Clinical Utility of Chromosome Genomic Array Testing for Unclassified and Advanced-Stage Renal Cell Carcinomas

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• **Context.**—Cytogenetic analysis provides a useful adjunct to traditional pathology in the categorization of renal cell carcinomas (RCCs), particularly in morphologically ambiguous cases, but it has disadvantages, including cost.

• **Objective.**—To define the clinical scenarios in which this technology has direct clinical applications.

• **Design.**—DNA was isolated from paraffin-embedded tissue from 40 selected cases of RCC. Chromosome genomic array testing was performed using the OncoScan.

• **Results.**—Of 23 cases of unclassified renal tumors, 19 (83%) were reclassified with incorporation of cytogenetic and histologic features, including 10 as clear cell RCC, 2 as collecting duct carcinoma, 2 as papillary RCC, and 1 as novel TFEB-amplified tumor lacking TFEB translocation. Of 5 tumors with “hybrid” oncocytoic features, 3 were reclassified as an eosinophilic variant of chromophobe RCC and 1 as oncocytoma. Appropriate staging in 2 patients was determined by identifying distinct, nonshared cytogenetic profiles. Of 11 cases of metastatic clear cell RCC, 7 (63%) had cytogenetic features associated with a poor prognosis.

• **Conclusions.**—We identified 5 scenarios in which chromosome genomic array testing has direct clinical utility: (1) to investigate unclassified RCCs, (2) to understand tumors with “hybrid” features and “collision” tumors, (3) to determine appropriate staging in questions of bilateral tumors and/or metastases, (4) to identify chromosomal aberrations in metastatic clear cell RCCs associated with a worse prognosis, and (5) to identify new entities. This has practical value in our institution, where a molecular profile diagnostically separating morphologically difficult to classify clear cell, papillary, chromophobe, and unclassified RCC influences treatment recommendations and clinical trial eligibility.

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Despite the powerful tools provided by morphology and immunohistochemistry, 4% to 5% of renal cell carcinomas (RCCs) remain unclassified.1 At our institutions, unclassified RCC represents 2% to 3% of cases per year, or 4% to 5% of cases per year when inclusive of low-grade oncocytoic neoplasms. Accurate classification has prognostic and therapeutic implications, and an unclassified RCC presents challenges for the pathologist, treating oncologist, and patient. However, there is growing complexity in RCC classification, with the recent inclusion of molecular-based diagnoses.2–3 Multiple new entities have been reported at a rapid rate in recent years,4 based in part on use of various molecular techniques, including TFE3 fluorescence in situ hybridization (FISH),5–7 immunohistochemistry and genetic testing related to metabolic pathway abnormalities,8–10 and other molecular studies,11–15 as well as ongoing histopathologic scrutiny and correlation with clinical setting.16–20 The clinical ramifications of a better understanding of these entities as well as clear cell RCC (CCRCC) are, hopefully, distinct treatment pathways with optimal use of new therapeutics. However, the appropriate clinical use of some of these molecular-based assays is not always clear, and cost and time constraints may limit use.

Cytogenetic analysis provides a useful adjunct diagnostic tool for traditional categorization of RCC, particularly in morphologically ambiguous cases. In addition to challenges in diagnostic classification, better clinical and pathologic prognostic and predictive markers are needed,21 particularly in CCRCC. Histopathologic prognostic variables include grade, stage, necrosis, and sarcomatoid and rhabdoid features, but additional prognostic information, such as that gained from cytogenetics, may better inform clinical decision-making. OncoScan is a microarray assay that...
provides genome-wide copy number and loss of heterozygosity (LOH) data from formalin-fixed, paraffin-embedded (FFPE) tissue. In this study, we aimed to determine clinical scenarios where the utility of chromosome genomic array testing (CGAT) would be most appropriate and provide maximum benefit in patient care.

MATERIALS AND METHODS

This study was conducted with approval from the Institutional Review Boards of the University of Washington, Seattle, and the University of Chicago Medical Centers, Chicago, Illinois. From 2009 to 2017, 40 RCCs, including unclassified RCC (n = 18), eosinophilic tumors with “hybrid” histologic and immunohistochemical features, sarcomatoid dedifferentiation, and rhabdoid features; and patient contents. A total of 4 of 5 unclassified RCCs (80%) were reclassified with incorporation of cytogenetic and histologic features, including 10 CCRCCs (43%), 2 collecting duct carcinomas (9%), 2 solid variants of PRCC (9%), and 1 novel -amplified tumor lacking TFE3 translocation (4%; data not shown). All ancillary tests were performed in the Clinical Laboratory Improvement Amendments (CLIA)-certified diagnostic laboratories of 2 institutions. The panels of antibodies in various combinations included carbohydrate IX, vimentin, cytokeratin 7 (CK7), CKIT, P504S, TFE3, S100A1, CD10, EMA, INI1, PA2X, PA2X8, AE1/AE3, CAM5.2, CK20, HMWCK, Melan-A, HMB-45, synaptophysin, chromogranin, and E-cadherin. All cases were reviewed by 3 pathologists with expertise in genitourinary pathology and renal pathology.

