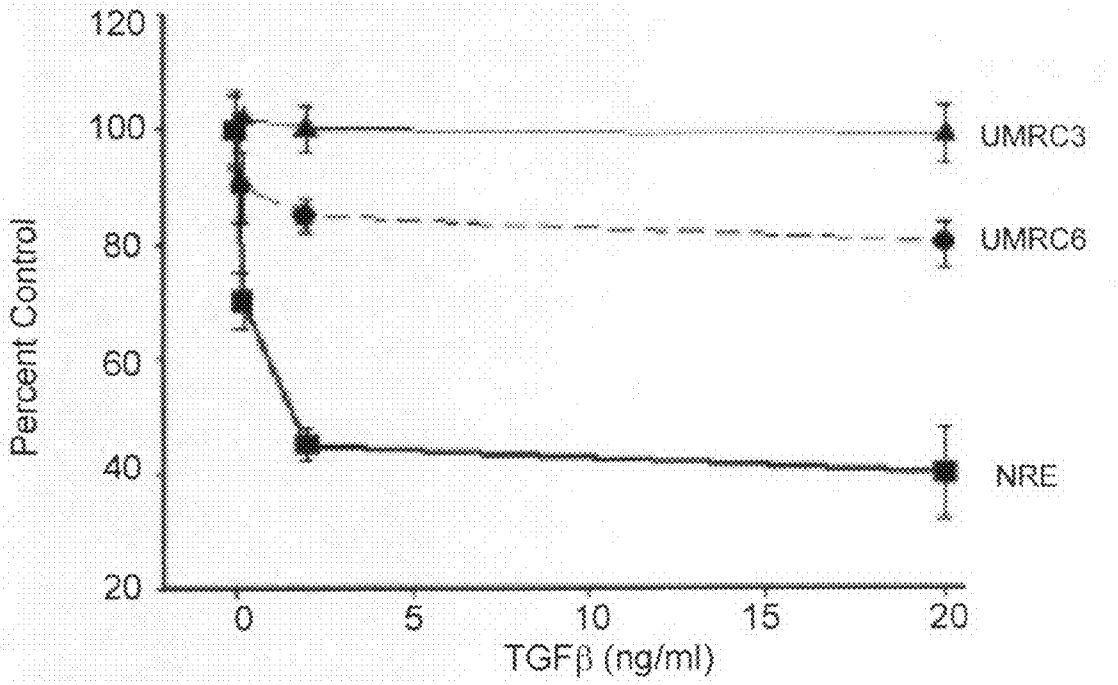

FIG. 15A

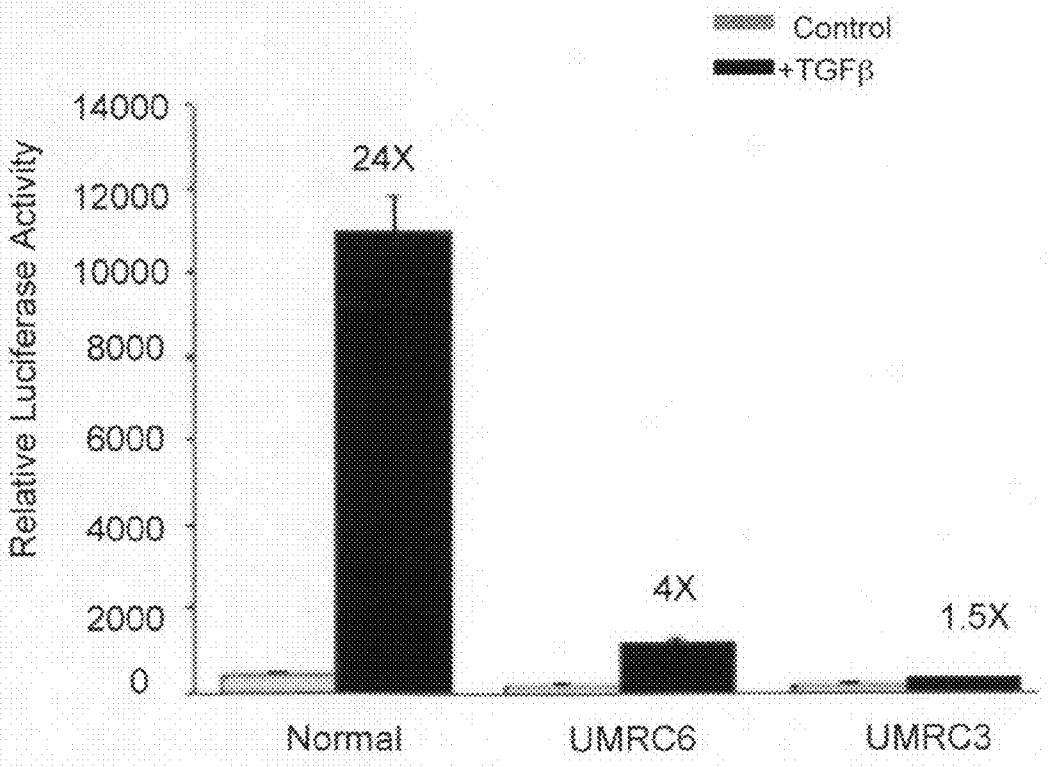

FIG. 15B

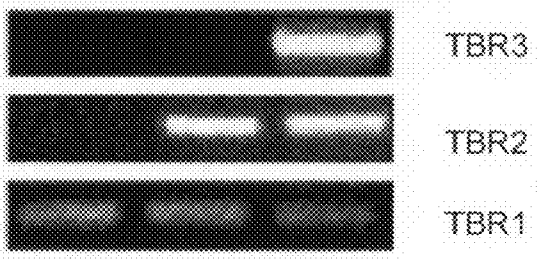

FIG. 16A

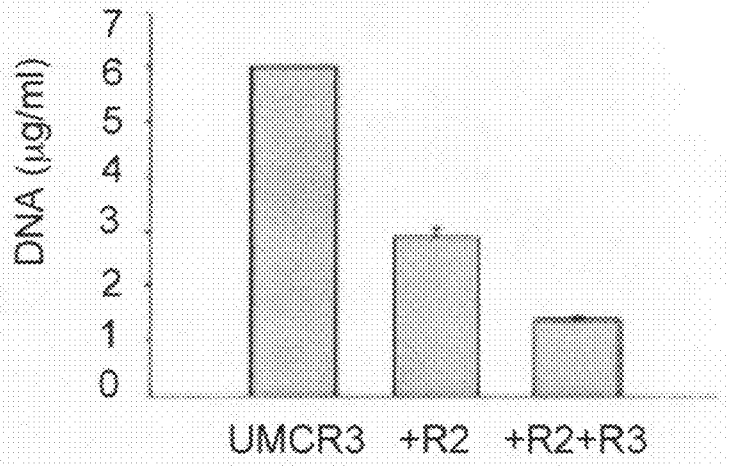

FIG. 16B

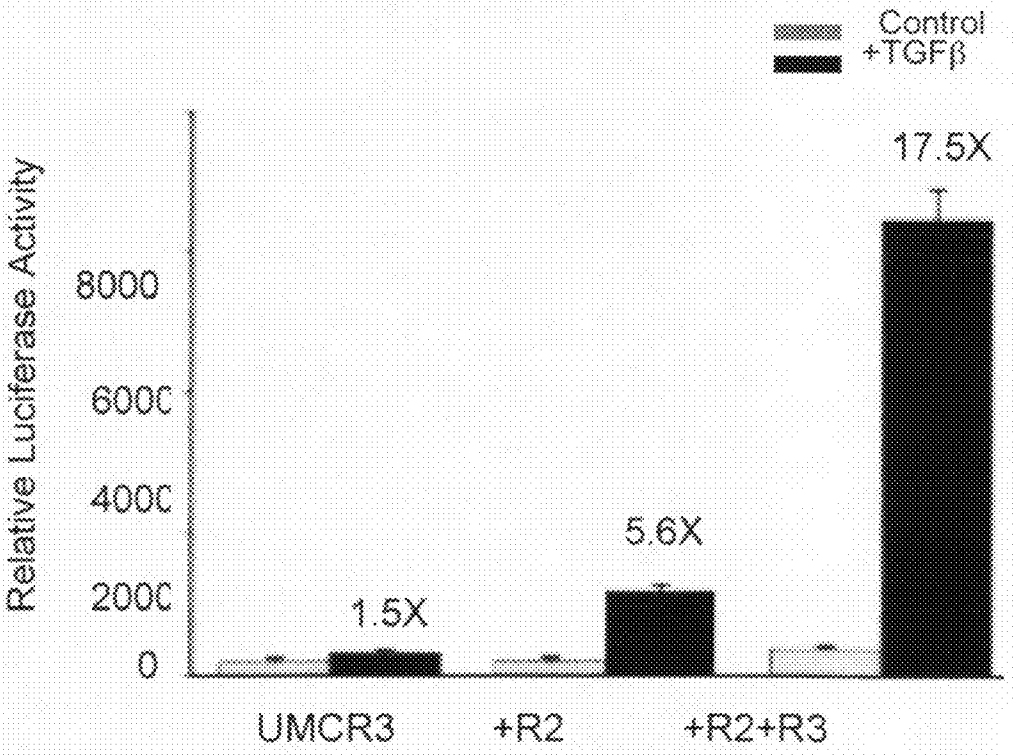

FIG. 16C

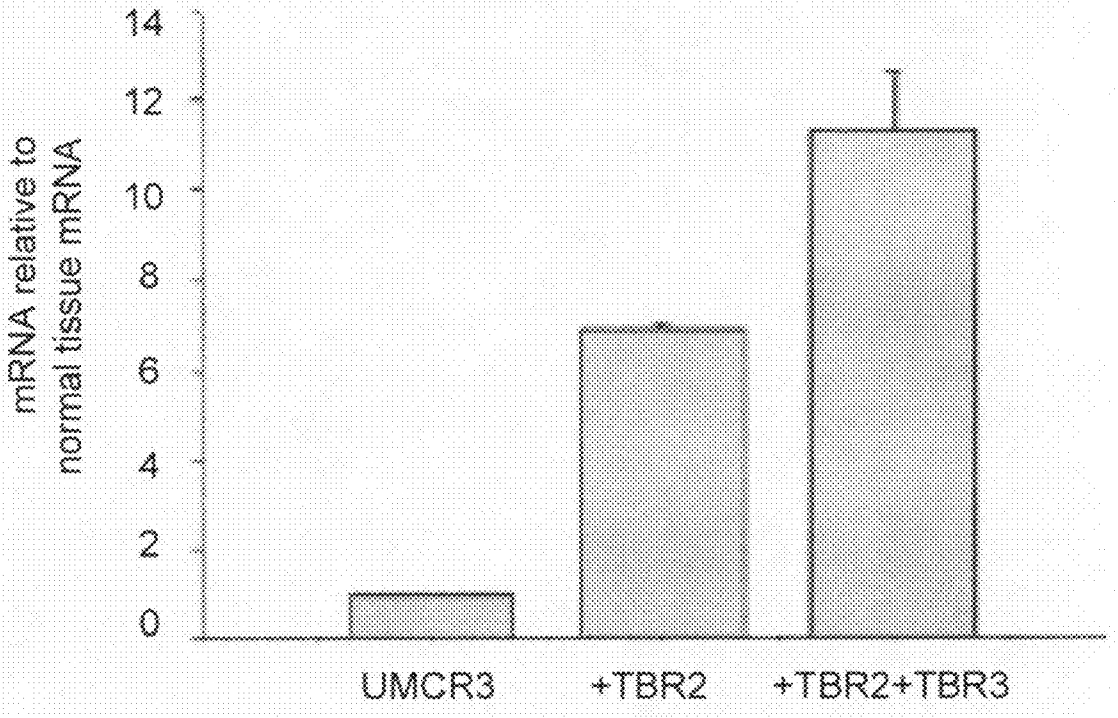

FIG. 16D

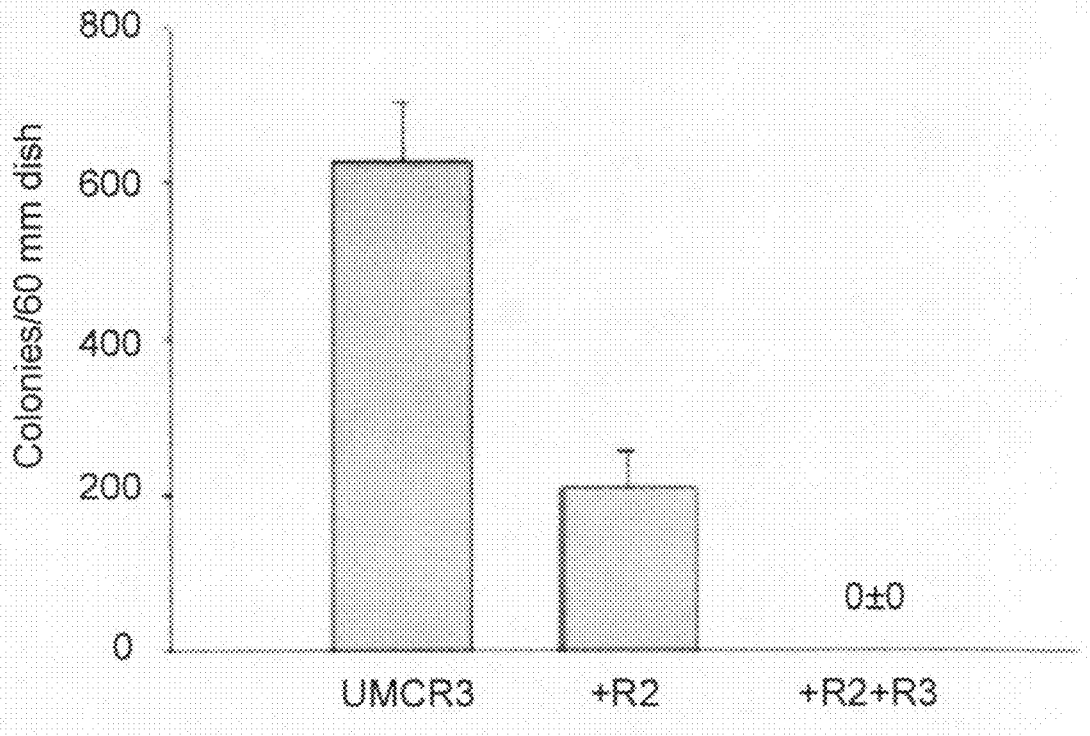

FIG. 16E

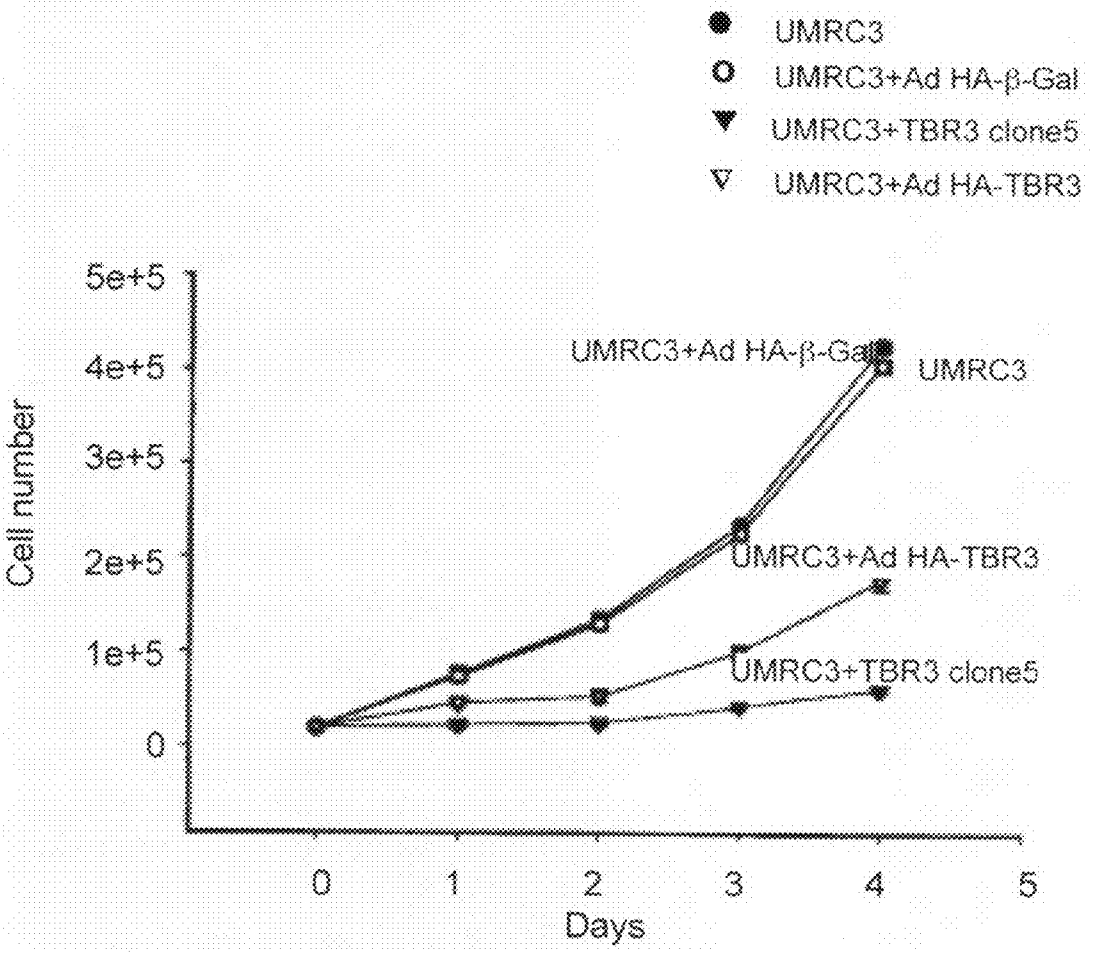

FIG. 17A

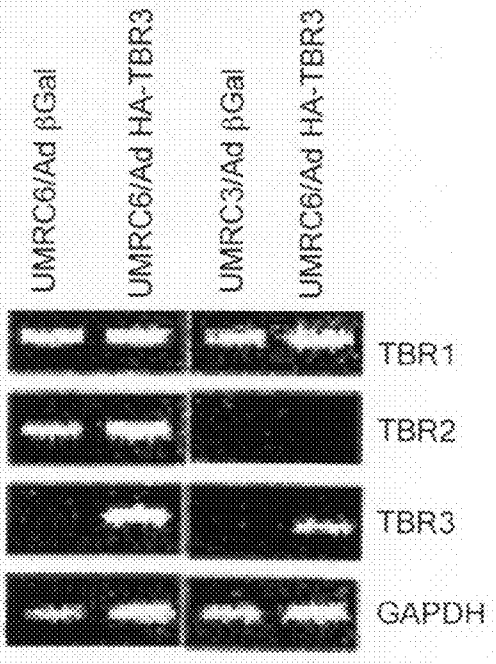

FIG. 17B

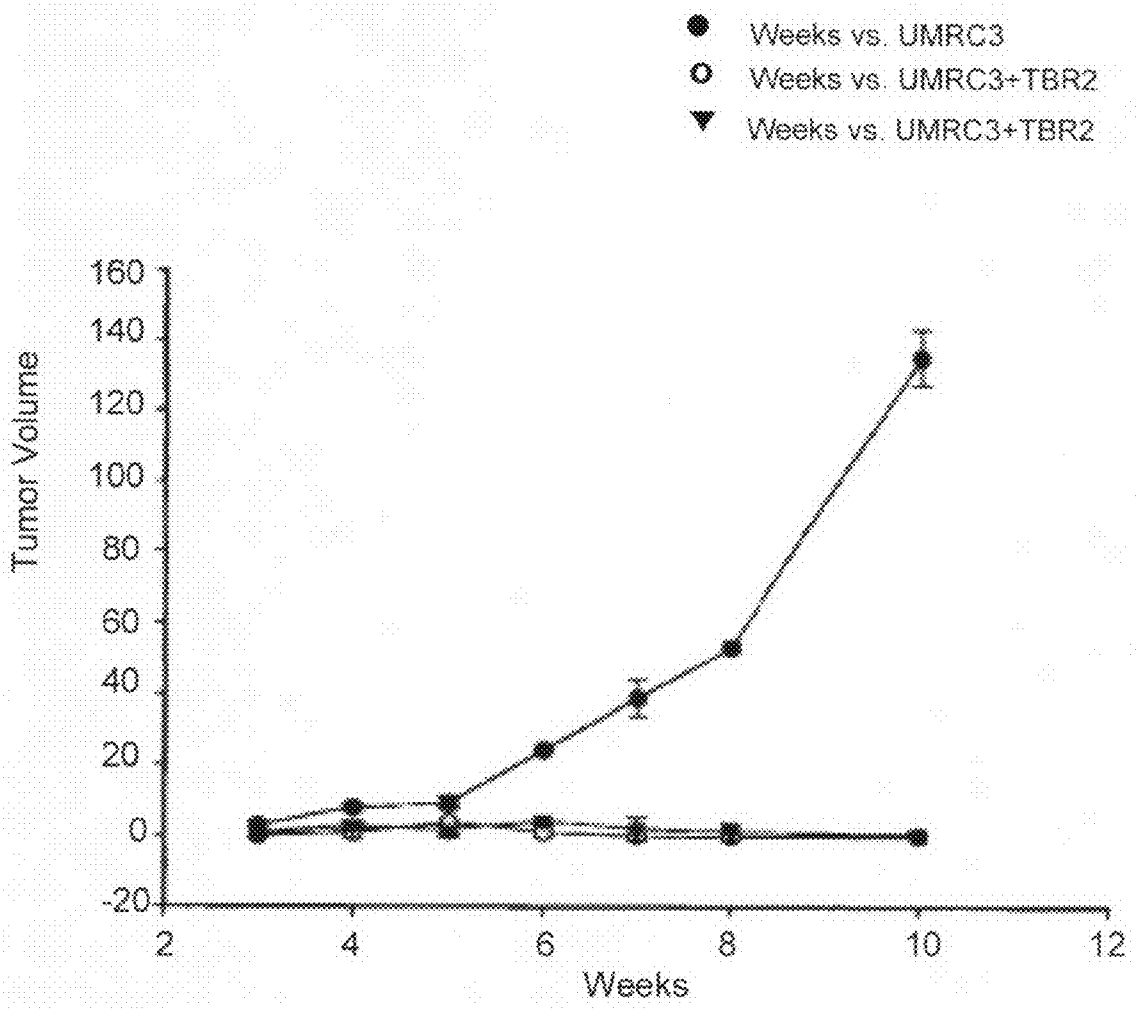
Weeks vs. UMRC3
Weeks vs. UMRC3+TBR2
Weeks vs. UMRC3+TBR2
Tumor Volume
160
140
120
100
80
60
40
20
0
-20
2
4
6
8
10
12
Weeks

FIG. 18

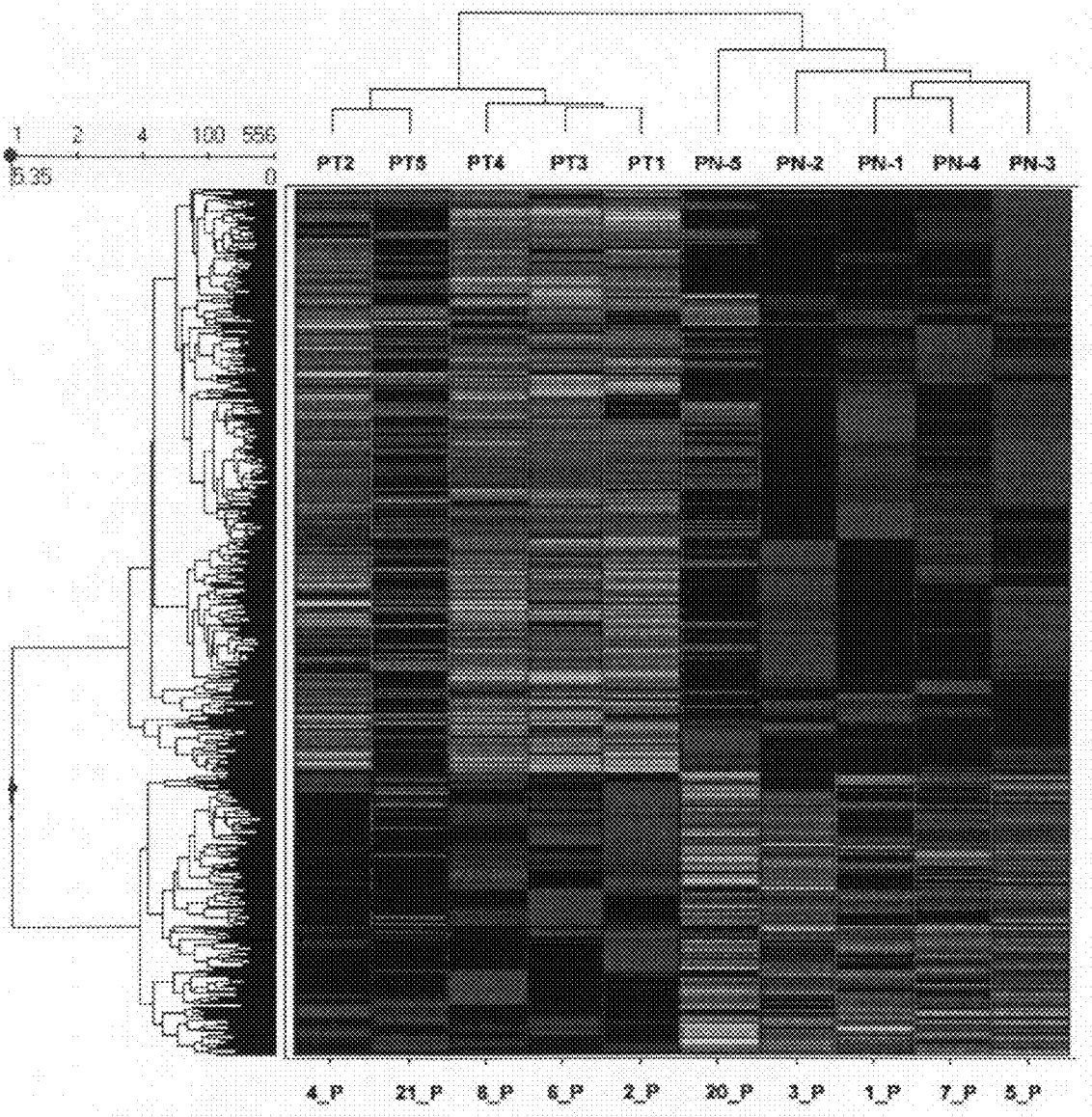
1
2
4
100
556
5.35
0
PT2
PT5
PT4
PT3
PT1
PN-5
PN-2
PN-1
PN-4
PN-3
4_P
21_P
8_P
6_P
2_P
20_P
3_P
1_P
7_P
5_P

FIG. 19

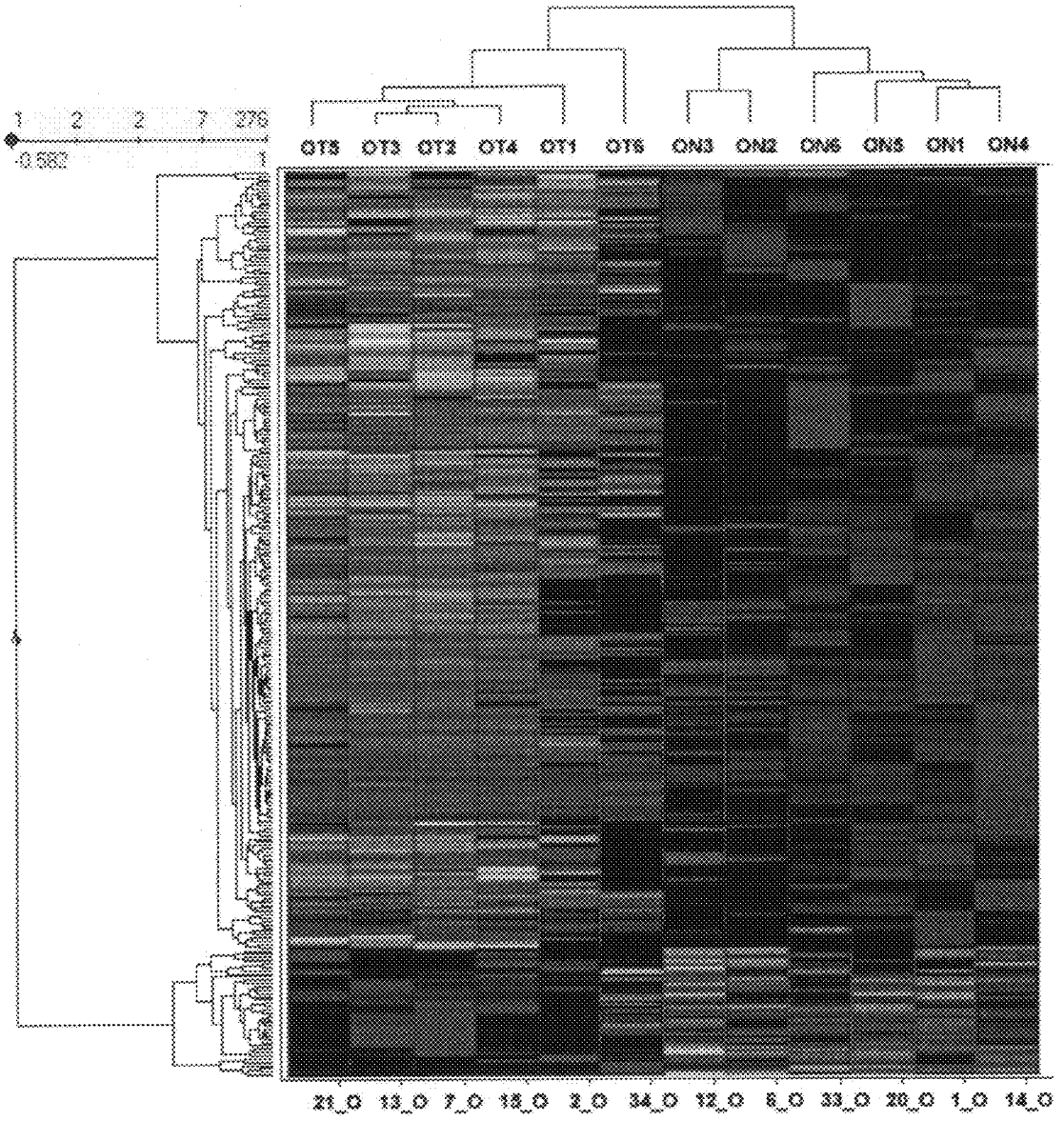
