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DIFFERENTIAL EXPRESSION IN CLEAR CELL RENAL CELL CARCINOMA IDENTIFIED BY GENE EXPRESSION PROFILING

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Abstract

Objective—Gene expression profiling has been shown to provide prognostic information regarding patients with a solitary, sporadic RCC. There is no reliable way to differentiate synchronous renal metastases from bilateral primary tumors in patients with bilateral RCC. We present data using a custom kidney cancer cDNA array that can predict outcomes in patients with unilateral and bilateral RCC.

Methods—Fresh frozen tissue from 38 clear cell RCC (cRCC) was analyzed using a cancer cDNA array containing 3966 genes relevant to cancer or kidney development. Median follow-up was 5.3 years; cancer had recurred in 12 (43%) patients and 11 (39%) patients were deceased at last follow-up.

Results—Using a training dataset of 8 tumors, a 44-gene expression profile (GEP) distinguishing aggressive and indolent cRCC was identified. Of 29 single cRCC, 16 were predicted to be indolent and 13 aggressive by GEP. Recurrence-free survival at 5 years was 68% and 42% in these 2 groups ($P=.032$). cRCC classified as indolent or aggressive according to SSIGN score had 5-year recurrence-free survival of 78% and 42%, respectively ($P=.021$). In a cox proportional hazards analysis, GEP was not an independent predictor of recurrence-free survival after accounting for SSIGN score. GEP classification correlated with cancer-specific survival at 5 years in 4 of 4 patients with metachronous cRCC, but only 2 of 4 patients with bilateral synchronous cRCC.

Conclusions—GEP using a kidney cancer-relevant cDNA array can differentiate between aggressive and indolent cRCC. GEP results may be most useful in unilateral cRCC when results are discordant with predictions of tumor behavior based on standard clinicopathologic features. In addition, GEP can provide prognostic information that may help characterize tumors of unknown clinical stage, such as bilateral metachronous cRCC.

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Abbreviations: RCC, renal cell carcinoma; cRCC, clear cell RCC; GEP, gene expression profile; IRB, Institutional Review Board; RFS, recurrence-free survival; CI, 95% confidence interval; SDF-1, stromal cell-derived factor 1; EGFR, epidermal growth factor receptor

Keywords

Carcinoma; Renal Cell; Microarray; Prognostic algorithm (nomogram); Gene expression profile

Introduction

Renal cell carcinoma (RCC) is a heterogeneous entity as classified genetically, histologically, and clinically. Clear cell RCC (ccRCC) is the most common histologic subtype, accounting for about 70% of all RCCs. Compared with the less common papillary and chromophobe subtypes, ccRCC is also the most lethal, with up to 40% of patients dying of metastatic disease. Several clinical algorithms currently exist to predict the risk of disease progression in patients with RCC.^{1–3}

Gene expression profiling is a high-throughput means to assess the molecular phenotype of cancers. Several groups have used commercial microarray analyses, such as AffymetrixTM, to characterize the genes expressed by the major renal epithelial neoplasms (cRCC, papillary RCC, chromophobe RCC, and oncocytoma) and to predict clinical outcomes in these patients.^{4–10} In combination with patient and tumor characteristics, molecular data has recently been shown to improve upon the accuracy of the RCC predictive algorithms that are currently available for patients with a single tumor.^{11,12}

For patients with bilateral synchronous or metachronous cRCC, there is currently no reliable way to differentiate renal metastases from multiple primary tumors. Clinical history and histologic features of the tumors can suggest the biologic potential of these tumors, but the exact clinical stage is unknown in the absence of nodal or distant metastases. Information regarding the likely clinical aggressiveness of these tumors can enable clinicians to better manage these situations, in which systemic treatment could be given if recurrence is likely. We have identified a set of 44 cancer- and kidney-specific genes that are differentially-expressed in aggressive and indolent cRCC. We have used this gene expression profile (GEP) to predict the clinical outcomes in 29 patients with unilateral cRCC and 9 patients with bilateral or metastatic cRCC.