Genomic DNA was isolated from FFPE tissue specimens using the QIAamp DSP DNA FFPE Tissue Kit (Qiagen, Valencia, California). The CGAT testing was validated as previously described and performed using the OncoScan FFPE assay kit (Thermo Fisher Scientific, Waltham, Massachusetts). In brief, OncoScan contains 220,000 single-nucleotide polymorphism probes and is specified to provide high coverage/resolution for determining copy number aberrations and LOH in genomic regions encompassing cancer genes. Samples were processed according to the manufacturer's recommendation. The ONCHP files were generated using the OncoScan Console Software (Thermo Fisher Scientific) and were visualized with Nexus Expression OncoScan software (BioDiscovery, Elserondo, California) and Chromosome Analysis Suite (Thermo Fisher Scientific). Based on the comparison with calls from a normal cohort and the database of benign copy number variants, copy number aberrations greater than 500 kb in size, and copy-neutral LOH greater than 10 Mb are considered abnormal based on the established performance characteristics of the assay validated in our Clinical Laboratory Improvement Amendments–certified diagnostic laboratory.

Guidelines for cytogenetic classification were derived from a literature review of multiple sources, including genetic profiles from 2016 World Health Organization classification. Reclassification was made with careful consideration of the entire CGAT findings and rereview of the morphology; CGAT findings were not interpreted in isolation. In general, we classified as CCRCC when the carcinoma had a deletion of 3p, or a gain of 5q with deletions of at least 2 of the chromosomes 4p, 6q, 8p, 9p, or 14q. We considered reclassification as PRCCs when tumors lacked 3p loss, and had a gain of at least 3 of the chromosomes 3q, 7, 8, 12, 16, 17, and 20. Cases reclassified as chromophobe RCC had multiple monosomies, with deletions of at least 2 of the chromosomes 1, 2, 6, 10, 13, or 17. Collecting duct carcinoma was diagnosed in the presence of multiple chromosomal losses: −1, −4, −6, −14, −15, −18, and −22. Tumors with the sole genetic abnormalities of −Y, −1, and/or −14 were reassigned as oncocytophoma. Cases that remained unclassified were those in which morphologic, immunohistochemical, and CGAT findings could not be integrated into a single diagnosis. Statistical analyses were performed with GraphPad Prism 7 (La Jolla, California).

RESULTS

Clinicopathologic Features of Unclassified Cases

The pertinent clinicopathologic data and most notable morphologic features are summarized in the Table. The cohort of 23 included 18 men (73%) and 5 women, with a median age of 56 years (range, 33–88 years). Tumor size ranged from 1.2 to 19 cm in the greatest dimension (median, 8.3). The pathologic stages were distributed as follows: 7 of 23 cases (30%) were pT1, 1 case (4%) was pT2, 12 cases (52%) were pT3, 1 case (4%) was pT4, and 2 biopsies (9%) of primary tumor were not staged. Lymph node metastases were present in 10 of 23 cases (43%), and 11 patients (48%) developed distant metastases. At a median follow-up time of 36.5 months (range, 10–371 months), follow-up was available in 21 of 23 patients (91%). Of these 21 patients, 5 (24%) had no evidence of disease, 10 (48%) were alive with disease, and 6 (28%) had died of disease.