1 2 2 7 376
-0.582 1
OT5 OT3 OT2 OT4 OT1 OT6 ON3 ON2 ON6 ON5 ON1 ON4
21_O 13_O 7_O 18_O 3_O 34_O 12_O 6_O 33_O 20_O 1_O 14_O

FIG. 20

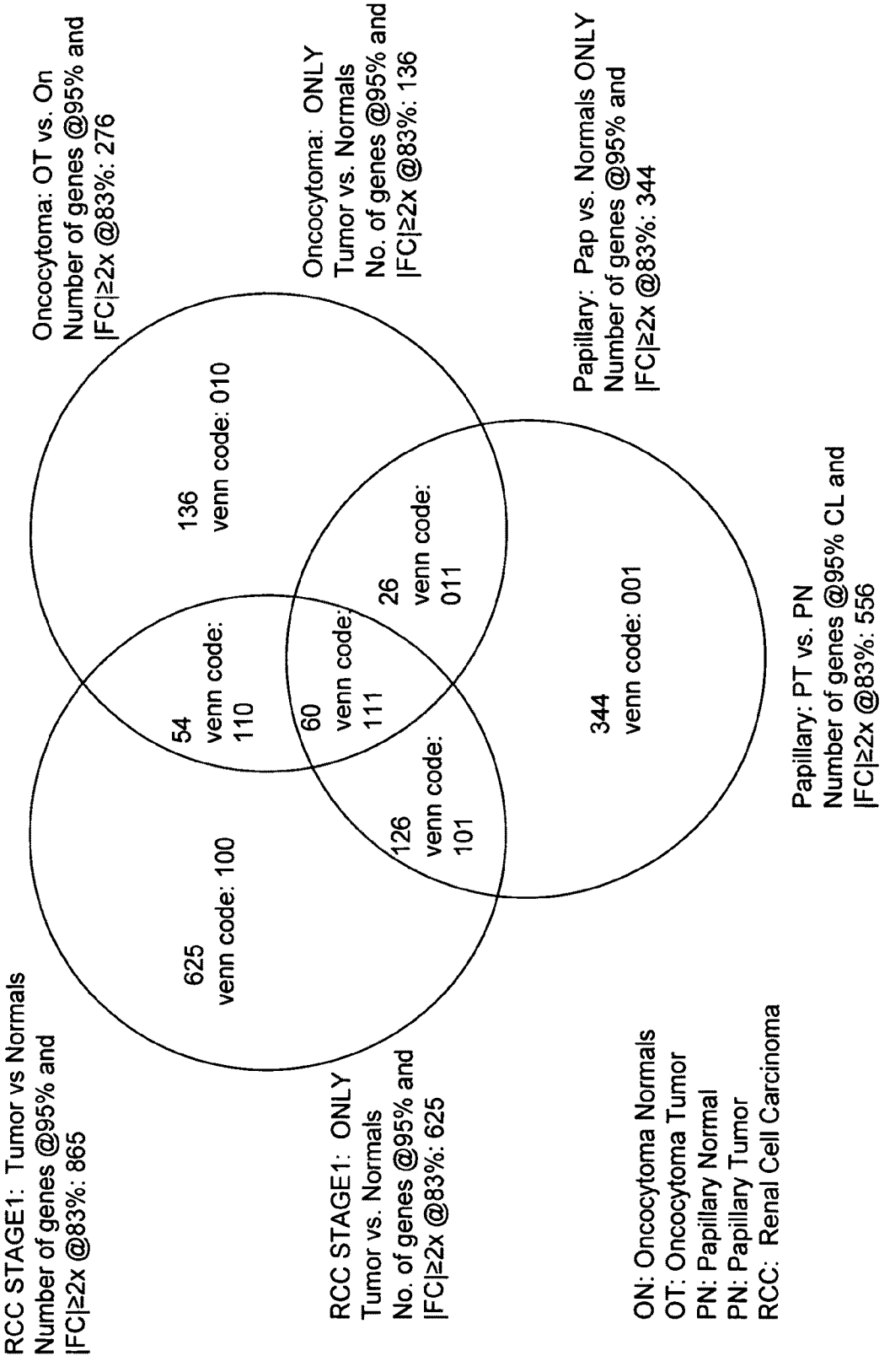
RCC STAGE1: Tumor vs Normals
Number of genes @95% and
|FC|≥2x @83%: 865
Oncocytoma: OT vs. On
Number of genes @95% and
|FC|≥2x @83%: 276
Oncocytoma: ONLY
Tumor vs. Normals
No. of genes @95% and
|FC|≥2x @83%: 136
Papillary: Pap vs. Normals ONLY
Number of genes @95% and
|FC|≥2x @83%: 344
Papillary: PT vs. PN
Number of genes @95% CL and
|FC|≥2x @83%: 556
RCC STAGE1: ONLY
Tumor vs. Normals
No. of genes @95% and
|FC|≥2x @83%: 625
625
venn code: 100
54
venn code:
110
136
venn code: 010
60
venn code:
111
126
venn code:
101
26
venn code:
011
344
venn code: 001
ON: Oncocytoma Normals
OT: Oncocytoma Tumor
PN: Papillary Normal
PN: Papillary Tumor
RCC: Renal Cell Carcinoma