Materials and Methods

Clinical information regarding patients

Thirty-eight specimens were collected from patients undergoing partial or radical nephrectomy between 1999 and 2001 at Cleveland Clinic under Institutional Review Board (IRB) approval. Clinical, pathologic, and follow-up data regarding all patients was obtained from review of the medical record and contact with patients or surviving family members as a part of an IRB-approved kidney cancer registry. All-cause mortality was assessed using the Social Security Death Index.¹³

Sample preparation and analysis of gene expression profile

Total RNA (5 µg) was extracted with Trizol reagent (Invitrogen) according to the manufacturer's instructions. Whole-cell extracts were prepared after 2 washes with ice-cold phosphate-buffered saline with a lysis buffer containing 1% Triton X-100 as the detergent, supplemented with protease and phosphatase inhibitors (leupeptin, aprotinin, phenylmethylsulfonyl fluoride, sodium orthovanadate, and pepstatin). Pooled RNA from 13- to 24-week-old human fetal kidneys (Stratagene, La Jolla, CA) was used as a common reference in the cDNA array experiments. GeneSpring software (SiliconGenetics, Redwood City, CA) was used for data analyses. Intensities below 100 were raised to 100 and nonlinear

normalization (LOESS) was done for each array to determine the abundance of each transcript in a tumor sample as a fold change with respect to its abundance in the human fetal kidney sample (common reference).

Cancer cDNA array construction

The GEP of cRCC was analyzed using a cancer cDNA array that contained 2 probes for each of 4608 unigenes or unclustered expressed sequence tags corresponding to 9216 cDNA clones.¹⁴ Excluding internal controls, 3966 genes relevant to cancer or kidney development were analyzed including: (a) 1800 genes implicated in cancer progression or metastasis^{15,16} and AffymetrixTM cancer G110 array (<http://www.affymetrix.com/analysis/index.affx>); (b) 1809 genes involved in kidney development (<http://golgi.ana.ed.ac.uk/kidhome.html>); (c) 1400 potential downstream targets of p53 transcriptional regulation; (d) 950 genes with AU-rich elements in their 3' and/or 5' untranslated region¹⁷; (e) 1245 cytokine responsive genes, genes for zinc finger proteins, or genes implicated in apoptosis. Construction of the array was described previously.^{14,17}

Array data extraction and normalization

For each gene, the expression signal for each tumor sample and its background, as well as the signal of the reference and its background, were available. The expression of each gene was background corrected by subtraction, and those with low intensities were adjusted by adding a small quantity (100). Expression of each array was then normalized by locally (at the print tip level) weighted polynomial regression (local LOESS) on the base 2 logarithm of the ratio of the intensity of sample over reference. The logarithm (M) was averaged over the 2 spots of each gene and the mean was used for subsequent analysis using statistical software R (Version 2.4).

A total of 41 cDNA arrays from 38 cRCC specimens were used in the study, including 3 replicates of one sample and 2 replicates from another. The study plan was to identify genes that best discriminated between 4 indolent and 4 aggressive cRCC that were selected based on near certainty of clinical classification status and without knowledge of specific gene expression, and then use these genes to classify the samples.

Statistical analysis

Supervised classification was performed using a classification and regression trees (CART) based program, RandomForest using statistical software R. 1,000,000 trees were built to discriminate the 8 tumors, and the frequency of use of each gene was ranked (as votes), resulting in a gene list based on importance. Each tree was constructed using a random sample of the 3966 genes and the importance identified from the error rate of genes not selected in the random sample, i.e., each tree is based on its own training set and test set.

Genes with >1.5-fold difference in expression between aggressive and indolent cRCC were used in an unsupervised classification of the testing dataset; heatmaps were created based on 2,500,000 trees constructed during the classification of the 29 tumors. Kaplan-Meier curves were made according to tumor classification by GEP prediction with and without clinical prediction according to SSIGN score. Statistical significance ($p < 0.05$) was determined using the Wilcoxon test. Cox proportional hazards models were constructed for recurrence-free survival (RFS) and overall survival using GEP prediction as a binary variable and SSIGN score as a continuous variable.