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### Summary of Clinical and Histopathologic Findings of Unclassified Renal Cell Carcinomas (RCCs; n = 23)*

<table>
<thead>
<tr>
<th>Case No.</th>
<th>Sex</th>
<th>Age, y</th>
<th>Tumor size, cm</th>
<th>pT</th>
<th>pN</th>
<th>pM</th>
<th>Nested</th>
<th>Papillary</th>
<th>Tubular</th>
<th>Solid</th>
<th>Clear Cell</th>
<th>Oncocytic cell</th>
<th>Spindle Cell</th>
<th>Scant Cytoplasm</th>
<th>ISUP Grade</th>
<th>Notable Pathologic Features</th>
<th>Outcome</th>
<th>Follow-up, mo</th>
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<td>2: HOCT-like with trabecular and solid patterns</td>
<td>NED</td>
<td>22</td>
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Sum: 5:18 56 8.3 43% 48% 65% 57% 52% 78% 78% 87% 13% 26% 36.5

Abbreviations: AWD, alive with disease; DOD, died of disease; HLRCC, hereditary leiomyomatosis and RCC; HOCT, hybrid oncocytic tumor; IHC, immunohistochemistry; ISUP, International Society of Urologic Pathologists; NA, not available; NED, no evidence of disease; SDH, succinate dehydrogenase-deficient RCC; TFE3, Xp11 translocation RCC involving TFE3 gene.

* Summary data are provided in medians where applicable.
morphology and minimal cytogenetic abnormalities, and this demonstrated **TFE3** gene rearrangement, allowing its reclassification as microphthalmia transcription factor family RCC, despite negative TFE3 immunostaining. Thus, our overall rate of reclassification increased to 20 of 23 (87%) at the conclusion of this study. Results are detailed below and in Figure 1 according to presence or absence of 3p loss and/or LOH, and separately for “hybrid” oncocytoic tumors.

**3p Loss and/or 3p LOH Was Common in Unclassified RCCs, and Most but Not All of These Cases Represented CCRCC.**—Of 23 cases of unclassified RCC, 14 (61%) had loss of 3p and/or LOH at 3p. Upon rereview of the morphology, 10 of these cases (71% of those with 3p loss and/or LOH) could be reclassified as CCRCC; 7 subsequently reclassified CCRCCs (70%) also had +5q, a common abnormality in CCRCC. The morphologic spectrum of these reclassified CCRCC cases is presented in Figure 2.

The 4 cases with 3p loss or LOH not reclassified as CCRCC had morphologic features too unusual for a confident diagnosis of CCRCC, had combined cytogenetic and morphologic features more compatible with a different diagnosis, and/or had CGAT findings suggesting that the 3p abnormality was in a minor clone (Figure 3: non-CCRCC tumors classified by CGAT; and Figure 4, A and B). Specifically, 2 cases (Nos. 8 and 11) had variable morphology and differential diagnoses, including collecting duct carcinoma, PRCC type 2, mucinous tubular and spindle cell carcinoma, hereditary leiomyomatosis–associated RCC, or metastasis; cytogenetic findings of −1p, −14, −15, −21, or −22 were most consistent with collecting duct carcinoma (Figure 3, A and B). One case (No. 16) had a newly described amplification of **TFEB** (6p21) that was not associated with **TFEB** translocation; this carcinoma also had −3p, likely representing a minor clone/low-level disease population, but it was not classified as CCRCC because of morphology and the finding of **TFEB** amplification (Figure 3, C and D). One case remained unclassified because of extreme morphologic heterogeneity and nonspecific CGAT abnormalities, including −3p, +3q, +5, +7, and +11 (case No. 3; Figure 4, A and B; Figure 4, A through F, shows cases that remained unclassified after CGAT).

**Unclassified RCC Without 3p Abnormalities by CGAT Were Diverse.**—Two cases were classified as solid variant of PRCC based in part on CGAT findings of multiple trisomies (+2, +7, +8, +10, +12, +16, +17, and +20; Figure 3, E and F). In one of these cases (No. 6) the morphologic differential diagnosis had included neuroendocrine cell neoplasm due to solid compact architecture and small cells, t(6;11) RCC due to focally biphasic appearance and globular hyaline material, and juxtaglomerular cell tumor (which would have been expected to have −9 and −11). The second case (No. 18) was characterized by solid and infiltrative growth with clear to spindle cells and sarcomatoid dedifferentiation. Because of cytoplasmic clearing this case was originally classified as CCRCC, but it developed metastatic disease resistant to interleukin 2 (IL2) therapy.