FIG. 21

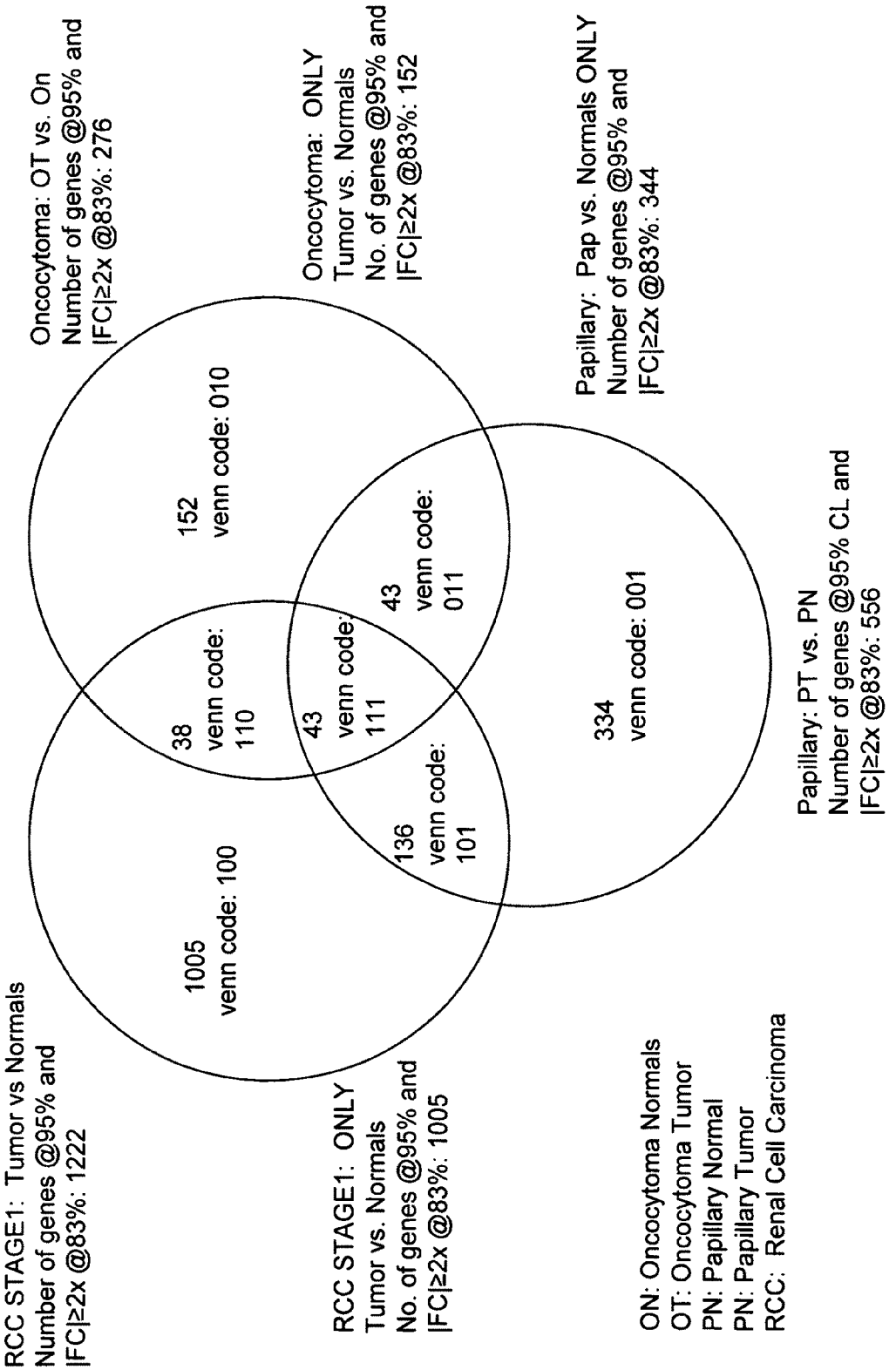
RCC STAGE1: Tumor vs Normals
Number of genes @95% and
|FC|≥2x @83%: 1222
Oncocytoma: OT vs. On
Number of genes @95% and
|FC|≥2x @83%: 276
Oncocytoma: ONLY
Tumor vs. Normals
No. of genes @95% and
|FC|≥2x @83%: 152
Papillary: Pap vs. Normals ONLY
Number of genes @95% and
|FC|≥2x @83%: 344
Papillary: PT vs. PN
Number of genes @95% CL and
|FC|≥2x @83%: 556
RCC STAGE1: ONLY
Tumor vs. Normals
No. of genes @95% and
|FC|≥2x @83%: 1005
1005
venn code: 100
38
venn code:
110
152
venn code: 010
43
venn code:
111
43
venn code:
011
136
venn code:
101
334
venn code: 001
ON: Oncocytoma Normals
OT: Oncocytoma Tumor
PN: Papillary Normal
PN: Papillary Tumor
RCC: Renal Cell Carcinoma

FIG. 22

METHODS FOR DETECTING, DIAGNOSING AND TREATING HUMAN RENAL CELL CARCINOMA

CROSS-REFERENCE TO RELATED APPLICATION

This is a continuation application under 35 U.S.C. §120 of nonprovisional application U.S. Ser. No. 10/938,973, filed Sep. 10, 2004, now abandoned, which claims benefit of provisional application U.S. Ser. No. 60/539,838, filed Jan. 28, 2004, now abandoned, and of provisional application U.S. Ser. No. 60/502,038, filed Sep. 10, 2003, now abandoned, the entirety of all of which are hereby incorporated by reference.

BACKGROUND OF THE INVENTION

1. Field of the Invention

The present invention relates generally to the field of cancer research. More specifically, the present invention relates to gene expression profiling for human renal cell carcinoma.

2. Description of the Related Art

Renal cell carcinoma (RCC) represents a major health issue. The American Cancer Society predicts 31,900 new cases will be diagnosed in the United States alone in the year 2003, with 11,900 people dying of the disease. When clinically localized or even locally advanced, renal cell carcinoma can be surgically resected for cure using a variety of approaches. With metastatic progression, however, renal cell carcinoma is incurable, and existing systemic therapies are largely ineffective in impacting disease response or patient survival. The lack of effective systemic therapy for metastatic renal cell carcinoma is, in part, due to a fundamental lack of understanding of the molecular events that result in cellular transformation, carcinogenesis, and progression in human kidney.

The advent of gene array technology has allowed classification of disease states at molecular level by examining changes in all mRNAs expressed in cells or tissues. Gene expression fingerprints representing large numbers of genes may allow precise and accurate grouping of renal cell carcinoma. Moreover, large scale gene expression analysis have the potential of identifying a number of differentially expressed genes in renal cell carcinoma compare to normal renal epithelial cells. These genes or markers may further be tested for clinical utility in the diagnosis and treatment of renal cell carcinoma.

Thus, the identification of novel renal cell carcinoma markers to be used for detection, diagnosis and development of effective therapy against the disease remains a high priority. The prior art is deficient in understanding the molecular differences between renal cell carcinoma and normal renal epithelium. The present invention fulfills this need in the art by providing gene expression profiling for these two types of tissues.

SUMMARY OF THE INVENTION

The present invention identifies genes with a differential pattern of expression between different subtypes of renal cell carcinomas (RCC) and normal renal epithelium. These genes and their products can be used to develop novel diagnostic and therapeutic markers for the treatment of renal cell carcinomas.

Genomic profiling of conventional renal cell carcinoma tissues and patient-matched normal kidney tissue samples was carried out using stringent statistical analyses (ANOVA with full Bonferroni corrections). Subtypes of renal cell carcinoma include stage I, II, III, and IV (reflecting differences in tumor size, lymph node and organ metastasis), stage I papillary renal cell carcinoma, and benign oncocytoma. Hierarchical clustering of the expression data readily distinguished normal tissue from renal cell carcinomas. The identified genes were verified by real-time FCR and immunohistochemical analyses.

Different subtypes of conventional renal cell carcinomas can be diagnosed either by drawing blood and identifying secreted gene products specific to renal cell carcinoma or by doing a biopsy of the tissue and then identify specific genes that are altered when renal cell carcinoma is present. An example of when this may be especially important is in distinguishing the deadly conventional renal cell carcinomas (account for 85% of all renal cell carcinomas) from renal oncocytoma (benign renal cell carcinoma) as well as identifying the histologic subtypes of papillary and sarcomatoid renal cell carcinoma. Identification of specific genes will also help in determining which patients will have a good prognosis verses that of a poor prognosis. In addition, subsets of genes identified in the present invention can be developed as targets for therapies that could cure, prevent, or stabilize the disease. Thus, results from the present invention could be used for diagnosis, prognosis, and development of therapies to treat or prevent renal cell carcinoma.

In one embodiment, there are provided methods of detecting conventional or clear cell renal cell carcinoma based on over-expression and/or down-regulation of a number of genes disclosed herein. In another embodiment, conventional or clear cell renal cell carcinoma is detected based on decreased expression of type III TGF-β receptor.

In yet another embodiment, there are provided methods of detecting stage I conventional or clear cell renal cell carcinoma based on over-expression and/or down-regulation of a number of genes disclosed herein.

The present invention also provides methods of detecting stage II conventional or clear cell renal cell carcinoma based on over-expression and/or down-regulation of a number of genes disclosed herein.

The present invention also provides methods of detecting papillary renal cell carcinoma or benign oncocytoma based on over-expression and/or down-regulation of a number of genes disclosed herein.

In another embodiment, there is provided a method of targeting conventional or clear cell renal cell carcinoma cells based on generating antibodies or small molecules directed against a cell surface molecule over-expressed in conventional renal cell carcinoma cells.

In yet another embodiment, there is provided a method of treating conventional or clear cell renal cell carcinoma by replacing down-regulated tumor suppressor gene in conventional renal cell carcinoma.

Other and further aspects, features, and advantages of the present invention will be apparent from the following description of the presently preferred embodiments of the invention. These embodiments are given for the purpose of disclosure.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. **1**A shows hierarchical clustering of genes expressed in normal renal cortex (12 patient tissue samples) verse stage I conventional renal cell carcinoma (6 patient tissue samples). Red indicates that a gene is highly expressed and green is indicative of low expression. Four hundred eighty eight genes were depicted in FIG. **1**A. FIG. **1**B shows hierarchical clustering of genes expressed in normal renal cortex (12 patient

tissue samples) verse stage II conventional renal cell carcinoma (6 patient tissue samples). Red indicates that a gene is highly expressed and green is indicative of low expression. Six hundred twenty eight genes were depicted in FIG. **1**B. FIG. **1**C shows hierarchical clustering of genes selected from a Venn analysis in which the chosen genes were expressed in common in both stage I and II at a 99% confidence level. One hundred eighty eight genes were depicted in FIG. **1**C. C, cancer cells; N, normal cells; S1, stage 1; S2, stage 2.

FIG. **2** shows TGF-β1 mRNA expression in stages I-IV renal cell carcinoma as measured by real time PCR TGF-β1 mRNA levels were up-regulated in all stages of renal cell carcinoma as compared to normal tissue counterparts.

FIG. **3** shows TGF-α mRNA expression in stages I-IV renal cell carcinoma as measured by real time PCR. TGF-α mRNA levels were up-regulated in all stages of renal cell carcinoma as compared to normal tissue counterparts.

FIG. **4** shows adrenomedulin mRNA expression in stages I-IV renal cell carcinoma as measured by real time PCR. Adrenomedulin mRNA levels were up-regulated in all stages of renal cell carcinoma as compared to normal tissue counterparts.

FIG. **5** shows TGF-β2 mRNA expression in stages I-IV renal cell carcinoma as measured by real time PCR. TGF-β2 mRNA levels were not altered between normal and tumor matched samples.

FIG. **6** shows TGF-β3 mRNA expression in stages I-IV renal cell carcinoma as measured by real time PCR. TGF-β3 mRNA levels were not altered between normal and tumor matched samples.

FIG. **7** shows tumor suppressor gene Wilms Tumor 1 (WT1) mRNA expression in stages I-IV renal cell carcinoma as measured by real time PCR. WT1 mRNA levels were down-regulated in all stages of renal cell carcinoma as compared to normal tissue counterparts.

FIG. **8** shows von Hippel Lindau mRNA expression in stages I-IV renal cell carcinoma as measured by real time PCR. A small percentage of tumor tissues demonstrated attenuated von Hippel Lindau mRNA levels when compared to matched normal tissue

FIG. **9** shows calbindin mRNA expression in stages I-IV renal cell carcinoma as measured by real time PCR. Calbindin mRNA was completely lost in all stage I renal cell carcinoma. $p<0.05$ compared to matched control. *Stage I tumor: 0±0; stage III tumor: 0.0009±0.0004; stage IV tumor: 0.003±0.0004/

FIG. **10** shows MUC1 mRNA expression in stages I-IV renal cell carcinoma as measured by real time PCR. MUC1 mRNA levels were down-regulated in all tumor tissues as early as stage I. *$p<0.05$ compared to matched control.

FIGS. **11**A-**11**B show stepwise loss of type III αreceptor (TBR3) and type II TGF-β receptor (TBR2) mRNA expression during renal cell carcinogenesis and progression in patient tissue samples. FIG. **11**A shows gene array data from 10 patients—five diagnosed with localized renal cell carcinoma and five with metastatic disease. '+' ($P<0.05$) indicates statistical difference for TBR3 mRNA levels as compared to normal tissue and '*' ($P<0.28$) indicates statistical difference for TBR2 mRNA levels as compared to normal controls. Data are expressed as mean±s.e. FIG. **11**B shows real-time RT-PCR verification of TBR1, TBR2, and TBR3 mRNA levels of tissue samples described in FIG. **11**A. Data are expressed as mean±s.d.

FIG. **12** shows immunohistochemistry of patient tissue demonstrating loss of type III αreceptor (TBR3) expression (top row) in all tumors, loss of type II αreceptor (TBR2) expression (middle row) in patients diagnosed with metastatic tumors, and no change in type I αreceptor (TBR1) protein expression (bottom row).

FIG. **13** demonstrates down-regulation of TGF-β-regulated genes in human tumor tissues by real-time PCR. Genes known to be up-regulated by α are suppressed in tumor tissues. Down-regulation of collagen IV type 6, fibulin 5, and connective tissue growth factor (CTGF) mRNA in tumor tissues were compared to matched normal tissue controls. Values were normalized to 18 s mRNA. Each matching tumor value was compared to its respective normal control. The mean±s.d. was calculated for each sample group with n values of 10-15 matched samples.

FIGS. **14**A-**14**B show tumor cell lines that lose type III αreceptor (TBR3) and type I TGF-β receptor (TBR2) expression. FIG. **14**A shows semi-quantitative RT-PCR measurements of mRNA levels of TBR1, TBR2, and TBR3 for UMRC3, UMRC6 and normal renal epithelial (NRE) cells. FIG. **14**B shows immunohistochemistry of protein expression for TBR1, TBR2, and TBR3 (×40 magnification).

FIGS. **15**A-**15**B show loss of type III TGF-β receptor (TBR3) and type II αreceptor (TBR2) expression in renal tumor cell lines correlate with loss of TGF-β-regulated growth inhibitory and transcriptional responses. FIG. **15**A shows cell proliferation was inhibited as assessed by DNA content 3 days after αtreatment. Percent of each respective untreated control was used for comparisons. Transient transfection using 3TP/Ix along with a renilla luciferase control demonstrates loss of responsiveness to 2 ng/ml TGF-β1 with loss of TGF-β receptor expression (FIG. **15**B). Firefly luciferase activity was normalized using the ratio of firefly luciferase/renilla luciferase. Data are expressed as mean±s.d.

FIG. **16**A demonstrates RT-PCR derived mRNA expression of type III αreceptor (TBR3), type II αreceptor (TBR2), and type I αreceptor (TBR1) in UMRC3 cells and cells stably transfected with TBR2 and TBR3. FIG. **16**B shows UMRC3 cells stably transfected with type II TGF-β receptor (UMRC3+TBR2) or type II and type III TGF-β receptor (UMRC3+TBR2+TBR3) demonstrated attenuated cell proliferation following the administration of exogenous TGF-β1 as compared to that of UMRC3 cells. FIG. **16**C shows UMRC3 cells, UMRC3+TBR2 cells, and UMRC3+TBR2+TBR3 stable cell lines transfected with 3TP/lux were treated with or without TGF-β and examined for luciferase activity. FIG. **16**D shows real-time PCR measuring mRNA levels for collagen IV type 6 in UMRC3, UMRC3+TBR2 cells, and UMRC3+TBR2+TBR3 cells in the presence of 2 ng/ml TGF-β1 for 24 h. FIG. **16**E shows colony formation assay demonstrates that UMRC3+TBR2+TBR3 cells have completely lost anchorage-independent growth, while attenuated growth in UMRC3+TBR2 cells occurs as compared to that of UMRC3 cells. The number of colonies were stained and counted after 45 days of growth. Data are expressed as mean±s.d.

FIG. **17**A shows growth inhibition after re-expressing human type III TGF-β receptor (TBR3) in UMRC3 cells. UMRC3 cells were stably transfected with TBR3 or infected using an adenoviral vector expressing TBR3. Cells were plated in culture dishes at 20,000 cells/well. Cell number was determined at the indicated times using a Coulter cell counter. FIG. **17**B shows RT-PCR data demonstrating the mRNA expression levels of type I, II, or III TGF-β receptors (TBR1, TBR2, TBR3) in UMRC3 cells in the presence or absence of the adenoviral vector expressing TBR3. Unmodified UMRC3 cells only express TBR1.

FIG. **18** shows re-expression of human type II or III TGF-β receptors (TBR2 or TBR3) inhibits tumor growth in nude mice. One million UMRC3 cells stably transfected with human type II or type III TGF-β receptors were implanted

into nude mice ectopically and tumor growth was measured weekly. Tumor volume (mm^3) was calculated by width×length×height×0.5236.

FIG. **19** shows hierarchical clustering of genes expressed in normal renal cortex verse stage I papillary renal cell carcinoma. Red indicates that a gene is highly expressed and green is indicative of low expression.

FIG. **20** shows hierarchical clustering of genes expressed in normal renal cortex verse benign oncocytoma. Red indicates that a gene is highly expressed and green is indicative of low expression.

FIG. **21** shows venn analysis of gene distribution among stage I renal cell carcinoma (RCC), oncocytoma and stage I papillary renal cell carcinoma.

FIG. **22** shows venn analysis of gene distribution among stage II renal cell carcinoma (RCC), oncocytoma and stage I papillary renal cell carcinoma.

DETAILED DESCRIPTION OF THE INVENTION

High-throughput technologies for assaying gene expression, such as high-density oligonucleotide and cDNA microarrays, offer the potential to identify clinically relevant genes differentially expressed between normal and tumor cells. The present invention discloses a genome-wide examination of differential gene expression between renal cell carcinomas (RCC) and normal renal epithelial cells.