Results

Clinical information regarding the 29 patients with single renal tumors is listed in Table 1. Nineteen patients (66%) were male and 6 (21%) had decreased performance status; median age at surgery was 58 years (interquartile range: 48–68). Median tumor size was 6.0 cm (3.3–8.0) and 38% were incidentally-detected. Surgery was partial (34%) or radical (66%) nephrectomy; adrenalectomy, regional lymphadenectomy, and/or vena caval thrombectomy were performed in selected cases. Pathologic tumor stage was T1 (n=10), T2 (n=8), or $\geq$ T3 (n=11). Two patients (6%) had lymph node metastases and 5 (15%) had distant metastases. Thirteen deaths (45%) and 12 cancer recurrences (41%) were detected following nephrectomy; median follow-up in living patients was 5.3 years (5.2–5.5).

Microarray analysis was performed on each kidney tumor using a custom cDNA array that included 3966 genes relevant to cancer or kidney development. A training dataset of 8 cRCC with near-certain classification status was used to generate a GEP that distinguished between indolent and aggressive cRCC. The 4 indolent tumors (C03, C07, C37, C51) were pT1 tumors that were <5.5 cm in size and had not recurred during at least 5 years of follow-up. The 4 aggressive tumors (C30, C39, C47, C56) were ≥ 6 cm in size and presented with metastatic disease. A total of 2,500,000 RandomForest constructs was utilized to generate a ranked list of the differentially-expressed genes in the indolent and aggressive cRCC's. Supervised classification generated a list of 44 genes with an average difference in expression between indolent and aggressive cRCC of at least 1.5-fold (Table 2). The resulting GEP was able to distinguish indolent cRCC from aggressive cRCC (Figure 1A). Also shown as an indication of the reproducibility of the microarray experiments is the tight clustering of the GEP from 3 replicates from tumor C86 and the 2 replicates from tumor C03 (Figure 1B).

The 24 up-regulated and 20 down-regulated genes in the aggressive RCC group were then used to classify the remaining unilateral cRCC (testing dataset). A dendrogram showing the GEP of each of the 29 cRCC reveals 16 indolent cRCC and 13 aggressive cRCC (Figure 1C). In order to determine whether the predicted clinical behavior of cRCC was accurately predicted by the GEP, Kaplan-Meier curves depicting the cumulative recurrence-free survival (RFS) were constructed for tumors categorized as indolent or aggressive based on GEP (Figure 2A). The estimated cumulative probability of RFS at 5 years for cRCC predicted to be indolent or aggressive based solely on molecular prediction was 67% (95% CI: (47%, 96%)) and 44% (95% CI: (23%, 83%)) respectively (P=0.032) (Table 4).

Predicted RFS was also assigned to each patient using the SSIGN score, a predictive algorithm based solely on standard clinicopathologic features of cRCC (Table 1).³ SSIGN was used because it is the only algorithm that includes patients with localized and metastatic cRCC. Kaplan-Meier curves were constructed to compare patients with tumors predicted to be indolent or aggressive based on SSIGN score, using a cut-off of 3 points that corresponds to a predicted 5-year RFS of 87.8% (Figure 2B). The estimated 5-year cumulative probability of RFS was 78% (95% CI: 55%, 100%) or 42% (95% CI: 24%, 74%) for cRCC predicted to be indolent or aggressive cRCC based solely on SSIGN score (P=0.021). Combination of the GEP prediction with the SSIGN prediction allowed for discrimination of tumors into low, intermediate, and high-risk groups (Figure 2C, P=0.017). Tumors predicted to be indolent by both GEP and SSIGN (low risk), aggressive by either GEP or SSIGN (moderate risk), or aggressive by both GEP and SSIGN (high risk) were associated with an estimated 5-year cumulative probability of RFS of 75% (95% CI: 50, 100%), 63% (95% CI: 38%, 100%) and 34% (95% CI: 13%, 80%), respectively. In a Cox proportional hazards model of RFS, GEP was associated with a hazard ratio of 2.9 (95% CI: 0.89, 9.2, P=0.07). However, GEP was not an independent predictor of RFS (P=0.6) after accounting for SSIGN score as a continuous