Figure 1. **Summary of cytogenetic findings of unclassified renal cell carcinomas (RCCs; N = 23).** *Case No. 10 was reclassified later as microphthalmia-associated transcription factor (MiTF) family RCC after fluorescence in situ hybridization analysis showing TFE3 translocation. Abbreviations: CCRCC, clear cell RCC; CDC, collecting duct carcinomas; ChRCC, chromophobe RCC; EV, eosinophilic variant; LOH, loss of heterozygosity; PRCC, papillary RCC; URCC, unclassified RCC.
Figure 2. Morphologic spectrum of renal cell carcinomas (RCCs) classified after chromosome genomic array testing as clear cell RCC. Case No. 2 exhibited alveolar and diffuse discohesive growth of highly atypical rhabdoid cells, and lacking areas of conventional clear cell RCC morphology (A). Case No. 13 demonstrated abundant discohesive eosinophilic cells in nests or large solid sheets, but lacking rhabdoid dedifferentiation (B). Two additional cases (Nos. 12 and 15) came from younger patients (both age 45 years) and exhibited morphology highly suspicious for translocation RCC because of alveolar and papillary formation, discohesive clusters of atypical cells with clear and oncocytic cytoplasm, and high-grade nuclei (C and D). Case No. 14 had tubulopapillary architecture devoid of any cytoplasmic clearing (E). All cells were uniformly eosinophilic, with large nuclei and very conspicuous nucleoli, concerning for hereditary leiomyomatosis–associated RCC. Case No. 17 was composed of pure eosinophilic cells arranged into large fronds, papillary structures, and solid sheets mimicking type 2 papillary RCC (F). CAIX was only focally expressed within necrotic areas (hematoxylin-eosin, original magnification x200).
Figure 3. Unclassified renal cell carcinomas (RCCs) classified as non-clear cell subtypes after chromosome genomic array testing (CGAT). Two cases (Nos. 8 and 11) were classified as collecting duct carcinoma after CGAT (A and B). Case No. 8 showed features of adenocarcinoma with elongated high-grade nuclei, areas of tubulopapillary formation, and cribriforming (A). Case No. 11 contained clear cell solid areas admixed with tubular and microcystic foci composed of smaller amphophilic cells (B). No desmoplastic response was noted in either of these 2 cases. Case No. 16 (C and D) represents the recently described novel entity of TFEB-amplified RCC without TFEB translocation. Morphologically it was very heterogeneous, displaying solid, acinar, tubular, and papillary architecture with focal cytoplasmic clearing (C) and notable biphasic morphology with papillae lined by large atypical cells and smaller cells apparently confined to the interpapillary lumen (D). Two cases with CGAT profiles consistent with papillary RCC (E and F) but morphologically very diverse. Case No. 6 exhibited solid growth with tight nests of small cells with round, hyperchromatic nuclei and scant cytoplasm (E). Some cells were arranged in small nodules around amorphous hyaline material. This tumor was reminiscent of either biphasic morphology of TFEB RCC or neuroendocrine tumor. Another case (No. 18) of CGAT-confirmed papillary RCC showed diffuse growth of clear cells without noticeable papillary architecture, and it was originally classified as clear cell RCC but developed metastatic disease resistant to interleukin 2 therapy (F) (hematoxylin-eosin, original magnification ×200).
No papillary formation was present in either of these 2 cases. Of note, focal gains in MET locus counts were not seen in this or other cases of unclassified RCC.

Two cases without 3p abnormalities by CGAT remained unclassified. One case (No. 1) had morphologic features of solid and cystic eosinophilic tumor, with papillary formation and focal areas of discohesive polygonal cells reminiscent of epithelioid angiomylolipoma (Figure 4, C and D). The CGAT showed multiple abnormalities on chromosome 1, likely resulting from complex rearrangements: loss of the entire chromosome (monosomy 1) is estimated to be present in 50% of cells; in addition, a large segment on 1q21q32 (51 Mb) was gained, and 2 focal amplifications were seen in 1p36 (6 Mb) and 1q32 (8 Mb). Another case (No. 10) had features of papillary or translocation RCC with prominent papillary formation and cytoplasmic clearing but indeterminate immunohistochemical and CGAT profile (Figure 4, E). This patient experienced local recurrence and developed metastatic disease following therapy for presumed CCRCC. Upon case rereview and CGAT analysis a break-apart TFE3 FISH was performed and showed Xp11 translocation.