Currently, there are no proven molecular markers useful clinically for the diagnosis, staging, or prognosis of sporadic renal cell carcinoma. The present invention detects genes that have differential expression between renal cell carcinomas and normal renal epithelial cells. The known function of some of these genes may provide insight into the biology of renal cell carcinomas while others may prove to be useful as diagnostic or therapeutic markers against renal cell carcinomas. Subtypes of renal cell carcinomas disclosed herein include stage I, II, III, and IV renal cell carcinomas (reflecting differences in tumor size, lymph node and organ metastasis), stage I papillary renal cell carcinoma, and benign oncocytoma.

The present invention provides methods of detecting conventional renal cell carcinoma based on determining the expression level of a number of genes that are found to have 2-fold or higher differential expression levels between tumor and normal tissue. In general, biological samples (e.g. tissue samples, serum samples, urine samples, saliva samples, blood samples or biopsy samples) are obtained from the individual to be tested and gene expression at RNA or protein level is compared to that in normal tissue. The normal tissue samples can be obtained from the same individual who is to be tested for renal cell carcinoma. It will be obvious to one of ordinary skill in the art that gene expression can be determined by DNA microarray and hierarchical cluster analysis, real-time PCR, RT-PCR, or northern analysis, whereas secreted gene products can be measured in blood samples by standard procedures.

In one embodiment, there is provided a method of detecting conventional or clear cell renal cell carcinoma based on differential expression of one or more of the following genes or proteins: TGF-β1, TGF-α, adrenomedulin, fibroblast growth factor 2 (FGF2), vascular epidermal growth factor (VEGF), osteonectin, follistatin like-3, inhibin beta A, spondin 2, chemokine X cytokine receptor 4 (CXCR4), fibronectin, neuropilin 1, frizzled homolog 1, insulin-like growth factor binding protein 3, laminin alpha 3, integrin beta 2, semaphorins 6A, semaphorins 5B, semaphorins 3B, caspase 1, sprouty 1, CDH16, PCDH9, compliment component 1-beta, compliment component 1-alpha, compliment component 1-gamma, CD53, CDW52, CD163, CD14, CD3Z, CD24, RAP1, angiopoietin 2, cytokine knot secreted protein, MAPKKKK4, 4-hydroxyphenylpyruvate dioxygenase, pyruvate carboxyknase 2, 11-beta-hydroxysteroid dehydrogenase 2, GAS1, CDKN1, nucleolar protein 3, interferon induced protein 44, NR3C1, vitamin D receptor, hypothetical protein FLJ14957 (Affy#225817_at), metallothionein 2A, metallothionein-If gene, metallothionein 1H, secreted frizzled related protein 1, connective tissue growth factor, and epidermal growth factor.

In another embodiment, there is provided a method of detecting conventional renal cell carcinoma by examining the expression level of type III TGF-β receptor, wherein decreased expression of type III TGF-b receptor indicates the presence of renal cell carcinoma. In general, the expression level of type III TGF-β receptor can be determined at the mRNA or protein level.

The present invention also provides methods of detecting stage I conventional renal cell carcinoma, stage II conventional renal cell carcinoma, stage I papillary renal cell carcinoma, or benign oncocytoma based on over-expression or down-regulation of a number of genes identified in the present invention. The present invention discloses a number of genes that are up- or down-regulated specifically in these subtypes of renal cell carcinoma. Determining the expression levels of these genes would provide specific diagnosis for these different subtypes of renal cell carcinoma.

For example, stage I conventional renal cell carcinoma can be detected based on (i) over-expression of one or more genes listed in Table 1, (ii) down-regulation of one or more genes listed in Table 2, or (iii) over-expression of one or more genes listed in Table 1 and down-regulation of one or more genes listed in Table 2. Similarly, stage II conventional renal cell carcinoma can be detected based on (i) over-expression of one or more genes listed in Table 3, (ii) down-regulation of one or more genes listed in Table 4, or (iii) over-expression of one or more genes listed in Table 3 and down-regulation of one or more genes listed in Table 4.

The present invention also discloses a number of genes that are up- or down-regulated in both stage I and stage II conventional renal cell carcinoma (Tables 5 and 6 respectively). These genes would also provide diagnosis for stage I or stage II conventional renal cell carcinoma. Hence, stage I or stage II conventional renal cell carcinoma can be detected based on (i) over-expression of one or more genes listed in Table 5, or (ii) down-regulation of one or more genes listed in Table 6.

In another embodiment, stage I papillary renal cell carcinoma can be detected based on (i) over-expression of one or more genes listed in Table 8, (ii) down-regulation of one or more genes listed in Table 9, or (iii) over-expression of one or more genes listed in Table 8 and down-regulation of one or more genes listed in Table 9.

In yet another embodiment, benign oncocytoma can be detected based on (i) over-expression of one or more genes listed in Table 10, (ii) down-regulation of one or more genes listed in Table 11, or (iii) over-expression of one or more genes listed in Table 10 and down-regulation of one or more genes listed in Table 11.

In still yet another embodiment, there are provided methods of utilizing genes over-expressed on the cell surface of renal carcinoma tissue to develop antibodies or other small molecules for the purpose of specifically targeting the renal tumor cells. The present invention discloses a number of genes that are up-regulated in stage I renal cell carcinoma (RCC), stage II RCC tumor, stage I papillary RCC, and benign oncocytoma. Antibodies or small molecules directed against proteins encoded by these genes can be linked with a

therapeutic drug to deliver drug to the tumor tissue, or be linked with dye, nanoparticle or other imaging agents for cancer imaging. Some of the novel genes identified herein for the first time include, but are not limited to, the following genes: calcitonin receptor-like (206331_at; 210815_s_at); receptor (calcitonin) activity modifying protein 2 (RAMP2; 205779_at); endothelin receptor type B (206701_x_at); beta 2 integrin (202803_s_at); alpha 5 integrin (201389_at); chemokine X cytokine receptor 4 (CXCR4); fibronectin; neuropilin 1 (212298_at; 210510_s_at); CD24; CD14; Cd163; CD53; Compliment Componenet 1-beta, 1-alpha, and 1-gamma; CDH4; integrin beta2; ADAM28; FK506 binding protein; collagen Valpha2; tumor necrosis factor receptor superfamily, member 6; tumor necrosis factor receptor superfamily, member 5; tumor necrosis factor (ligand) superfamily, member 13b; tumor necrosis factor receptor superfamily, member 12A; and the FGF receptor.

In another embodiment, there is provided a method of treating conventional or clear cell renal cell carcinoma. The method involves replacing tumor suppressor genes (e.g., via gene therapy) whose expression is down-regulated in tumor tissues or introducing a molecule that induces the down-regulated gene to be re-expressed in the tumor. The present invention discloses a number of genes that are down-regulated in stage I renal cell carcinoma (RCC), stage II RCC tumor, stage I papillary RCC, and benign oncocytoma. Some examples of down-regulated genes identified in stage I and/or II RCC tumors include, but are not limited to, CDKN1, secreted frizzled related protein 1, semaphoring 6D, semaphoring 3B, CDH16, TNF alpha, calbindin D28, defensin beta1, beta-catenin interacting protein 1, GAS1, vitamin D receptor, Kruppel-like factor 15. This method of treatment can be combined with other therapies to provide combinatorial therapy.

The genes that are found to have altered expression in stage I and stage II renal cell carcinoma would also be useful for determining patient prognosis. These genes or gene products (i.e., proteins) would have the unique characteristic of being altered in tumor verses normal samples in a subset of patients. For example, basic transcription element binding protein 1 is down-regulated in 7 out of 12 renal cell carcinoma tumors. Other examples include CD164, decreased 5/12; Map kinase kinase kinase 7, increased 6/12; Endoglin, increased 7/12; SERPIN A1, increased 6/12; Metalloprotease 11 (MMP11), increased 7/12; Integrin 3 alpha, increased 4/12; carbonic anhydrase II, decreased 7/12; protein tyrosine kinase 2, increased 4/12; fibroblast growth factor 11, increased 6/12; fibroblast growth factor 2, increased 7/12; VEGF B, increased 5/12.

Moreover, the levels of change may be a useful determinant of patient outcome and/or rationale for strategy of treatment course. An example of this is found for chemokine (C—X—C motif) ligand 14 (CXCL14, 222484_s_at). Six patients with stage I and six patients with stage II renal cell carcinoma were analyzed by genomic profiling. A patient with stage I renal cell carcinoma has CXCL14 mRNA expression levels of 19862 and 24.49 in his normal tissue and tumor tissue respectively. This patient would be predicted to have a poor prognosis or poor response to therapy based upon this result along with other gene predictors. On the other hand, a patient with stage II RCC has CXCL14 mRNA expression levels of 20435 and 18557 in his normal tissue and tumor tissue respectively. This patient would be predicted to have a good prognosis and good response to chemotherapy.

The following examples are given for illustrating various embodiments of the invention and are not meant to limit the present invention in any fashion. One skilled in the art will appreciate readily that the present invention is well adapted to carry out the objects and obtain the ends and advantages mentioned, as well as those objects, ends and advantages inherent herein. Changes therein and other uses which are encompassed within the spirit of the invention as defined by the scope of the claims will occur to those skilled in the art.

EXAMPLE 1

Tissue Banking

Renal tissue (normal and tumor) was transported to a sterile hood on ice and under sterile conditions. Tissue was dissected under the direction of a pathologist. The tissue was frozen in liquid nitrogen for isolation of RNA, DNA, and protein or processed to establish primary cell cultures. The tissue was fixed in formalin for immunohistochemistry and in situ hybridization and RNAlater (Ambion) for RNA isolation. Primary normal renal epithelial (NRE) cell cultures were established using standard collagenase/Dnase techniques to digest tissue and isolate single cells. NREs were easily isolated and grew well in culture for up to 10 passages. These cells were further analyzed for homogeneity with regard to epithelial population using appropriate immunohistochemical markers such as vimentin, cytokeratin, and megalin.

EXAMPLE 2

Genomic Gene Array and Microarray Data Analysis

Gene expression profiling was performed using Affymetrix HU95A oligonucleotide gene arrays (>12,600 genes) or HG-U133 A&B GeneChip® oligonucleotide microarrays (33,000+ probe sets). Total RNA (Trizol®, Ambion) was extracted from patient-matched normal renal cortex and tumor tissue from patients diagnosed with local disease confined to the kidney. Alternatively, the investigators analyzed metastatic disease defined by lesions in lymph nodes, adrenal, or other organs. Data were analyzed by a combination of two-dimensional ANOVA, Affymetrix MAS5.0®, and hierarchical cluster analysis using Spotfire®. Procedure that were used to identify altered expression of large sets of genes, as well as other issues concerning microarray analyses can be found in a recent review article by Copland et al. (2003).

EXAMPLE 3

Real-Time PCR

Applied Biosystems' assays-by-design or assays-on-demand 20× assay mix of primers and TaqMan® MGB probes (FAM® dye-labeled) for all target genes and predeveloped 18S rRNA (VIC® dye-labeled probe) TaqMan® assay reagent for internal control were used for real-time PCR measurements. These assays were designed to span exon-exon junctions so as not to detect genomic DNA and all primers and probes sequences were searched against the Celera database to confirm specificity. Validation experiments were performed to test the efficiency of the target amplification and the efficiency of the reference amplification. All absolute values of the slope of log input amount versus DC_T is less than 0.1.

Separate tubes (singleplex) for one-step RT-PCR was performed with 50 ng RNA for both target genes and endogenous controls using TaqMan® one-step RT-PCR master mix reagent kit (Applied Biosystems). The cycling parameters for one-step RT-PCR were: reverse transcription 48° C. for 30

min, AmpliTaq® activation 95° C. for 10 min, denaturation 95° C. for 15 s, and annealing/extension 60° C. for 1 min (repeat 40 times) on ABI7000®. Duplicate C_T values were analyzed with Microsoft Excel® using the comparative C_T(DDC$_T$) method as described by the manufacturer (Applied Biosystems). The amount of target (2^{-DDCT}) was obtained by normalizing to an endogenous reference (18smRNA) and relative to a calibrator (normal tissue).

EXAMPLE 4

Immunohistochemical Analyses of Protein Expression

For immunohistochemical analyses of type I TGF-β receptor (TBR1), type II TGF-β receptor (TBR2), and type Ill TGF-β receptor (TBR3) expression, patient-matched normal renal and tumor tissue samples were fixed in 10% neutral-buffered formalin and embedded in paraffin blocks. Consecutive sections were cut 5 um thick, deparaffinized, hydrated, and immunostained using antibodies recognizing human TBR1, TBR2, and TBR3 (1:100; Santa Cruz Biotechnology). Biotinylated secondary antibody (1:600; Santa Cruz Biotechnology) was detected using avidin-biotin-peroxidase detection according to the manufacturer's instructions (Vectastatin Elite ABC kit; Vector Lab). All slides were lightly counterstained with hematoxylin before dehydration and mounting.

For cell lines, cells were plated on glass coverslips in wells. Prior to the detection of TGF-β receptor expression as described above, cells were fixed onto the coverslips with 3% formalin.

EXAMPLE 5

Gene Expression Profiling of Renal Cell Carcinoma

Gene expression profiling was performed using Affymetrix oligonucleotide gene arrays. RNA was extracted from patient-matched normal renal cortical and tumor tissues from patients diagnosed with localized and metastatic renal cell carcinoma. Data were analyzed by a combination of two-dimensional ANOVA, Affymetrix MAS5.0®, and hierarchical cluster analysis using Spotfire® (reviewed in Copland et al., 2003).

A primary goal of microarray analysis is to discover hidden patterns of differential expression within a large data field. Normal renal cortical and primary tumor tissue with no metastasis were collected from patients diagnosed with local disease. Normal tissue, primary tumor, and metastatic tissue were also collected from patients diagnosed with metastatic disease. Comparison of patient-matched normal and tumor tissue allowed for the discovery of changes in mRNA levels between normal and tumor tissue, as well as local and metastatic disease.

Heatmaps with two-way dendograms depicting genes specifically altered in tumor tissue as compared to normal renal cortex are shown in FIG. **1**. FIG. **1**A shows hierarchical clustering of genes expressed in normal renal cortex verses stage I conventional renal cell carcinoma. FIG. **1**B shows hierarchical clustering of genes expressed in normal renal cortex verses stage II renal cell carcinoma. FIG. **1**C shows hierarchical clustering of genes selected from a Venn analysis in which the chosen genes were expressed in common in both stage I and II at a 99% confidence level.

TGF-β1, TGF-α and adrenomedulin mRNA levels were up-regulated in all stages of renal cell carcinoma as compared to normal tissue counterparts (FIGS. **2-4**), whereas TGF-β2 and TGF-β3 mRNA levels were not altered between normal and tumor matched samples (FIGS. **5-6**).

Tumor suppressor gene Wilms Tumor 1 (WT1) was down-regulated in all stages of renal cell carcinoma (FIG. **7**). A small percentage of tumor tissues demonstrated attenuated von Hippel Lindau mRNA levels when compared to matched normal tissue (FIG. **8**). Calbindin mRNA was completely lost (FIG. **9**) while MUC1 was greatly attenuated in stage I renal cell carcinoma (FIG. **10**).

The present analysis identifies 278 genes that were up-regulated in stage I renal cell carcinoma, whereas 380 genes were up-regulated in stage II renal cell carcinoma. Among these genes, 82 were up-regulated in both stages I and II renal cell carcinoma. One hundred fifty nine genes were down-regulated in stage I renal cell carcinoma, whereas 195 genes were down-regulated in stage II RCC. Among these genes, 82 were down-regulated in both stage I and II renal cell carcinoma.

Genes over-expressed and down-regulated in stage I renal cell carcinoma are listed in Table 1 and Table 2 respectively. Genes over-expressed and down-regulated in stage I renal cell carcinoma are listed in Table 3 and Table 4 respectively. Genes over-expressed in both stage I and II renal cell carcinoma are listed in Table 5. Genes down-regulated in both stage I and II renal cell carcinoma are listed in Table 6.