variable (HR: 1.6, 95% CI: 1.2–2.1, $P=0.0006$). Neither SSIGN score ($P=0.5$) nor GEP ($P=0.4$) was a predictor of overall survival.

Clinical information regarding 4 patients with metachronous renal cRCC, 4 patients with bilateral synchronous cRCC, and 1 patient with metastatic cRCC is summarized in Table 3. In these more complex clinical situations, GEP data were analyzed in order to determine whether they could aid in predicting outcomes in these patients with uncertain pathologic stage and disease course. The molecular prediction of clinical behavior was accurate for all 4 patients (100%) with metachronous cRCC; including 1 with GEP-aggressive cRCC that died with cancer recurrence and 3 with GEP-indolent cRCC that are alive at last-follow-up, 2 recurrence-free and 1 alive 7.9 years after the most recent of 4 surgeries for recurrent RCC (Table 3). GEP prediction correlated with clinical outcome in 2 of 4 patients (50%) with synchronous tumors; 2 patients with GEP-indolent tumors were alive and recurrence-free at more than 5 years of follow-up. A third patient was alive and without recurrence almost 7 years after bilateral partial nephrectomies despite having a GEP-aggressive cRCC. The fourth patient with bilateral synchronous cRCC and a GEP-indolent tumor died with metastatic cRCC, most likely related to a contralateral pT3b cRCC which was not available for GEP analysis. Finally, GEP analysis of a cRCC bone metastasis predicted an indolent clinical course in a patient alive 6.7 years after presentation with metastatic cRCC.

Discussion

Analysis of renal neoplasms using microarray techniques can provide diagnostic and prognostic information.^{4–10} Several groups have hypothesized that data derived from these advanced molecular investigations will provide clinically-useful information. However, few groups have investigated the utility of these data in the context of current prediction tools that are based on established clinical and pathologic risk factors. Most of the initial publications investigating cDNA arrays employed only univariate analysis or accounted for only some of the major cRCC risk factors, but the true value of a new prognostic factor is determined based on its ability to provide additional prognostic information above and beyond current factors. Lam et al demonstrated proof-of-principle for the superiority of a combination of molecular and clinical factors over either approach alone, combining immunohistochemical data obtained from tissue microarray analysis with the UCLA-integrated symptom score (UISS).¹¹ We have investigated the utility of a 44-gene GEP derived from analysis of a novel, kidney cancer-related cDNA microarray in sporadic and multifocal cRCC in conjunction with SSIGN score, a predictive algorithm based on the major clinicopathologic risk factors for cRCC recurrence. In addition, we explore a new use for cDNA data, namely to provide additional information for cRCC of uncertain clinical stage.

In the present study, we have demonstrated that a GEP comprised of 44 genes selected from among 3966 genes related to cancer and kidney development is able to distinguish between indolent and aggressive cRCC. The 44 genes include some factors identified in previous studies, as well as several other novel biomarkers. For example, the most highly over-expressed gene (2.7-fold) in our dataset, integrin alpha-2 (ITGA2) was also associated with poor survival in 2 previous cDNA studies and is the only gene in common between the gene lists of Zhao et al and our data.¹⁰ Integrins mediate cell-to-cell and cell-to-extracellular matrix interactions and are believed to play a primary role in tumor growth and metastasis. Stromal cell-derived factor 1 (SDF-1, CXCL12) is a CXC chemokine that binds to CXCR4, a ligand-receptor interaction which has been implicated in leukocyte trafficking and HIV infection. Repression of CXCR4 gene expression has been reported to be associated with poorer outcomes in 4 prior RCC studies,^{4–6,18} but we are the first to report altered expression of SDF-1 in association with RCC prognosis. Decreased expression of SERPINA5, a member of a large family of serine protease inhibitors, was associated with poorer survival in 5 prior cDNA studies,^{4,5,7,18} and