TABLE 1

Genes With Up-Regulated Expression In stage I Renal Cell Carcinoma

Genbank ID	Gene Symbol	Genbank ID	Gene Symbol
NM004356.1	CD81	NM004079.1	CTSS
NM002293.2	LAMC1	NM001784.1	CD97
NM000980.1	RPL18A	AF151853.1	PREI3
AK002091.1	MGEA5	NM000491.2	C1QB
NM005721.2	ACTR3	BC000125.1	TGFB1
NM002668.1	PLP2	NM004520.1	KIF2
NM021038.1	MBNL	NM000321.1	RB1
AF070656.1	YME1L1	NM012262.2	HS2ST1
NM021029.1	RPL36A	NM000560.1	CD53
NM002945.1	RPA1	NM005502.1	ABCA1
NM002480.1	PPP1R12A	AF285167.1	ABCA1
NM001349.1	DARS	BG170541	MET
NM005496.1	SMC4L1	NM021642.1	FCGR2A
AW163148	MARCKS	BE967532	KIAA0220
NM002356.4	MARCKS	NM006526.1	ZNF217
M68956.1	MARCKS	NM000570.1	FCGR3B
AI589086	LAPTM5	N26005	PPP1R3C
NM006762.1	LAPTM5	NM006153.1	NCK1
NM014267.1	SMAP	NM001549.1	IFIT4
NM000235.1	LIPA	NM003141.1	SSA1
NM000176.1	NR3C1	NM014705.1	KIAA0716
NM005737.2	ARL7	NM005197.1	CHES1
NM005737.2	ARL7	NM002907.1	RECQL
BC001051.1	ARL7	U43328.1	CRTL1
NM006169.1	NNMT	NM017925.1	FLJ20686
NM005862.1	STAG1	NM006773.2	DDX18
AI356412	LYN	U20350.1	CX3CR1
NM002350.1	LYN	NM005761.1	PLXNC1
BG107456	TRIP-Br2	NM004834.1	MAP4K4
NM021913.1	AXL	NM021644.1	HNRPH3
NM002194.2	INPP1	NM006640.1	MSF
NM019058.1	RTP801	NM004180.1	TANK
NM002110.1	HCK	AW148801	NAP1L1
NM030755.1	TXNDC	AB011118.1	KIAA0546
NM030984.1	TBXAS1	AU145005	SP3
NM014350.1	GG2-1	N80918	CG018
BC001312.1	P5	BF439472	ATP11A
U14990.1	RPS3	BE968801	RPL35A
D83043.1	HLA-B	AI985751	NAP1L1
AI888672	NAP1L1	AI735692	LST1
BC002387.1	NAP1L1	AA995910	ALOX5
M60334.1	HLA-DRA	M12679.1	HUMMHCW1A

TABLE 1-continued

Genes With Up-Regulated Expression In stage I Renal Cell Carcinoma

Genbank ID	Gene Symbol	Genbank ID	Gene Symbol
AF161522.1	C3orf4	AL133053.1	FLJ23861
BG256677	IFI16	X03348.1	NR3C1
M26880.1	UBC	AC005339	N/A
U17496.1	PSMB8	AK024836.1	HLA-C
AF141347.1	TUBA3	AC003999	SCAP2
L01639.1	CXCR4	AJ224869	CXCR4
NM005445.1	CSPG6	AL022067	PRDM1
AB030655.1	EFEMP2	AL110158.1	KIAA1078
AF165520.1	APOBEC3C	S81916.1	N/A
AF009670.1	ABCC3	M80469	N/A
AF020314.1	CMRF-35H	NM002860.1	PYCS
BC001606.1	NCF2	NM020198.1	GK001
BC005352.1	GG2-1	NM016304.1	C15orf15
AF281030.1	HRIHFB2122	AA102574	BAZ1A
BC001052.1	RECQL	NM024844.1	PCNT1
L32610.1	HNRPH3	NM015938.1	CGI-07
M23612.1	RASA1	NM018200.1	HMG20A
AF109683.1	LAIR1	NM025235.1	TNKS2
BC002841.1	HSA9761	NM015991.1	C1QA
D29640.1	IQGAP1	NM016090.1	RBM7
L25259.1	CD86	NM024554.1	PGBD5
M60333.1	HLA-DRA	NM017718.1	FLJ20220
U13698.1	CASP1	NM017923.1	FLJ20668
U90940.1	FCGR2C	NM030921.1	DC42
M90685.1	HLA-G	BC004470.1	ASC
M90684.1	HLA-G	AK021413.1	LARS
M90686.1	HLA-G	BF444916	FAD104
L22453.1	RPL3	BC004819.1	PLDN
U01351.1	NR3C1	AF247167.1	AD031
U62824.1	HLA-C	U39402.1	N/A
L07950.1	HLA-B	BC006112.1	DKFZP434B195
AF348491.1	CXCR4	BG388615	N/A
NM003079.1	SMARCE1	AB033007.1	KIAA1181
BE646386	EXO70	BG250721	N/A
AI972475	N/A	AK024221.1	C40
AA195999	MAPK1	BF477658	N/A
AL049397.1	N/A	BG251556	KIAA1949
BE895685	KIAA0853	AB033091.1	KIAA1265
M82882.1	ELF1	AK024350.1	AMOTL1
AB020633.1	KIAA0826	NM018440.1	PAG
AL031781	N/A	AW500180	N/A
BF209337	MGC4677	AW026543	N/A
AI709406	N/A	AI092770	N/A
AI806905	N/A	NM020679.1	AD023
AI392933	FLJ36090	AK024855.1	CTSS
AH42096	N/A	AK000119.1	N/A
AL137430.1	N/A	AW977527	PRDM1
AV724266	FLJ20093	BE671060	N/A
BF589359	N/A	AL037450	N/A
AW084125	CAPZA1	AI401535	N/A
N20927	RAP2B	AV683852	N/A
AI627666	LOC115548	BF055144	N/A
AV726322	N/A	AA352113	N/A
AI697657	LANPL	BF056209	N/A
BF002625	N/A	X60592	TMFRSF5
BF439533	N/A		

TABLE 2

Genes With Down-Regulated Expression In stage I Renal Cell Carcinoma

Genbank ID	Gene Symbol	Genbank ID	Gene Symbol
L38487	ESRRA	AK024386.1	GRHPR
NM004415.1	DSP	AL109716.2	N/A
NM005327.1	HADHSC	AK026411.1	ALDOB
NM003321.1	TUFM	M10943	N/A
NM002084.2	GPX3	AW088547	N/A
AI983043	N/A	NM018049.1	GNRPX
NM006066.1	AKR1A1	NM017900.1	AKIP
NM006384.2	CIB1	NM006548.1	IMP-2

TABLE 2-continued

Genes With Down-Regulated Expression In stage I Renal Cell Carcinoma

Genbank ID	Gene Symbol	Genbank ID	Gene Symbol
NM001685.1	ATP5J	NM025135.1	KIAA1695
NM014652.1	IMP13	NM016458.2	LOC51236
NM013410.1	AK3	NM022128.1	RBSK
NM016725.1	FOLR1	NM015974.1	CRYL1
NM021151.1	CROT	NM013333.1	EPN1
NM005951.1	MT1H	AA133341	C14orf87
NM005952.1	MT1X	AF226732.1	NPD007
AL080102.1	N/A	AF265439.1	MRPS15
BC000931.2	ATP5C1	AI743534	DKFZP564B1162
BC005398.1	DKFZP566D193	AB042647.1	B29
D87292.1	TST	AL522667	ORF1-FL49
AU151428	IDH2	BG255416	KIAA0114
BC000109.1	ILVBL	AF308301.1	MRPS26
AF333388.1	N/A	BE408081	N/A
NM005953.1	MT2A	AL521634	FLJ32452
BF217861	N/A	BF203664	MGC14288
AA594937	COBL	BE645551	MGC39329
AW052179	COL4A5	AW193698	TGFBR3
AI884867	LOC155066	BF540829	N/A
BF246115	N/A	W72455	FLJ25476
AW028110	KIAA0500	AI457453	N/A
AW242315	N/A	BF056892	N/A
AW080549	FUT3	AK024386.1	GRHPR
AW149846	GPX3	AL109716.2	N/A
AI038402	N/A	AA442776	N/A
AI051046	MGC4614	AI913600	N/A
AI659456	N/A	AW771908	N/A
AW664964	N/A	AI807887	N/A
AI631895	SGK2	AW102941	N/A
AI263078	FLJ31168	AW024656	N/A
BF057634	HOXD8	AB002342	PRKWNK1
AA746038	GPR110		

TABLE 3

Genes With Up-Regulated Expression In stage II Renal Cell Carcinoma

Genbank ID	Gene Symbol	Genbank ID	Gene Symbol
NM006096.1	NDRG1	NM002053.1	GBP1
NM006098.1	GNB2L1	NM000089.1	COL1A2
NM001780.1	CD63	NM021105.1	PLSCR1
NM003118.1	SPARC	NM002467.1	MYC
NM000291.1	PGK1	NM001284.1	AP3S1
NM003870.1	IQGAP1	AI825926	PLSCR1
AB032261.1	SCD	NM014736.1	KIAA0101
NM002629.1	PGAM1	AF161461.1	LEPROTL1
NM003564.1	TAGLN2	NM014873.1	KIAA0205
NM000310.1	PPT1	AI005043	N/A
NM003405.1	YWHAH	NM000416.1	IFNGR1
U82164.1	MIC2	NM004172.1	SLC1A3
NM002305.2	LGALS1	NM004207.1	SLC16A3
NM001096.1	ACLY	AI761561	HK2
NM002121.1	HLA-DPB1	Y09216.1	N/A
NM021038.1	MBNL	NM002922.1	RGS1
NM003651.1	CSDA	NM005990.1	STK10
AV685920	CAPZA2	NM014863.1	GALNAC4S-6ST
NM002654.1	PKM2	NM014737.1	RASSF2
NM001175.1	ARHGDIB	NM000418.1	IL4R
BC000182.1	ANXA4	BC000658.1	STC2
NM001153.2	ANXA4	NM003751.1	EIF3S9
NM001975.1	ENO2	NM002339.1	LSP1
NM006435.1	IFTTM2	NM004604.1	STX4A
NM001387.1	DPYSL3	NM006404.1	PROCR
BG398414	RPA1	AF275945.1	EVA1
NM004039.1	ANXA2	NM004221.1	NK4
NM005534.1	IFNGR2	NM004556.1	NFKBIE
AL136877.1	SMC4L1	NM004688.1	NMI
NM014876.1	KIAA0063	NM003332.1	TYROBP
NM024830.1	FLJ12443	NM015136.1	STAB1
NM005505.1	SCARB1	NM006019.1	TCIRG1

TABLE 3-continued

Genes With Up-Regulated Expression In stage II Renal Cell Carcinoma

Genbank ID	Gene Symbol	Genbank ID	Gene Symbol
NM003025.1	SH3GL1	NM004877.1	GMFG
NM013285.1	HUMAUANTIG	NM002317.1	LOX
NM005720.1	ARPC1B	NM025201.1	PP1628
AW157070	EGFR	NM014800.1	ELMO1
NM002835.1	PTPN12	L41944.1	IFNAR2
NM004428.1	EFNA1	NM007268.1	Z39IG
AW006290	SUDD	NM006994.2	BTN3A3
NM014791.1	MELK	AF091352.1	VEGF
NM014882.1	KIAA0053	AB035482.1	ICB-1
NM003864.1	SAP30	Z24727.1	TPM1
NM001558.1	IL10RA	M19267.1	TPM1
NM003264.1	TLR2	U13700.1	CASP1
NM014221.1	MTCP1	M27281.1	VEGF
AV756141	CSF2RB	BC005838.1	N/A
AI123251	LCP2	BC005858.1	FN1
NM006433.2	GNLY	BC005926.1	EVI2B
NM000861.2	HRH1	BE513104	YARS
NM001870.1	CPA3	AU147399	CAV1
NM003586.1	DOC2A	AK023154.1	HN1L
NM004271.1	MD-1	AK021757.1	KIAA0648
NM014932.1	NLGN1	H95344	VEGF
NM014947.1	KIAA1041	AB023231.1	FNBP4
NM000647.2	CCR2	AL523076	N/A
NM002562.1	P2RX7	NM030666.1	SERPINB1
NM006058.1	TNIP1	AB018289.1	KIAA0746
NM013447.1	EMR2	AW043713	SULF1
NM013416.1	NCF4	BE880591	EP400
NM001776.1	ENTPD1	AU158495	NOTCH2
NM020037.1	ABCC3	BE965029	N/A
NM006135.1	CAPZA1	AL564683	CEBPB
NM007036.2	ESM1	AA349595	RAB6IP1
AF034607.1	CLIC1	AI809341	PTPRC
BC000915.1	PDLIM1	AW205215	KIAA0286
AL162068.1	NAP1L1	BE349017	HA-1
NM006947.1	SRP72	AF070592.1	HSKM-B
L12387.1	SRI	AI769685	CARS
AF141349.1	N/A	AI935123	LOC113146
AF263293.1	SH3GLB1	BG255188	N/A
BC000389.1	TM4SF7	AI088622	PRKCDBP
AF007162.1	CRYAB	BE222709	N/A
D38616.1	PHKA2	AW007573	DKFZP586L151
AV717590	ENTPD1	BG332462	N/A
U87967.1	ENTPD1	AI862658	FEM1C
H23979	MOX2	AI934469	KIAA0779
AF063591.1	MOX2	AB018345.1	KIAA0802
BC005254.1	CLECSF2	W87466	LOC92689
BC000893.1	H2BFT	BE908217	ANXA2
L22431.1	VLDLR	NM005615.1	RNASE6
AI741056	SELPLG	BE300252	K-ALPHA-1
AF084462.1	RIT1	BF740152	MYO1F
U62027.1	C3AR1	AV711904	LYZ
M87507.1	CASP1	AW072388	N/A
J04132.1	CD3Z	AW190316	SHMT2
M31159.1	IGFBP3	NM005412.1	SHMT2
AF257318.1	SH3GLB1	NM006417.1	IFI44
BC001388.1	ANXA2	AL008730	C6orf4
AF130095.1	FN1	L16895	LOC114990
AF022375.1	VEGF	Z21533.1	HHEX
AA807529	MCM5	AK022955.1	DKFZp762L0311
AK026737.1	FN1	BF001267	N/A
X14355.1	N/A	AL558987	N/A
AK025608.1	KIAA0930	AA577672	LOC151636
AF183421.1	RAB31	BE620734	ZAK
NM002695.1	POLR2E	AI937446	N/A
AF288391.1	C1orf24	H99792	N/A
NM003730.2	RNASE6PL	BE966748	N/A
NM016359.1	ANKT	AI659418	MGC21854
NM014164.2	FXYD5	AI990891	DKFZp761K2222
NM022736.1	FLJ14153	AA827892	N/A
NM021158.1	C20orf97	AL135264	N/A
NM017792.1	FLJ20373	AI375753	N/A
NM020142.1	LOC56901	AA573502	TAP2
NM016448.1	RAMP	BG387557	CASP2
NM005767.1	P2Y5	AA554833	MAP1B
NM020169.1	LXN	AK026764.1	N/A

TABLE 3-continued

Genes With Up-Regulated Expression In stage II Renal Cell Carcinoma

Genbank ID	Gene Symbol	Genbank ID	Gene Symbol
NM022834.1	FLJ22215	AU146532	PDK1
NM018460.1	BM046	BE348597	N/A
NM024629.1	FLJ23468	AL577758	LOC133957
NM018641.1	C4S-2	AI133452	FGG
NM018295.1	FLJ11000	AU157224	N/A
NM024576.1	FLJ21079	AI742057	N/A
NM016582.1	PHT2	BE500942	N/A
NM003116.1	SPAG4	N25631	RFXANK
NM018454.1	ANKT	AU145366	N/A
NM018099.1	FLJ10462	AW270037	KIAA0779
NM007072.1	HHLA2	BF526978	N/A
NM022445.1	TPK1	AW182575	N/A
AW173623	TDE1	BF339831	MGC13114
AB044088.1	BHLHB3	AI056992	N/A
AF043244.1	NOL3	BE222668	N/A
AF133207.1	H11	BG165011	N/A
AF313468.1	CLECSF12	AI188445	MGC14289
AA191576	NPM1	BE551416	HAK
AI765383	KIAA1466	AI972498	a1/3GTP
BC003654.1	SLC27A3	AW662189	N/A
W60806	N/A	AA142842	N/A
AI335263	NETO2	BF939473	N/A
AI378406	EGLN3	AI681260	N/A
BC005400.1	FKSG14	AA551090	AP1S2
AI761520	CENTA2	AA045175	MS4A6A
BC000771.1	TPM3	W05495	N/A
BC000190.1	HSPC216	AI093231	N/A
BC002776.1	SEMA5B	AI565054	N/A
AF132203.1	SCD	AL553774	N/A
BC006107.1	ARHGAP9	AK023470.1	MGC15875
AK024263.1	N/A	AL157377	ENPP3
AK024846.1	SET7	AL139109	TEX11
BE878463	N/A	AK025631.1	POLH
AW304786	PTR4	AI873425	N/A
AI769269	N/A	BF541967	N/A
AI935334	N/A	AI686890	N/A
BF437747	SAMHD1	AI936034	ITGA4
AW300953	N/A	U88964	ISG20
H37811	N/A	AJ243797	TREX1
AA603344	SAMHD1	D29642	KIAA0053
AA742310	N/A	D87433	STAB1
AI248208	FLJ25804	AI129310	FLJ21562
AI962367	ECGF1		

TABLE 4

Genes With Down-Regulated Expression In stage II Renal Cell Carcinoma

Genbank ID	Gene Symbol	Genbank ID	Gene Symbol
NM012248.1	SPS2	AB019695.1	TXNRD2
NM002300.1	LDHB	M61900.1	PTGDS
BC000306.1	HADHSC	BF967998	N/A
NM001640.2	APEH	BF967998	N/A
NM005875.1	GC20	AL526243	KIAA0446
NM003365.1	UQCRC1	NM000532.1	PCCB
BF031714	HYA22	BE042354	LDHB
NM005808.1	HYA22	AI587323	ATP5A1
AF113129.1	ATP6V1A1	AW195882	ATPW
NM002402.1	MEST	H71135	ADH6
NM006844.1	ILVBL	AV659180	ALDOB
NM004636.1	SEMA3B	AK027006.1	TNRC9
NM002496.1	NDUFS8	AV693216	PLXNB1
NM006556.1	PMVK	BG398937	N/A
NM004255.1	COX5A	NM002489.1	NDUFA4
NM002225.2	IVD	NM003849.1	SUCLG1
NM004524.1	LLGL2	NM014019.1	HSPC009
AI950380	BCL7A	NM024952.1	FLJ20950
AB020707.1	WASF3	NM014185.1	MOG1
NM000481.1	AMT	NM018013.1	FLJ10159
NM012317.1	LDOC1	NM018373.1	SYNJ2BP

TABLE 4-continued

Genes With Down-Regulated Expression In stage II Renal Cell Carcinoma

Genbank ID	Gene Symbol	Genbank ID	Gene Symbol
NM006456.1	STHM	NM014067.2	LRP16
NM006614.1	CHL1	NM013261.1	PPARGC1
NM015393.1	DKFZP564O0823	NM021963.1	NAP1L2
AV729634	DNAJC6	NM018658.1	KCNJ16
NM002628.1	PFN2	NM014553.1	LBP-9
NM003500.1	ACOX2	AF112204.1	ATP6V1H
NM002655.1	PLAG1	AU145941	CDC14B
NM004393.1	DAG1	AF061264.1	MGC4825
NM003026.1	SH3GL2	BF941492	FLJ10496
NM002010.1	FGF9	AI984229	HSPC121
NM014033.1	DKFZP586A0522	N71923	FLRT3
NM004868.1	GPSN2	BC005050.1	NICN1
BC000649.1	UQCRFS1	AF172327.1	N/A
S69189.1	ACOX1	AF356515.1	HINT2
AF153330.1	SLC19A2	BE620739	RHOBTB3
AF094518.1	ESRRG	BF435123	N/A
M55575.1	BCKDHB	AW149498	BTBD6
BE044480	MGC32124	AW024437	LOC118491
BF382393	N/A	AW195353	N/A
AV751731	PNKP	BE044193	N/A
U55984	N/A	AI493303	FLJ31709
BF059512	DNER	AI636080	N/A
AK025934.1	Evi1	BF509031	ATP6V1G3
AL036088	SEMA6D	AW242920	N/A
BE964222	FLJ38482	BF002046	ANGPTL1
AW290940	N/A	BF130943	N/A
AL545998	N/A	AW452631	N/A
AW274874	N/A	AI792937	N/A
AI709389	N/A	AI810572	N/A
BF224092	MGC15854	BG165743	LOC112817
AU145805	N/A	AW466989	N/A
AW079843	MGC33338	R48991	N/A
AW138815	N/A	BF029215	MSI2
AW242286	N/A	D21851	LARS2
AW025023	N/A	Z83838	ARHGAP8
BE672659	N/A		

TABLE 5

Genes With Up-Regulated Expression In both stage I & stage II Renal Cell Carcinoma

Genbank ID	Gene Symbol	Genbank ID	Gene Symbol
NM005566.1	LDHA	NM014812.1	KIAA0470
NM000291.1	PGK1	AF208043.1	IFI16
NM001219.2	CALU	BC002654.1	TUBB-5
NM002966.1	S100A10	BC006379.1	K-ALPHA-1
NM000034.1	ALDOA	BC006481.1	K-ALPHA-1
NM002627.1	PFKP	AF000426.1	LST1
NM006082.1	K-ALPHA-1	AF000424.1	LST1
AI922599	VIM	BG500301	ITGB1
NM020474.2	GALNT1	AL516350	ARPC5
NM006406.1	PRDX4	M27487.1	HLA-DPA1
NM015344.1	LEPROTL1	M27487.1	HLA-DPA1
NM014755.1	TRIP-Br2	AW517686	ATP2B4
AI796269	NBS1	AL581768	K-ALPHA-1
NM005783.1	APACD	AA524505	TSGA
BF197655	N/A	Z78330	ACTR3
NM001233.1	CAV2	Z78330	ACTR3
NM002845.1	PTPRM	BG532690	ITGA4
NM014302.1	SEC61G	AW005535	RAP2B
U47924	CD4	NM007161.1	LST1
NM004106.1	FCER1G	AK026577.1	ALDOA
NM015474.1	SAMHD1	AI091079	SHC1
NM004915.2	ABCG1	AV713720	LST1
NM002432.1	MNDA	NM021103.1	TMSB10
NM005565.2	LCP2	NM016337.1	RNB6
NM005531.1	IFI16	NM013260.1	HCNGP
NM005849.1	IGSF6	NM021199.1	SQRDL
NM002189.1	IL15RA	NM018149.1	FLJ10587
NM004353.1	SERPINH1	NM016951.2	CKLF1

TABLE 5-continued

Genes With Up-Regulated Expression In both stage I & stage II Renal Cell Carcinoma

Genbank ID	Gene Symbol	Genbank ID	Gene Symbol
NM017760.1	FLJ20311	AB033038.1	FLJ10392
NM022349.1	MS4A6A	AI184968	C1QG
NM023003.1	TM6SF1	AL161725	FLJ00026
NM016184.1	CLECSF6	NM018440.1	PAG
NM031284.1	DKFZP434B195	AL553942	FLJ31951
BC002342.1	CORO1C	AI394438	N/A
AA775177	PTPRE	T64884	N/A
AL162070.1	CORO1C	T64884	N/A
AF253977.1	MS4A6A	AW511319	N/A
AF237908.1	MS4A6A	AI640834	RA-GEF-2
W03103	DDEF1	AI655467	N/A
AK022888.1	FENS-1	AL161725	FLJ00026
AI141784	N/A	T92908	N/A

TABLE 6

Genes With Down-Regulated Expression In Both stage I And stage II Renal Cell Carcinoma

Genbank ID	Gene Symbol	Genbank ID	Gene Symbol
NM004092.2	ECHS1	BC002449.1	FLJ13612
NM000270.1	NP	J02639.1	SERPINA5
NM002354.1	TACSTD1	BC002571.1	DKFZP564O243
AF017987.1	SFRP1	U03884.1	KCNJ1
NM003012.2	SFRP1	AF173154.1	HYAL1
NM000666.1	ACY1	AF130103.1	PBP
NM000191.1	HMGCL	AL117618.1	PDHB
NM015254.1	KIF13B	AF063606.1	N/A
NM000140.1	FECH	BC005314.1	N/A
U75667.1	ARG2	BF686267	PBP
NM000196.1	HSD11B2	AI742553	PRKWNK1
NM014636.1	RALGPS1A	D83782.1	SCAP
NM001441.1	FAAH	AB029031.1	TBC1D1
NM005978.2	S100A2	AK025432.1	KIAA0564
NM001678.1	ATP1B2	AL117643.1	N/A
NM001099.2	ACPP	AW772192	N/A
NM014731.1	ProSAPiP1	NM003944.1	SELENBP1
BF343007	N/A	AL049977.1	CLDN8
NM000035.1	ALDOB	AK023937.1	THEA
NM005950.1	MT1G	AK025084.1	TNRC9
NM002371.2	MAL	X03363.1	ERBB2
NM006984.1	CLDN10	AK026411.1	ALDOB
NM002567.1	PBP	NM016026.1	RDH11
NM000019.1	ACAT1	NM016286.1	DCXR
NM001692.1	ATP6V1B1	NM019027.1	FLJ20273
X77737.1	N/A	BG338251	RAB7L1
NM006226.1	PLCL1	NM006113.2	VAV3
NM000893.1	KNG	NM018075.1	FLJ10375
NM000412.2	HRG	NM013271.1	PCSK1N
NM001963.2	EGF	NM017586.1	C9orf7
NM003361.1	UMOD	NM016321.1	RHCG
NM000050.1	ASS	NM025247.1	MGC5601
NM001438.1	ESRRG	BC002449.1	FLJ13612
NM020632.1	ATP6V0A4	AI379517	N/A
AI632015	SLC12A1	AA058832	MGC33926
NM000701.1	ATP1A1	AW274034	N/A
NM031305.1	DKFZP564B1162	AI580268	NUDT6
AF130089.1	ALDH6A1	AI761947	DKFZP564B1162
AK025651.1	N/A	AI793201	N/A
W45551	MMP24	AK025898.1	N/A
W67995	FXC1	AB046810.1	C20orf23
AL136566.1	IBA2	AK024204.1	N/A
AF105366.1	SLC12A6	BF594722	N/A
AF284225.1	DMRT2	R88990	N/A
AA191708	N/A	N73742	N/A
AL355708.1	N/A	AI697028	FLJ90165
BE783949	FLJ10101	BF590528	N/A
AL529672	N/A	AI733359	N/A
AL568674	MYBBP1A	H20179	N/A
AU147564	CLMN	AA991551	MGC14839
AK000208.1	N/A	AI758950	SLC26A7

TABLE 6-continued

Genes With Down-Regulated Expression In Both stage I And stage II Renal Cell Carcinoma

Genbank ID	Gene Symbol	Genbank ID	Gene Symbol
AB051536.1	FLJ14957	AA911561	N/A
AI569747	TFDP2	AI769774	N/A
AK025562.1	N/A	AA669135	N/A
AI660243	TMPRSS2	AW136060	SLC13A2
N50413	N/A	AI733593	N/A
AI347918	N/A	BF739841	N/A
AL536553	GRP58	AA600175	N/A
BC000282.1	LOC89894	BF477980	N/A
BF106962	FAM3B	AI934557	N/A
AI051248	FLJ32115	BE326951	KNG
AI928242	N/A	AI632567	N/A
BG236006	N/A	BE300882	N/A
AI653107	N/A	BE855713	N/A
AI824037	FLJ25461	AA485440	DBP
R61322	N/A	AA915989	FLJ10743
AW071744	KCNJ10	AA085764	SIGIRR
BF059276	N/A		

EXAMPLE 6

Loss of TGF-β Receptor Expression Demonstrated by Gene Array and Real-Time PCR in Renal Cell Carcinoma

Expression of type I TGF-β receptor (TBR1), type II TGF-β receptor (TBR2), and type III TGF-β receptor (TBR3) mRNA were compared in normal renal tissue, primary renal cell carcinoma without metastasis, primary lesions of metastatic renal cell carcinoma, and metastatic lesions. A summary of gene array analysis was presented as average signal intensities in FIG. **11**A (mean±standard error). The signal intensity for TBR1 (cross-hatched bars) was relatively low, although TBR1 was scored as 'Present' in all samples. No significant changes in TBR1 expression were observed. TBR2 (gray bars) was abundantly expressed in normal epithelium and in primary lesions of nonmetastatic renal cell carcinoma. TBR2 was significantly reduced in primary lesions with metastatic disease ($P<0.028$ by ANOVA). TBR2 was even more reduced in metastatic lesions. TBR3 expression was high in normal epithelium, but was significantly reduced in each of the five primary tumors with nonmetastatic disease (black bars). TBR3 expression was also reduced in primary tumors with metastatic lesions and in metastatic lesions themselves.

These expression patterns were confirmed by real-time PCR (Tagman®) in the 10 patients used for gene array analysis. Means and standard errors for individual samples are shown in FIG. **11**B. All data were normalized to 18S rRNA and calibrated to target abundance in the paired normal tissues. TBR1 mRNA abundance did not change (cross-hatched bars), consistent with the gene chip data. TBR2 (gray bars) was not reduced in primary tumors without metastases, whereas TBR2 was significantly reduced in primary tumors with metastatic disease and in metastatic lesions. TBR3 was reduced in all tumors (black bars).

The investigators have subsequently completed real-time PCR analysis of TBR1, TBR2, and TBR3 expression in 16 primary tumors without metastases (plus paired normal epithelium) and nine samples of primary tumors with metastatic disease, paired metastatic lesions, and paired normal tissue. The data were consistent with those shown for the samples analyzed in FIG. **11**A. TBR3 expression was significantly reduced in all tumors; whereas TBR2 expression was reduced in only 1/16 primary tumors without metastatic lesions, but was reduced in primary tumors with metastatic lesions (8/9). These data show that loss of TBR3 is an early event in renal cell carcinoma, strongly suggesting that TBR3 plays a critical role in renal cell carcinoma carcinogenesis.

The loss of TBR3 mRNA expression was also correlated with TNM scores (T, histological score; N, lymph node number; M, number of organ metastases) from patient samples (data not shown). TBR3 mRNA expression was suppressed in the earliest stage, stage I, and was found to be suppressed in all tumor stages (I-IV). In addition, loss of TBR2 in the primary tumor is significantly associated with acquisition of the metastatic phenotype and clinically manifests as metastatic progression.

EXAMPLE 7

Attenuation of TGF-β-Mediated Signal Transduction in Human Renal Cell Carcinoma

Decreased type III TGF-β receptor (TBR3) mRNA expression in all tumors was associated with failure to detect TBR3 protein by immunohistochemistry (FIG. **12**). Type I TGF-β receptor (TBR2) protein was detected in localized tumor (primary, no mets), but was not detectable in primary tumors with metastatic disease or in corresponding metastatic lesions. Type I TGF-β receptor (TBR1) protein was detected in normal tissue and in all tumor samples.

The investigators hypothesized that these losses seen in TGF-β receptor expression would manifest as an attenuation of TGF-β mediated signal transduction, and would significantly alter the expression of TGF-β regulated genes. From the gene array data disclosed above, 13 known TGF-β/Smad-regulated genes were down-regulated in renal cell carcinoma (Table 7). Using mRNA from 35 patient-matched samples, the investigators verified loss of expression of three of these genes by comparing matched normal and tumor tissue. Real-time PCR was used to measure the expression of Collagen IV type 6, fibulin-5, and connective-tissue growth factor (CTGF). Collagen IV type 6 (gray bars) is an extracellular matrix protein that plays a critical role in the regulation of membrane integrity and cell signaling. Fibulin-5 is a recently discovered TGF-β-regulated gene, which has tumor suppressor activity. Fibulin-5 is an extracellular matrix protein that is believed to signal through interaction with integrins. CTGF is a secreted protein involved in angiogenesis, skeletogenesis, and wound healing. CTGF enhances TGF-β1 binding to TBR2, and CTGF and TGF-β collaborate to regulate the expression of extracellular matrix proteins during renal fibrosis. As summarized graphically in FIG. **13**, all the evaluated TGF-β-regulated genes were down-regulated in early tumor stages, suggesting that renal cell carcinoma undergoes loss of TGF-β responsiveness at an early stage. These data indicate that this loss of TGF-β sensitivity is due, primarily, to loss of type III TGF-β receptor (TBR3) in early tumor development and further loss of sensitivity in metastatic disease is mediated through subsequent loss of type II TGF-β receptor (TBR2).

TABLE 7

Known TGF-β-Regulated Genes Found To Be Down-Regulated In Localized Tumors By Gene Array Analysis

GenBank No.	Gene Name	Fold Attenuation
S81439	TGFβ-induced early growth factor (TIEG)	2.5
AF093118	Fibulin 5	4.0
U42408	Ladinin 1	15.4
U01244	Fibulin 1	4.8
J05257	Dipeptidase 1	7.7
D21337	Collagen, type IV, a6	3.6
X80031	Collagen, type IV, a3	2.4
M64108	Collagen, type XIV, a1	3.2
M98399	Collagen, type I receptor	4.2
L23808	Matrix metallo-proteinase 12	3.7
M35999	Integrin, b3	2.5
AI304854	$p27^{Kip1}$	2.1
J05581	Mucin 1	6.5

Data were analysed by a combination of two-dimensional ANOVA, Affymetrix MAS5.0, and hierarchical cluster analysis using Spotfire to identify genes that are down-regulated in local tumors versus that of normal renal cortex tissue.

EXAMPLE 8

TGF-β Receptor Expression in Renal Cell Carcinoma Cell Lines

Human renal cell carcinoma cell lines were identified that recapitulate the clinical observations of TGF-β receptor biology described above. UMRC6 cells were derived from a clinically localized human renal cell carcinoma (Grossman et al., 1985). As shown in FIG. **14**A, UMRC6 cells express type II TGF-β receptor (TBR2) mRNA, but not type III TGF-β receptor (TBR3). Immunohistochemical analysis (FIG. **14**B) confirms the presence of TBR2 protein and the absence of TBR3 expression. UMRC3 cells were derived from the primary tumor of a patient with metastatic renal cell carcinoma. This highly aggressive cell line lacks detectable TBR2 and TBR3 mRNA (FIG. **14**A) and protein (FIG. **14**B).

In addition to these relevant laboratory models, normal renal epithelial (NRE) tissue was harvested from nephrectomy specimens and established as primary cultures (Trifillis, 1999). As shown in FIGS. **14**A and **14**B, these primary cultures of NRE expressed TBR3, TBR2, and TBR1 mRNA and protein in vitro. NRE cells can be grown in culture for 10 passages and were easily isolated and characterized. NRE cells were characterized for cytokeratin expression and tubule-specific gene expression, for example, megalin (data not shown). Thus, there are relevant cell models in which TBR2 and TBR3 expression can be manipulated to examine the impact of TGF-β receptor biology on the carcinogenesis and progression of human renal cell carcinoma in vitro.

EXAMPLE 9

TGF-β Activity in Renal Cell Carcinoma Cell Lines

It is well known that TGF-β1 inhibits cell proliferation in epithelial cells. The present example demonstrates the effects of TGF-β on renal tumor cell proliferation.

DNA content of cells was used as a measure of cell proliferation. Cells were plated at 20,000 cells/well in 12-well plates. Cells were grown in 10% FBS:DMEM:penicillin: streptomycin. The following day, media were exchanged with appropriate treatment added to the media. On day 3 of treatment, cells were analyzed for DNA content using Hoechst reagent. DNA standard was used to correlate DNA content per well.

As shown in FIG. **15**A (squares), TGF-β1 inhibited the proliferation of normal renal epithelial cells in culture. URMC3 cells expressed neither type II or type III TGF-β receptors and, not surprisingly, were resistant to the inhibitory effects of TGF-β on cell proliferation (triangles, FIG. **15**A). UMRC6 cells expressed type II but not type III TGF-β receptors, and were partially resistant to TGF-β1 (circles, FIG. **15**A).

TGF-β transcriptional activity was also measured in the above cell models using transient transfection of the 3TP/lux reporter, which contains an AP-1/Smad3 response element from the PAI-1 promoter. This luciferase reporter construct demonstrates increased transcriptional activity in response to exogenous TGF-β-mediated signal transduction. 3TP/lux was transiently transfected along with SV/renilla luciferase (Promega) into cells using fugene (Roche) as the transfection agent. Cells were treated with or without TGF-β1 24 h after transfection and luciferase activity (Promega Luciferase Assay system and Lumat luminometer) was determined 24 h after TGF-β treatment. Firefly luciferase activity was normalized using the ratio of firefly luciferase/renilla luciferase. As shown in FIG. **15**B, normal renal epithelial cells were highly responsive to 2 ng/ml (80 pM) of TGF-β1. UMRC6 cells demonstrated significantly less luciferase activity in response to TGF-β1, and UMRC3 cells were entirely unresponsive.

EXAMPLE 10

Recapitulation of TGF-β Signaling Through Reintroduction of TGF-b Receptor Expression into Renal Cell Carcinoma

To test whether reintroduction of TGF-β receptor expression would result in re-establishment of TGF-β signal transduction and reacquisition of TGF-β cellular sensitivity, UMRC3 cells were engineered to express stably either type II TGF-β receptor (+TBR2) alone or type II plus type III TGF-β receptor (+TBR2+TBR3).

Plasmid construction and transfection were described as follows. The complete coding sequences for human type II TGF-β receptor (TBR2) was cloned into the EcoRI/XbaI site of pcDNA3/FLAG. The expression vector was stably transfected into UMRC3 cells using fugene as DNA carrier and genticin as selection antibiotic (Sigma, 1 mg/ml). Ten clones (UMRC3/TBR2) were selected and verified for TBR 2 mRNA and protein expression such as Western analysis using the FLAG antibody (data not shown). From these cell clones, one was to be selected that had equivalent protein expression of TBR2 to that of normal renal epithelial (NRE) and UMRC6 cells.