we report that down-regulation of a second family member (SERPINA7) is associated with poorer survival in cRCC. Finally, we found that epidermal growth factor receptor (EGFR) cDNA was induced 1.6-fold in aggressive cRCC. Previous data have suggested that an altered pattern of EGFR protein expression may be associated with cRCC pathologic features.^{19,20} Our own immunohistochemical analyses of 44 cRCC with at least 5-year follow-up found that increased membranous and cytosolic EGFR expression correlated with higher pathologic stage.²⁰ Thus, the findings of the current study appear to be consistent with other results obtained using different methodologies to evaluate these differentially-expressed genes at both the RNA and protein level. Moreover, we suggest that selected genes from the 44-gene GEP should be further examined by additional methodologies (RT-PCR, immunohistochemistry, and tissue microarray) in a larger subset of patients in order to further characterize them as prognostic factors and, potentially, targets for systemic therapy for advanced RCC.

Kaplan-Meier estimates revealed a significant difference in the recurrence-free survival of cRCC predicted to demonstrate indolent or aggressive behavior based on GEP alone. In addition, in combination with SSIGN score, the GEP prediction was also able to define an intermediate-risk group that included patients with tumors predicted to be aggressive by either GEP prediction or SSIGN score (but not both). This suggests that molecular expression data can identify patients at higher risk of recurrence from those predicted to have low-risk disease based on standard clinical factors. It should be noted, however, that GEP was not an independent predictor of RFS in a cox proportional hazard model incorporating SSIGN score as a continuous variable. In the current study, SSIGN was chosen because it is the only predictive algorithm that applies directly to patients with localized cRCC and cRCC metastatic to lymph nodes or distant organs.

Finally, GEP can help to characterize tumors of uncertain clinical stage, including patients with bilateral metachronous renal tumors. GEP prediction correlated perfectly with clinical outcome in 4 of 4 patients with metachronous renal cRCC. GEP prediction did not perform as well in the setting of bilateral synchronous cRCC, perhaps because not all renal tumors were available for analysis. Synthesis of information from tumors from each kidney may have provided improved results, but unfortunately tumor material was not available for analysis in the current study.

In conclusion, we describe a genetic profile based on 44 genes that can distinguish between cRCC with indolent and aggressive clinical behavior. In combination with clinical prediction tools, this gene-expression profile can identify patients at intermediate or high risk for disease progression. These data provide proof-of-principle to support the use of molecular data not only in unilateral cRCC, but also in more complex situations, in which this information can help to clarify the clinical stage of patients with multiple renal tumors.

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Figure 1.

Gene expression profiling (GEP) of conventional RCC. (A) A GEP consisting of 44 genes that differ in expression by >1.5-fold is developed using data from 4 indolent and 4 aggressive cRCC based on known clinical outcome. (B) GEP classification is reproducible (see replicates for C3 and C86). (C) Using the 44-gene GEP, 29 unilateral cRCC and 9 cRCC of uncertain clinical status are classified molecularly into indolent or aggressive groups independent of clinical outcome.

Figure 2.

Estimated cumulative probability of recurrence free survival where patients are grouped according to GEP (A), SSIGN (B), or combination of SSIGN and GEP (C). Key: low-risk (blue), intermediate-risk (purple), and high-risk (red).