The type III TGF-β receptor (TBR3) coding sequence was PCR amplified from a plasmid expressing wild-type TBR3 in pSV7d (a gift from Dr C-H Heldin). TBR3 was then cloned into the EcoRI site of pcDNA4/TO/myc-His® (InVitrogen) in the sense and antisense (negative control) orientation. The orientation and sequence of TBR3 was verified. The antisense TBR3 (As TBR3) vector was used as a control. TBR3/pcDNA4/TO/myc-His and As TBR3/pcDNA4/TO/myc-His vectors were stably transfected into UMRC3/TBR2 cells. A clone was selected that demonstrated an equivalent expression of TBR3 mRNA to that of normal renal epithelial cells. As a control for UMRC3+TBR2 and UMRC3+TBR2+TBR3, wild-type UMRC3 were stably transfected with both pcDNA/FLAG and pcDNA4/TO/myc-His vectors.

As shown in FIGS. **16**A-**16**B, stable transfection of type II TGF-β receptor (TBR2) alone or type II plus type III TGF-β receptor (TBR2+TBR3) resulted in detectable levels of mRNA for each receptor on RT-PCR analysis. On examining the in vitro growth kinetics of these re-engineered cells, it was noted that reintroduction of TBR2 resulted in a twofold reduction in cell proliferation and reintroduction of both TBR2 and TBR3 resulted in a fourfold reduction in cell proliferation with the addition of exogenous TGF-β.

The investigators then examined TGF-β-mediated transcriptional activity as a consequence of TGF-β receptor re-expression. As shown in FIG. **16**C, reintroduction of TBR2 partially restored transcriptional responsiveness, as evidenced by a 5.6-fold increase in 3TP/lux activity after addition of TGF-β1. Reintroduction of both TBR2 and TBR3 into UMRC3 cells resulted in 17.5-fold increase in 3TP/lux activity after addition of TGF-β1.

To demonstrate reestablishment of TGF-β-regulated gene expression, collagen IV type 6 mRNA expression was examined by real-time PCR in these re-engineered cell lines in the presence of TGF-β1. As shown in FIG. **16**D, reexpression of TBR2 in UMRC3 cells results in a sevenfold increase in collagen IV type 6 mRNA levels over that of UMRC3 controls, while reintroduction of both TBR2 and TBR3 enhanced collagen IV type 6 mRNA expression 11-fold. These data are consistent with a number of published reports that indicate expression of TBR3 is essential for full TGF-β responsiveness.

UMRC3 cells have been shown to be tumorigenic in athymic nude mice (Grossman et al., 1985). Anchorage independent growth in soft agar is a well-established in vitro correlate of in vivo tumorigenicity. Colonies formation in soft agar was determined as follows. UMRC3 (pcDNA/FLAG and pcDNA4/T0/myc-His empty vectors), UMRC3+TBR2, or UMRC3+TBR2+TBR3 cells were plated at 1000 cells/60 mm dish in an agarose/FBS/media sandwich in the presence of 2 ng/ml TGF-β. No selection antibodies were added to the agarose media mixture. The cells were incubated for 45 days to insure that no colony formation would occur. Cells were then stained with 0.005% Crystal Violet, photographed, and assessed for number and size of colonies.

As shown in FIG. **16**E, UMRC3 cells demonstrated anchorage independent growth in soft agar. Reintroduction of TBR2 into UMRC3 cells significantly decreased the number and size of colonies that formed in soft agar. Reintroduction of both TBR2 and TBR3 completely abrogated the ability of UMRC3 cells to form colonies in soft agar, even after 45 days in culture. These data demonstrate that reintroduction of TBR2 resensitizes UMRC3 cells to the effects of exogenous TGF-β through reacquisition of TGF-β signal transduction. More interestingly, however, reintroduction of TBR3 in the presence of TBR2 into UMRC3 cells significantly enhanced TGF-β-regulated gene transcription, growth inhibition, and loss of anchorage-independent growth over that seen with reintroduction of TBR2 alone. These data clearly show that renal cell carcinoma cells are TGF-β resistant. Loss of TBR3 expression occurs early and appears to be associated with a relatively less aggressive state that is partially TGF-β responsive. Loss of TBR2 results in frank TGF-β resistance and is associated with acquisition of a more aggressive phenotype.

FIGS. **17**-**18** demonstrate that re-expression of type II or type Ill TGF-β receptor in the highly metastatic human renal cell carcinoma cell line UMRC3 inhibited cell proliferation in cell culture and tumor growth in a nude mouse model. The TGF-β receptors were either re-expressed in a stable vector system or as an adenoviral vector. For clinical purposes, it would be envisioned to treat patients with an adenovirus expressing one or both of the TGF-β receptors to block tumor growth or cause tumor regression.

EXAMPLE 11

Stepwise Sequential Loss of Type III and Type II TGF-β Receptor Expression in Renal Cell Carcinoma

With genomic profiling in human renal cell carcinoma, the data presented above demonstrated a stepwise sequential loss of type III and type II TGF-β receptor expression in association with renal cell carcinogenesis and progression. These findings were confirmed by both immunohistochemistry and real-time PCR in patient-matched tissue samples. This clinical observation was brought to the laboratory to identify relevant in vitro models. Using these models, it was demonstrated that loss of type III TGF-β receptor expression resulted in incremental desensitization to TGF-β and attenuation of TGF-β signaling. Subsequent loss of type II TGF-β receptor resulted in complete loss of TGF-β sensitivity. With in vitro modulation of TGF-β receptor expression, it was demonstrated that reconstitution of the TGF-β signaling pathway resulted in significant growth inhibition and loss of the aggressive phenotype.

These experiments are unique in that clinically relevant observations, which are derived from the evaluation of gene expression in normal renal cortical tissue, localized renal cell carcinoma and metastatic renal cell carcinoma, were brought to the laboratory for validation and experimental manipulation in relevant in vitro models. Other investigators have examined human renal cell carcinoma cell lines and identified alterations in the expression of TGF-β signaling pathway intermediaries, but those observations have not been validated in the clinical biology of renal cell carcinoma. To the investigators' knowledge, few studies have methodically examined the expression of all three TGF-βreceptors in patient samples at the protein and mRNA level in an effort to correlate TGF-β receptor expression to disease-specific states of renal cell carcinoma (i.e. localized versus metastatic tumor). A major strength of the present study is that the investigators recognized distinct disease states in renal cell carcinoma, associated them with specific alterations in the TGF-β signaling pathway, and then validated and manipulated the clinical observations in the laboratory.

Although the mechanisms are not well understood, it is clear that TGF-β regulates a large number of diverse biological functions, including cell proliferation, differentiation, cell adhesion, apoptosis, extracellular matrix production, immune regulation, neuroprotection, and early embryonic development. In epithelial cells, the effect of TGF-β is generally to inhibit proliferation, promote cellular differentiation, and regulate interactions with the extracellular matrix. As a direct consequence, aberrations in TGF-β signaling can have a dramatic impact on cellular processes that are critically associated with neoplastic and malignant transformation. Given the well-documented observation that the end result of TGF-β signaling is largely growth inhibitory, it makes intuitive sense that cancer cell would develop mechanisms to escape TGF-β sensitivity. To date, these mechanisms have not been elucidated in human renal cell carcinoma.

Based on the data presented above, the investigators hypothesize that this escape from the growth-inhibitory effects of TGF-β is mediated through the stepwise sequential loss of type III and type II TGF-β receptor expression. To the investigators' knowledge, no one has linked sequential loss of these two types of receptors to carcinogenesis and metastatic

progression in oncology. This is the first time that stepwise loss of a single transduction pathway has been associated with important biologic sequelae in a human cancer.

Results presented in the present invention demonstrate that loss of type III TGF-β receptor expression is an early event in renal cell carcinoma biology and that this loss has important sequelae with regard to renal cell carcinoma carcinogenesis and progression. All clinical samples of localized renal cell carcinoma demonstrated loss of type III TGF-β receptor, but had normal expression of type I and type II TGF-β receptors. Replication of this clinical observation in in vitro models demonstrated significant loss of TGF-β sensitivity, manifest as a significant reduction in the growth inhibitory effects of TGF-β1 and significantly reduced TGF-β-mediated transcription. Interestingly, cell lines derived from localized RCC retained type II TGF-β receptor expression and therefore, still demonstrated sensitivity, albeit reduced, to TGF-β. Only with metastatic progression and loss of type II TGF-β receptor expression does the cell become completely resistant to the effects of TGF-β. The investigators hypothesize that this retained, but attenuated, TGF-β signaling seen in local tumors must convey some as yet unrecognized biologic benefit for local tumors that is no longer required, and therefore discarded, with metastatic progression. In fact, this loss of type II TGF-β receptor expression may be an absolute integral component in the cascade of intracellular events that lead to the development of metastatic potential. In keeping with this hypothesis, it has been shown that loss of type I TGF-b receptor expression was one of 40 integral alterations of gene expression to predict for poor prognosis of patients diagnosed with renal cell carcinoma.

In summary, the above results demonstrate a clear link between loss of type III TGF-β receptor expression to a human disease state. Reduced type III TGF-β receptor (TBR3) expression has been reported in human breast tumor cell lines, suggesting that loss of TBR3 expression may be a more ubiquitous phenomena in carcinogenesis, rather than an isolated finding in human RCC biology. The fact that the investigators found down-regulation of TBR3 in every renal cell carcinoma specimen studied to date (35 patients) and that re-expression of TBR3 (in the presence of re-expressed TBR2) completely abolish growth on soft agar suggests an important role for TBR3 in normal renal epithelial homeostasis that must be abrogated for renal cell carcinogenesis and progression to occur. Little attention has been given to TBR3 in normal cell biology or the changes in expression that occur with carcinogenesis and progression. Observations from the present invention would suggest that TBR3 plays an important functional role in signaling and that loss of expression is an important event in the acquisition of the tumorigenic and metastatic phenotype

EXAMPLE 12

Genomic Profiling for Stage I Papillary Renal Cell Carcinoma and Benign Oncocytoma

FIG. **19** shows hierarchical clustering of genes over-expressed or down-regulated (with at least 2 fold differences) in stage I papillary renal cell carcinoma verses normal renal cortex. Genes over-expressed and down-regulated in stage I papillary renal cell carcinoma are listed in Table 8 and Table 9 respectively. FIG. **20** shows hierarchical clustering of genes over-expressed or down-regulated (with at least 2 fold differences) in benign oncocytoma verses normal renal cortex. Genes over-expressed and down-regulated in benign oncocytoma are listed in Table 10 and Table 11 respectively. FIG. **21** shows venn analysis of gene distribution among stage I renal cell carcinoma (RCC), oncocytoma and stage I papillary renal cell carcinoma. Genes with at least 2-fold differences in expression were filtered at 95% confidence level (CL) in the following 3 t-tests: stage I RCC vs. normal; oncocytoma vs. normal; and stage I papillary renal cell carcinoma vs. normal. Six hundred twenty five genes were present only in stage I RCC (95% CL), 136 genes were present only in oncocytoma (95% CL), 344 genes were present only in stage I papillary renal cell carcinoma (95% CL), and 60 genes were common to stage I RCC, oncocytoma and stage I papillary renal cell carcinoma. FIG. **22** shows venn analysis of gene distribution among stage II renal cell carcinoma (RCC), oncocytoma and stage I papillary renal cell carcinoma. Genes with at least 2-fold differences in expression were filtered at 95% confidence level (CL) in the following 3 t-tests: stage II RCC vs. normal; oncocytoma vs. normal; and stage I papillary renal cell carcinoma vs. normal. One thousand and five genes were present only in stage II RCC (95% CL), 152 genes were present only in oncocytoma (95% CL), 334 genes were present only in stage I papillary renal cell carcinoma (95% CL), and 43 genes were common to stage II RCC, oncocytoma and stage I papillary renal cell carcinoma.

TABLE 8

Genes With Up-Regulated Expression In stage I Papillary Renal Cell Carcinoma

Genbank ID	Gene Symbol	Genbank ID	Gene Symbol
NM_003505	FZD1	AC004382	DKFZP434K046
AL035683	B4GALT5	NM_000248	MITF
R56118	N/A	NM_022154	SLC39A8
NM_014575	SCHIP1	AI436813	N/A
AI694320	ZNF533	AF007162	CRYAB
BC031322	N/A	NM_015392	NPDC1
BF346665	N/A	AL136585	DKFZp761A132
BC004283	LOC283639	AB040120	SLC39A8
AF302786	GNPTAG	NM_138473	SP1
AU121975	PAICS	AU144387	182-FIP
NM_016315	GULP1	NM_022763	FAD104
AL541302	SERPINE2	AI093231	APBB1IP
BG391217	C9orf80	NM_000235	LIPA
NM_000700	ANXA1	AI817079	EXOC7
N30188	N/A	NM_004385	CSPG2
NM_003651	CSDA	NM_024801	TARSH
AI830227	FLII	BF218922	CSPG2
U20350	CX3CR1	BF590263	CSPG2
NM_005692	ABCF2	NM_001233	CAV2
U34074	AKAP1	AB020690	PNMA2
AB056106	TARSH	AW188198	TNFAIP6
AU151483	CDH6	NM_007115	TNFAIP6
BC026260	TTC3	AI742838	DOCK11
AL133001	SULF2	AW117264	N/A
NM_003358	UGCG	AF016266	TNFRSF10B
NM_001282	AP2B1	NM_013952	PAX8
AF322067	RAB34	AA771779	ZFP90
NM_001540	HSPB1	W72333	FLJ21657
N58363	STATIP1	H23979	MOX2
AF072872	FZD1	BG542521	PPM2C
BF247552	SLC38A1	AF063591	MOX2
X69397	CD24	BF247383	BMPR2
BC000251	GSK3B	NM_005114	HS3ST1
BF691447	B4GALT5	BE466145	N/A
AB046817	SYTL2	BC005352	TNFAIP8
AF255647	DKFZP566N034	AC002045	LOC339047
BF344237	N/A	BC040558	D2LIC
AW242720	LOC143381	U13699	CASP1
AA115485	MGC3222	NM_002718	PPP2R3A
NM_006588	SULT1C2	BF476502	MPPE1
NM_000546	TP53	BC034275	LOC253982
N92494	JWA	AF279145	ANTXR1
W74580	MGC3222	AV724216	NDRG4
AF131749	PSK-1	BG165613	N/A
AW026491	CCND2	NM_018205	LRRC20

TABLE 8-continued

Genes With Up-Regulated Expression In stage I Papillary Renal Cell Carcinoma

Genbank ID	Gene Symbol	Genbank ID	Gene Symbol
NM_012410	PSK-1	NM_022083	C1orf24
NM_002800	PSMB9	NM_006169	NNMT
BF512748	JAK3	AF141347	TUBA3
AA404269	PRICKLE1	NM_000064	C3
M33376	AKR1C1	AV710838	BCDO2
AF035321	DNM1	AI417917	EHD2
NM_002862	PYGB	AI681260	LILRB1
AF132000	DKFZP564K1964	NM_000389	CDKN1A
L07950	HLA-C	AF288391	C1orf24
AF114011	TNFSF13	NM_002627	PFKP
BF674052	VMP1	NM_001975	ENO2
AI922599	VIM	NM_030786	SYNCOILIN
AF044773	BANF1	NM_006169	NNMT
NM_015925	LISCH7	AI417917	EHD2
NM_001684	ATP2B4	NM_006868	RAB31
AI123348	CHST11	L03203	PMP22
NM_001304	CPD	AF199015	VIL2
NM_006762	LAPTM5	AI873273	SLC16A6
NM_000211	ITGB2	NM_017821	RHBDL2
AA995910	ALOX5	BF740152	MYO1F
NM_018965	TREM2	AA954994	N/A
AL353715	STMN3	AI458735	MGC26717
BC019612	C20orf75	NM_003254	TIMP1
AF086074	N/A	AI688631	N/A
NM_005045	RELN	AK026037	N/A
AI935123	C14orf78	BG327863	CD24
AL550875	C7orf28B	NM_016008	D2LIC
L27624	TFPI2	AI394438	LOC253981
AL574096	TFPI2	AA947051	D2LIC
AA005141	MET	AI819043	N/A
D86983	D2S448	AI378044	UGCG
AW439242	C6orf68	NM_024576	OGFRL1
AB000221	CCL18	M76477	GM2A
NM_002121	HLA-DPB1	NM_002214	ITGB8
U17496	PSMB8	AI879381	ADCK2
U05598	AKR1C1	NM_000152	GAA
BF342851	D2S448	H15129	MEIS4
BF311866	PTGFRN	L42024	HLA-C
NM_001449	FHL1	NM_002178	IGFBP6
AA954994	N/A	AI761561	HK2
Y13710	CCL18	AA722799	DCBLD2
BG170541	MET	NM_003255	TIMP2
AB037813	DKFZp762K222	NM_000107	DDB2
D28124	NBL1	AV699565	CTSC
NM_021103	TMSB10	NM_000861	HRH1
AI949772	N/A		

TABLE 9

Genes With Down-Regulated Expression In stage I Papillary Renal Cell Carcinoma

Genbank ID	Gene Symbol		
AF232217	N/A	NM_003877	SOCS2
AI823572	MGC45438	AI768894	CGN
AU154994	SLC13A3	AW772192	N/A
AW979271	N/A	AF094518	ESRRG
AF064103	CDC14A	T40942	ANGPTL3
AI524125	PCDH9	NM_001146	ANGPT1
AI733474	GPR155	AI242023	N/A
AI767756	HS6ST2	BF970431	N/A
NM_000412	HRG	NM_005670	EPM2A
NM_021614	KCNN2	AW071744	KCNJ10
M13149	HRG	AI928242	TFCP2L1
H17038	N/A	AI769774	LOC155006
NM_002010	FGF9	AW274034	USP2
AI635774	EMCN	NM_004633	IL1R2
AW007532	IGFBP5	NM_003289	TPM2
NM_004070	CLCNKA	BF512388	C10orf58
NM_014621	HOXD4	BC005830	ANXA9
AI733593	N/A	NM_000362	TIMP3