Table 1
Pathologic information about 29 patients undergoing nephrectomy for a single conventional RCC

Histologic Type	Pathologic Size (cm)	Nuclear Grade	Histologic Necrosis	Vascular Invasion	2002 TNM Stage	Clinical Prediction of RFS	Molecular Prediction by GEP	Follow-up (yrs)	Status	Cancer Recurrence	Years to Recurrence
eRCC	2.0	2	0	0	pT1aNxM0	99%	I	5.48	Alive	No	
eRCC	1.7	1	0	0	pT1aNxM0	99%	I	5.32	Alive	No	
eRCC	3.0	2	0	0	pT1aNxM0	99%	I	1.11	Dead	No	
eRCC	4.5	2	0	0	pT1bNxM0	99%	I	5.11	Alive	No	
eRCC	3.2	2	0	0	pT1aNxM0	99%	A	0.38	Dead	No	
eRCC	3	3	0	0	pT1aNxM0	99%	I	4.89	Dead	Yes	3.99
eRCC [†]	6.0	3	0	0	pT1bNxM0	88%	I	5.15	Alive	No	
eRCC [†]	3.0	3	1	0	pT1aNxM0	88%	I	5.31	Alive	No	
eRCC	7.6	2	0	0	pT2NxM0	88%	A	5.42	Alive	No	
eRCC	5.2	3	0	0	pT1bNxM0	88%	I	5.01	Dead	No	
eRCC	8.5	2	0	0	pT2NxM0	88%	I	4.43	Alive	Yes	2.45
eRCC	7.5	2	0	0	pT3aNxM0	79%	A	6.25	Alive	No	
eRCC	10	2	0	1	pT3bNxM0	79%	I	4.44	Dead	Yes	4.44
eRCC	7	2	0	1	pT3cN0M0	79%	I	5.07	Alive	No	
eRCC	7.0	2	1	0	pT2NxM0	65%	I	6.15	Alive	No	
eRCC	6	4	0	1	pT1bNxM0	65%	A	5.44	Alive	No	
eRCC	3.4	3	1	1	pT3bNxM0	65%	I	0.01	Dead	Yes	0.00
eRCC	9.0	3	1	0	pT2NxM0	54%	I	5.33	Alive	No	
eRCC	9	3	1	0	pT2NxM0	54%	I	5.87	Alive	No	
eRCC	6.0	2	1	1	pT3cNxM0	54%	A	0.03	Dead	No	
eRCC	13	3	1	0	pT2NxM0	54%	I	0.65	Dead	Yes	0.65
eRCC	4.5	4	1	0	pT3aN0M0	41%	A	5.41	Alive	No	
eRCC	4.0	4 [*]	1	1	pT3bN0M0	41%	A	0.26	Dead	Yes	0.26
eRCC	6	3	0	1	pT3bNxM1	20%	A	5.16	Alive	Yes	0.00
eRCC	10.0	4 [*]	1	1	pT2N2M0	7%	A	0.48	Dead	Yes	0.12
eRCC	9.0	4	0	1	pT2N0M1	7%	A	5.15	Alive	Yes	0.00
eRCC	0.5	4 [*]	1	1	pT3bN0M1	7%	A	1.34	Dead	Yes	0.00

Histologic Type	Pathologic Size (cm)	Nuclear Grade	Histologic Necrosis	Vascular Invasion	2002 TNM Stage	Clinical Prediction of RFS	Molecular Prediction by GEP	Follow-up (yrs)	Status	Cancer Recurrence	Years to Recurrence
cRCC	7	3	1	0	pT3aN0M1	7%	A	0.65	Dead	Yes	0.00
cRCC	6.9	3	1	0	pT3aN2M1	7%	A	4.04	Dead	Yes	0.00

na cava) thrombectomy; LN, regional lymph node sampling; Ad, adrenalectomy

tors were subtyped as unclassified RCC by standard histopathologic analysis, but grouped with I cRCC by molecular analysis, and have therefore

Table 2
Genes that differ in expression level between aggressive (A) and indolent (I) conventional RCC