TABLE 9-continued

Genes With Down-Regulated Expression In stage I Papillary Renal Cell Carcinoma

Genbank ID	Gene Symbol		
NM_020632	ATP6V0A4	NM_001438	ESRRG
AI697028	FLJ90165	AU146204	ENPP6
AA897516	PTGER4	AA775681	FLJ23091
NM_024307	MGC4171	AI393205	ACY-3
J02639	SERPINA5	AF017987	SFRP1
NM_000085	CLCNKB	NM_005951	MT1H
AA058832	MGC33926	NM_005950	MT1G
BF059276	N/A	NM_021805	SIGIRR
BC043647	LOC284578	AA557324	CYP4X1
AL161958	THY1	BF528646	DKFZP564I1171
AL121845	KIAA1847	AW340112	LOC401022
AY079172	ATP6V0D2	R73554	IGFBP5
AA928708	CYP8B1	AI826437	N/A
H71135	ADH6	AV720650	KIAA0888
NM_000102	CYP17A1	AA780067	HS3ST3B1
Z92546	SUSD2	NM_000640	IL13RA2
AL558479	THY1	AI806338	TBX3
BC005314	ALDOB	NM_003155	STC1
NM_173591	FLJ90579	AA931562	N/A
BF510426	N/A	AI694325	N/A
AF331844	SOST	AF205940	EMCN
X77737	SLC4A1	NM_001290	LDB2
NM_004392	DACH1	NM_016242	EMCN
BC001077	LOC87769	AW014927	CALB1
AA218868	THY1	AI758950	SLC26A7
BF478120	RECQL5	AK024256	KIAA1573
BC041158	CYP4A11	BF726212	ANK2
AI623321	MTP	AI985987	SCNN1G
AI796189	PAH	AW242408	UPP2
NM_021161	KCNK10	NM_000860	HPGD
NM_000163	GHR	BF447963	KIAA0962
AL136880	ESPN	BF941499	GPR116
NM_024426	WT1	AW242409	N/A
M61900	PTGDS	BF509031	ATP6V1G3
AW963951	SIAT7C	NM_000934	SERPINF2
AW340588	MAN1C1	BF248364	AF15Q14
AI263078	SLC23A3	AL534095	FLJ23091
BF130943	PPAPDC1	NM_004929	CALB1
AI732596	N/A	AI222435	N/A
AA603467	ZNF503	NM_005397	PODXL
R41565	N/A	AI090268	N/A
AI951185	NR2F1	AI300520	STC1
NM_002609	PDGFRB	BC006236	MGC11324
NM_006984	CLDN10	NM_024609	NES
BG413612	N/A	NM_002591	PCK1
D64137	CDKN1C	NM_005410	SEPP1
AK026344	PEPP2	AB020630	PPP1R16B
AI670852	PTPRB	AF022375	VEGF
AI693153	GABRB3	NM_016246	DHRS10
NM_001393	ECM2	AA873542	SLC6A19
N93191	PR1	U95090	PRODH2
BC005090	AGMAT	D26054	FBP1
NM_000717	CA4	AI732994	MGC13034
D38300	PTGER3	NM_000151	G6PC
AI650260	N/A	AK025651	PNAS-4
BC024226	IFRG15	AF161441	N/A
BC006294	DHRS10	AF161454	APOM
NM_003039	SLC2A5	NM_022129	MAWBP
AI675836	SORCS1	AI733515	MGC52019
NM_005276	GPD1	NM_001443	FABP1
NM_014298	QPRT	AI433463	MME
M10943	MT2A	AL049313	N/A
NM_005952	MT1X	BF195998	ALDOB
NM_002450	MT1X	NM_022829	SLC13A3
NM_002910	RENBP	NM_000035	ALDOB
BF246115	MT1F	NM_007287	MME
AF078844	MT1F	NM_003399	XPNPEP2
AF170911	SLC23A1	NM_000196	HSD11B2
AF333388	MT1H	BF431313	N/A
NM_003500	ACOX2	NM_004844	SH3BP5
AA995925	N/A	NM_003206	TCF21
NM_001218	CA12	AI311917	DPYS
BF432333	FLJ31196	AA843963	PRLR
NM_001385	DPYS	NM_017753	PRG-3
NM_003052	SLC34A1	NM_006633	IQGAP2

TABLE 9-continued

Genes With Down-Regulated Expression In stage I Papillary Renal Cell Carcinoma

Genbank ID	Gene Symbol		
NM_000778	CYP4A11	NM_001133	AFM
AL136551	SESN2	T90064	N/A
NM_000792	DIO1	BF696216	N/A
NM_016725	FOLR1	NM_004413	DPEP1
NM_019101	APOM	Z98443	FLJ38736
NM_014270	SLC7A9	NM_018456	EAF2
AF124373	SLC22A6	AW771563	N/A
NM_016327	UPB1	NM_014495	ANGPTL3
NM_024734	CLMN	AI074145	KMO
NM_016527	HAO2	NM_000896	CYP4F3
NM_003645	SLC27A2	NM_001072	UGT1A6
AB051536	FLJ14957	AI631993	N/A
NM_025149	FLJ20920	NM_000277	PAH
BC005939	PTGDS	M74220	PLG
AL574184	HPGD	AI935789	UMOD
NM_000161	GCH1	NM_002472	MYH8
H57166	N/A	BC020873	CLCNKA
NM_000597	IGFBP2	NM_000550	TYRP1
NM_000790	DDC	AA806965	BTNL9
NM_004668	MGAM	NM_020163	LOC56920
NM_021027	UGT1A6	NM_004490	GRB14
AF348078	GPR91	AA788946	COL12A1
NM_016347	NAT8	AW242315	N/A
AF338650	PDZK3	AI735586	LOC152573
BE221817	CNTN3	R88990	N/A
NM_004476	FOLH1	NM_003278	TNA
NM_004615	TM4SF2	NM_007180	TREH
NM_023940	RASL11B	AW173045	TBX2
AI742872	SLC2A12	U28049	TBX2
BC001196	HS6ST1	NM_001395	DUSP9
AW195353	TFCP2L1	NM_000336	SCNN1B
NM_003122	SPINK1	U43604	N/A
NM_144707	PROM2	BC029135	N/A
AI653981	L1CAM	NM_005414	SKIL
AI796169	GATA3	BQ894022	PDE1A
M96789	GJA4	NM_013335	GMPPA
N74607	AQP3	NM_003221	TFAP2B
NM_014059	RGC32	BF057634	HOXD8
AI572079	SNAI2	AA523172	N/A
AI056877	N/A	AF319520	ARG99
NM_006206	PDGFRA	NM_002885	RAP1GA1
AW771314	MGC35434	NM_003361	UMOD
NM_016955	SLA/LP	NM_000142	FGFR3
AI569804	LOC375295	NM_000893	KNG1
NM_001584	C11orf8	BC029135	N/A
BG261252	EVI1	NM_147174	HS6ST2
NM_006226	PLCL1	NM_000218	KCNQ1
NM_001172	ARG2	U03884	KCNJ1
AL050264	TU3A	X83858	PTGER3
BC003070	GATA3	BF439270	N/A
AL120332	MGC20785	AA911235	MST1
NM_000459	TEK	NM_000955	PTGER1
AW242836	LOC120224	NM_022844	MYH11
AI926697	Gup1	BC042069	N/A
NM_000486	AQP2	NM_005518	HMGCS2
AI870306	IRX1	NM_001963	EGF
AW264204	CLDN11	AI632015	SLC12A1
BF431989	THRB	AF339805	N/A
AI459140	N/A	BF106962	FAM3B
NM_001864	COX7A1	NM_005019	PDE1A
AI471866	SLC7A13	AU146305	PDE1A
AI653107	NRK	NM_000663	ABAT
NM_004466	GPC5	AU119437	LOC144997
BF195936	KRT18L1	BC036095	DRP2
NM_022454	SOX17	R49295	N/A
AW299531	HOXD10	AI623202	PRDM16
AL137716	AQP6	AW452355	N/A
AI332407	SFRP1	AA563621	HSPB6
AL565812	PTN	X15217	SKIL
AI452457	LOC199920	AK095719	N/A
AI281593	DCN	AI056187	N/A
M21692	ADH1B	AI668598	N/A
AI660243	TMPRSS2	AI700882	SLC13A3
AI754423	FLJ38507	NM_000963	PTGS2
AA759244	FXYD4	AW051712	N/A

TABLE 9-continued

Genes With Down-Regulated Expression In stage I Papillary Renal Cell Carcinoma

Genbank ID	Gene Symbol		
U75667	ARG2	AL832099	MGC33190
NM_000930	PLAT	AK057337	LOC145820
AF083105	SOX13	AW300204	SLC30A8
NM_013231	FLRT2	NM_005856	RAMP3
BI825302	PR1	AI458003	CYYR1
NM_003012	SFRP1	AK026877	N/A
AF138300	DCN	AI632567	N/A
AU155612	N/A	U91903	FRZB
BG435302	EBF	AF352728	FLJ12541
NM_005978	S100A2	BM128432	IGFBP5
NM_000900	MGP	NM_003102	SOD3
AK026748	DKFZP761M1511	BE676272	TACC1
J03208	DBT	AI692180	PPFIBP2
NM_002345	LUM	AL544576	LOC92162
NM_006623	PHGDH	NM_017688	BSPRY
AF063606	my048	AU146310	N/A
NM_001647	APOD	AI912976	RASGRF2
AI935541	N/A	U83508	ANGPT1
NM_005558	LAD1	L47125	GPC3
AW138125	PRODH2	NM_000663	ABAT

TABLE 10

Genes With Up-Regulated Expression In Benign Oncocytoma

Genbank ID	Gene Symbol	Genbank ID	Gene Symbol
NM_005114	HS3ST1	AF178532	BACE2
AA650558	GNAS	AI521166	LOC283104
BF062244	LIN7A	AA005023	NOD27
NM_030674	SLC38A1	AV725364	GPRC5B
NM_014766	SCRN1	AW195581	GPSM2
BC002471	CPLX1	BG503479	B4GALT6
AF183421	RAB31	BF031829	DSG2
AK022100	KIAA0256	AW975728	SLC16A7
BF508244	AKR1C2	NM_022495	C14orf135
BG772511	N/A	AA703159	N/A
AB037848	SYT13	BF247552	SLC38A1
AK055769	N/A	NM_001673	ASNS
T58048	N/A	NM_024622	FLJ21901
NM_012105	BACE2	AI565054	N/A
AA992805	LEF1	AW058459	LOC134285
AK026420	DMN	NM_001233	CAV2
NM_024812	BAALC	BC036550	N/A
AI057226	N/A	BE464483	N/A
AW138767	ELOVL7	NM_002512	NME2
NM_013233	STK39	AF178532	BACE2

TABLE 11

Genes With Down-Regulated Expression In Benign Oncocytoma

Genbank ID	Gene Symbol	Genbank ID	Gene Symbol
BF593625	SYK	AW274034	USP2
AI310001	FLJ22789	NM_147174	HS6ST2
NM_006206	PDGFRA	AA074145	PRODH
NM_003740	KCNK5	AL049176	CHRDL1
AW138125	PRODH2	NM_020353	PLSCR4
NM_000336	SCNN1B	NM_024803	TUBAL3
BC005314	ALDOB	D16931	ALB
AI796189	PAH	NM_019076	UGT1A10
NM_013363	PCOLCE2	AF138303	DCN
NM_004466	GPC5	D13705	CYP4A11
AI627531	N/A	NM_000587	C7
U28055	MSTP9	R49295	N/A
NM_152759	MGC35140	NM_000385	AQP1
AW052159	N/A	AI669229	RARRES1
NM_017712	PGPEP1	U36189	C5orf13
AI961231	TOX	AL110135	FLJ14753

TABLE 11-continued

Genes With Down-Regulated Expression In Benign Oncocytoma

Genbank ID	Gene Symbol	Genbank ID	Gene Symbol
AI767962	BNC2	AW271605	N/A
AF350881	TRPM6	BF358386	N/A
AU146418	N/A	NM_016270	KLF2
BE875072	N/A	AA905508	LOC128153
AI653981	L1CAM	NM_021630	PDLIM2
AI634662	SLC13A3	AA915989	TBC1D13
NM_000486	AQP2	AL565812	PTN
AW206292	AQP2	AI990790	N/A
AI572079	SNAI2	BC041158	CYP4A11
AI694118	N/A	NM_138474	N/A
NM_000142	FGFR3	NM_002899	RBP1
U78168	RAPGEF3	AK024256	KIAA1573
AI913600	UNQ846	AW779672	SLC17A1
W93847	MUC15	NM_021161	KCNK10
NM_004616	TM4SF3	BF196891	TPMT
AI935789	UMOD	AY028896	CARD10
NM_007180	TREH	NM_018456	EAF2
AL110152	CD109	NM_017806	FLJ20406
AW051599	N/A	X59065	FGF1
AI796169	GATA3	AI650353	DACH1
AF017987	SFRP1	AW771563	N/A
BE550027	DKFZp761N1114	BF431313	N/A
AA535065	KIAA1847	NM_000896	CYP4F3
NM_003361	UMOD	BC005090	AGMAT
AI263078	SLC23A3	U24267	ALDH4A1
M13149	HRG	AI090268	N/A
AF278532	NTN4	AW014927	CALB1
AI632015	SLC12A1	AL023553	PIPPIN
NM_000412	HRG	AL049313	N/A
NM_000893	KNG1	AK021539	NCAG1
BG398937	KNG	AI220117	MGST1
AL049977	CLDN8	NM_020300	MGST1
N74607	AQP3	NM_022568	ALDH8A1
AW071744	KCNJ10	BE874872	FAM20C
AW015506	AQP2	NM_004668	MGAM
AI927000	SOSTDC1	BF033242	CES2
AI471866	SLC7A13	BC004542	PLXNB2
NM_001099	ACPP	NM_000204	F
NM_005074	SLC17A1	NM_004525	LRP2
AA995925	N/A	AA442149	MAF
AF352728	FLJ12541	NM_000049	ASPA
BF343007	TFAP2A	AI830469	TFEC
NM_016929	CLIC5	NM_003759	SLC4A4
AA911235	MST1	AF169017	FTCD
AA639753	N/A	AF170911	SLC23A1
NM_004887	CXCL14	AA865601	LOC123876
AW771565	AIM1	AA863031	MGC32871
AI264671	N/A	AW136060	SLC13A2
BF510426	N/A	NM_003041	SLC5A2
AV728958	TLN2	NM_021924	MUCDHL
T90064	N/A	AW299568	N/A
AA218868	THY1	AI927941	N/A
NM_003104	SORD	AI433463	MME
AJ292204	AGXT2	AL365347	SLC7A8
AI056359	MAPT	AA502331	PRAP1
AL568422	DZIP1	NM_024709	FLJ14146
AF339805	N/A	AF289024	FTCD
NM_000163	GHR	NM_017614	BHMT2
AI042017	NPL	NM_016347	NAT8
AW340457	N/A	NM_000277	PAH
BF431199	DEHAL1	NM_000316	PTHR1
BF432254	MGC15937	NM_001091	ABP1
AI368018	GPD1	NM_000790	DDC
AF144103	CXCL14	BF217861	MT1E
NM_016725	FOLR1	BF447963	KIAA0962
NM_000050	ASS	NM_001081	CUBN
AA693817	N/A	NM_018484	SLC22A11
NM_004929	CALB1	AW192692	N/A

TABLE 11-continued

Genes With Down-Regulated Expression In Benign Oncocytoma

Genbank ID	Gene Symbol	Genbank ID	Gene Symbol
NM_000592	C4A	BF000045	TINAG
AL574184	HPGD	BC005830	ANXA9
AA676742	DMGDH	NM_025257	C6orf29
AI631993	N/A	NM_020973	GBA3
AI566130	AK3	NM_001977	ENPEP
AW024233	GLYAT	AI632692	N/A
AA873542	SLC6A19	BI825302	PR1
AK026966	AK3	L12468	ENPEP
NM_022829	SLC13A3	AL571375	SCD4
NM_005950	MT1G	AL136858	ZMYND12
AV700405	MGC52019	NM_024027	COLEC11
AI733515	MGC52019	NM_014934	DZIP1
NM_000860	HPGD	BG496631	FBI4
U95090	PRODH2	NM_018265	FLJ10901
NM_001385	DPYS	AI770035	UPB1
BG401568	SLC16A9	AF177272	UGT2B28
NM_000846	GSTA1	NM_004392	DACH1
BF195998	ALDOB	N95363	CDKN1C
NM_004413	DPEP1	AF261715	FOLH1
NM_000151	G6PC	NM_000042	APOH
NM_006744	RBP4	NM_001393	ECM2
NM_013410	AK3	R88990	N/A
NM_000035	ALDOB	AA557324	CYP4X1
AK026411	ALDOB	AF116645	ALB
AL135960	CYP4A11	BC015993	MGC27169
M74220	PLG	AL558479	THY1
NM_001713	BHMT	NM_000785	CYP27B1
AW614558	SLC39A5	AW051926	AMN
Z92546	SUSD2	AA928708	CYP8B1
NM_000778	CYP4A11	BE407830	KIFC3
NM_000792	DIO1	AI431643	RRAS2
AI222435	N/A	AF001434	EHD1
D26054	FBP1	BC005894	FMO2
AW025165	SLC22A8	NM_006798	UGT2A1
NM_007287	MME	BF217861	MT1E

The following references were cited herein:

Copland et al., Recent Prog. Horm. Res. 58:25-53 (2003).

Copland et al., Oncogene 22:8053-62 (2003).

Grossman et al., J. Surg. Oncol. 28:237-244 (1985).

Trifillis, Exp. Nephrol. 7:353-359 (1999).

What is claimed is:

1. A method of detecting a renal cell cancer comprising the steps of:

obtaining one or more biological samples comprising renal tissue or renal cells from an individual;

determining an RNA gene expression level of secreted frizzled related protein 1; and

performing statistical analysis on the expression level of said gene as compared to that expressed in normal biological samples comprising renal tissue or renal cells, wherein statistically down-regulated gene expression levels would indicate said individual has papillary or clear cell renal cell cancer.

2. The method of claim **1**, wherein statistically down-regulated secreted frizzled related protein 1 gene expression levels would indicate said individual has papillary renal cell cancer.

3. The method of claim **1**, wherein statistically down-regulated secreted frizzled related protein 1 gene expression levels would indicate said individual has clear cell renal cell cancer.

* * * * *