Up-Regulated in Aggressive cRCC					
Rank	Name	Gene Description	UniGene	Genbank	Fold Change (A/I)
1	ITGA2	integrin, alpha 2 (cd49b, alpha 2 subunit of vla-2 receptor)	Hs.482077	AA463257	2.704
2	SLC39A6	solute carrier family 39 (zinc transporter), member 6	Hs.79136	H29407	2.128
3	RAB5A	rab5a, member ras oncogene family	Hs.475663	H11564	2.111
4	LAMA4	laminin, alpha 4	Hs.213861	R43734	2.029
5	TRA1	tumor rejection antigen (gp96) 1	Hs.192374	AA598758	1.984
6	GMFB	glia maturation factor, beta	Hs.151413	H22652	1.980
7	DAZAP2	daz associated protein 2	Hs.369761	R19889	1.972
8	IL8	Interleukin 8	Hs.624	AA102526	1.962
9	LAMC1	laminin, gamma 1 (formerly lamb2)	Hs.497039	AA599005	1.952
10	UBE2C	ubiquitin-conjugating enzyme e2c	Hs.93002	AA430504	1.928
11	PRKCA	protein kinase c, alpha	Hs.531704	AA030029	1.907
12	CAPG	capping protein (actin filament), gelsolin-like	Hs.516155	AA486942	1.771
13	HERPUD1	homocysteine-inducible, ER stress-inducible, UB-like domain member 1	Hs.146393	AI675465	1.695
14	UBE2B	ubiquitin-conjugating enzyme e2b (rad6 homolog)	Hs.385986	AA598492	1.694
15	CTNND1	catenin (cadherin-associated protein), delta 1	Hs.166011	AA024656	1.648
16	yh96g12.s1	Human cDNA clone IMAGE:137638, 3' mRNA sequence	Data not found	R37224	1.631
17	KCNQ1DN	kcnq1 downstream neighbor	Hs.127821	AA828167	1.628
18	yv17a01	Human cDNA clone IMAGE:242952, 3' mRNA sequence.	Data not found	H95638	1.622
19	FH	fumarate hydratase	Hs.498239	AA026917	1.593
20	EGFR	Epidermal growth factor receptor	Hs.488293	R35665	1.578
21	UGP2	udp-glucose pyrophosphorylase 2	Hs.516217	AA044390	1.571
22	LGALS8	lectin, galactoside-binding, soluble, 8 (galectin 8)	Hs.4082	R96337	1.571
23	Flj43227	cDNA flj43227 fis, clone hchon2000212	Hs.527051	H50345	1.560
24	UBE4A	ubiquitination factor e4a (ufd2 homolog, yeast)	Hs.75275	AA447528	1.529
Down-Regulated in Aggressive cRCC					
20	TNNT1	troponin t1, skeletal, slow	Hs.534085	AA868929	-1.511
19	CYP2E1	cytochrome p450, family 2, subfamily e, polypeptide 1	Hs.12907	H50500	-1.529
18	FLJ10979	hypothetical protein flj10979	Hs.461819	AA281793	-1.532

Up-Regulated in Aggressive cRCC

Rank	Name	Gene Description	UniGene	Genbank	Fold Change (A/I)
17	HIBCH	3-hydroxyisobutyryl-coenzyme a hydrolase	Hs.236642	AA055335	-1.557
16	CRABP1	cellular retinoic acid binding protein 1	Hs.346950	AA421218	-1.559
15	ATP5S	atp synthase, h+ transporting, mitochondrial f0 complex, subunit s (factor b)	Hs.438489	W53000	-1.584
14	GRN	Granulin	Hs.514220	AA496452	-1.591
13	CD33	cd33 antigen (gp67)	Hs.83731	AI344474	-1.605
12	TFDP2	transcription factor dp-2 (e2f dimerization partner 2)	Hs.379018	AA465444	-1.625
11	PEX6	peroxisomal biogenesis factor 6	Hs.107410	AA460646	-1.656
10	PDIR	for protein disulfide isomerase-related	Hs.477352	AA404394	-1.688
9	CXCL12	chemokine (c-x-c motif) ligand 12 (stromal cell-derived factor 1)	Hs.522891	AA447115	-1.699
8	COL17A1	collagen, type xvii, alpha 1	Hs.117938	AA128561	-1.714
7	NPY	neuropeptide y	Hs.1832	AI675889	-1.740
6	ARHGEF16	rho guanine exchange factor (gsf) 16	Hs.87435	AA482070	-1.746
5	SERPINA7	serine (or cysteine) proteinase inhibitor, clade a (alpha-1 antiproteinase, antitrypsin), member 7	Hs.76838	T64901	-1.792
4	SAH	sa hypertension-associated homolog (rat)	Hs.160976	W01011	-1.804
3	NRG1	Neuregulin 1	Hs.453951	H24357	-1.842
2	NEK2	nima (never in mitosis gene a)-related kinase 2	Hs.153704	W93379	-1.846
1	CHI3L2	chitinase 3-like 2	Hs.514840	AA481250	-2.114

Table 3 Clinical and microarray data in patients with metachronous renal cRCC, bilateral synchronous cRCC, and metastatic cRCC

Prior Pathologic Stage	Prior Clinical Prediction of 5y RFS	Current Surgery (Interval)	Current Pathology	Current Clinical Prediction of 5y RFS	Suspected Clinical Scenario	GEP Status Observed	Status	Years After Diagnosis
pT2NxM0 8 cm, g2	79%	L OPN (7y)	pT1aNxM0 1.6 cm, g3 (10 tumors)	99%	Metastases from large primary or additional primary tumors	I	ANED	1.3
≥pT2NxM0	<88%	R OPN (6y)	pT1aNxM0 2.7 cm, g1	99%	Metastasis from large primary or second indolent primary tumor	I	ANED	11.4
≥pT2NxM0	<88%	L OPN (2y)	pT3aNxM0 8.5 cm, g3	88%	Metastasis or second tumor	A	DWD	0.1
p3aN0M1 12.0, 6 cm L 2.7, 2.1 cm R all g2	24%	R ORN (4.4y)	pT3aN0M1 4.2 cm, g3; and 1.5 cm nodule in fat	24%	Local recurrence	I	AWD	7.9
p3bNxM0 10.5 cm, g3	65%	L OPN (2m)	pT1aNxM0 2.8 cm, g3	99%	Metastasis or indolent second primary tumor	I	DWD	2.2
pT3aNxM0 1.7 cm, g2	95%	L OPN (3m)	pT1aNxM0 2.0 cm, g1	99%	Metastasis or indolent second primary tumor	I	ANED	6.9
pT1aNxM0 3.9 cm, g2	99%	L OPN (2m)	pT1bNxM0 6.5 cm, g2	88%	Metastasis or second primary tumor	A	ANED	6.7
≥pT2NxM0	<88%	L OPN (6m)	pT1aNxM 2.5 cm, g2	99%	Metastasis or indolent second primary tumor	I	ANED	5.1
pTxNxM1	ND	Bone metastectomy	pTxNxM1	ND	Metastasis	I	AWD	6.7

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P (partial); N (nephrectomy); cRCC, clear cell RCC; A, aggressive; I, indolent; ND, not determined; Ad, adrenalectomy; XRT, ceased with disease recurrence; AWD, alive with disease recurrence

Estimated cumulative probability of recurrence-free survival according to clinical and molecular prediction of cRCC aggressiveness

Table 4

	N	Cumulative Probability of Survival at 5 Years (95% CI)
Microarray		
Indolent	16	0.67 (0.47, 0.96)
Aggressive	13	0.44 (0.23, 0.83)
SSIGN Score		
Indolent (≤ 3)	11	0.78 (0.55, 1)
Aggressive (> 3)	18	0.42 (0.24, 0.74)
SSIGN Score and Microarray		
Low Risk	9	0.75 (0.50, 1)
Intermediate Risk	9	0.63 (0.38, 1)
High Risk	11	0.34 (0.13, 0.80)