CGAT Revealed Diagnostic Cytogenetic Underpinnings in “Hybrid” Oncocytic Tumors.—Chromosome genonomic array testing proved particularly useful in identifying the eosinophilic variant of chromophobe RCC or oncocytoma in cases of “hybrid” morphology (n = 5; Figure 5). Three cases (Nos. 19, 22, and 23) were classified as eosinophilic variant of chromophobe RCC, subsequent to identification of multiple monosomies (−1, −2, −6, −7, −10, −13, and/or −16) by CGAT, thereby excluding oncocytoma and type 2 PRCC (Figure 5, B through D). One of these cases (No. 23) represented an oncocytic neoplasm with histologic and immunohistochemical features shared between oncocytoma and eosinophilic variant of chromophobe RCC. This tumor was focally positive for both CK7 and S100A1 in addition to diffuse immunoreactivity with CKIT, AMACR, and CAIX, thus also concerning for PRCC and CCRCC (Figure 5, D through F). The histologic pattern (trabecular and solid with uniform cells) raised the possibility of a carcinoid tumor; however, a lack of synaptophysin and chromogranin immunoreactivity combined with PAX8 positivity provided evidence against that consideration. CGAT showed distinctive features of chromophobe RCC—an abnormal genome with multiple monosomies, including chromosomes 1, 7, 10, and 16—as well as a large deletion in 11q—and a diagnosis of chromophobe RCC, eosinophilic variant, was therefore rendered. One of the hybrid cases (No. 20) had loss of chromosome 1 material by CGAT and lacked cytogenetic findings to support a diagnosis of chromophobe or PRCC; this case was classified as oncocytoma (Figure 5, A). An additional case (No. 21) had oncocytic and clear cell features; CGAT revealed no quantitative cytogenetic abnormalities, and SDH gene analysis was negative (Figure 4, F), and the case remained unclassified.

CGAT Can Provide Definitive Information When Pathologic AJCC Stage Is Uncertain

Microarray analysis was employed in a case of bilateral CCRCC to determine if the carcinomas represented metastases or independent neoplasms. Chromosome genomic array testing revealed that the right kidney CCRCC showed a main clone of 3pterp14− (terminal deletion starting from band p14 on the short arm of chromosome 3), −14, and copy-neutral LOH of 19p with a secondary clone showing 1q+, +2, +5, +7, +15, and 19q+. The left-sided CCRCC, by contrast, had a main clone with −Y, 3p26p12−, +7, and +12, with a secondary clone of −9 and −14. These dissimilar CGAT results provided evidence for independent tumors rather than metastatic disease. In another case, a patient with CCRCC presented with a dermal pleomorphic spindle cell neoplasm, concerning for metastatic RCC with sarcomatoid features. However, CGAT analysis of the CCRCC demonstrated −Y, 3p−, 5q+, 6q−, 8q+, and −14, whereas the dermal neoplasm showed a main clone with −4, −5, −6, +7, −9, −10, 11p−, 12p−, −13, 17p−, and −22, with homozygous deletions of PTEN (64 kb on 10q23) and RB1 (1.2 Mb on 13q14). The secondary clone had deletions of Yq11−, 1q+, 3p−, 3q−, −8, and 12q−. This genomic profile was distinct from that of the RCC, suggesting an independent neoplasm rather than a metastasis.

“Collision” Tumor

One case that had distinct CCRCC and type 1 PRCC areas suggestive of a “collision” tumor was evaluated. The CCRCC area demonstrated deletion of 3p, the genomic hallmark of CCRCC, and gain of 5q and loss of 14q, also common in CCRCC. The area of PRCC showed extensive copy number alterations, including gains of 3q, 7, and 17, common findings in type 1 PRCC, as well as gains of 12, 16, and 20, which often occur in an advanced state of PRCC. The genomic profile of the PRCC tumor area appeared distinct from the area of CCRCC, although the finding of copy-neutral LOH of 3p in the region of PRCC raised the possibility of shared underlying genetic mechanisms involving genes on 3p.

In CCRCC With Metastases, CGAT Provided Prognostic Information

Of 11 cases of CCRCC with metastasis, CGAT performed on the primary RCC revealed that 10 (91%) had loss of chromosomal material on 3p, and 8 (73%) had cytogenetic features associated with a poor prognosis, including −14q, −9p, and/or −4p,2,32 Nine of these cases (81%) were pT3, 1 was pT2, and 1 was pT1. Five patients (45%) had metastatic disease at presentation, including lymph node metastases (n = 2) and distant metastases (n = 3). All 5 of these patients had cytogenetic features associated with a poor prognosis, compared with 3 of 6 patients with an unfavorable cytogenetic profile but localized disease at presentation (P = .18, Fisher exact test). Interestingly, 1 case of previously treated CCRCC with classic morphology and dense inflammation had CGAT features of PRCC (+7, +17, no −3p).

DISCUSSION

In summary, we identified 5 situations in which OncoScan chromosome genomic array testing had direct clinical utility in kidney tumors: (1) to investigate unclassified RCCs, (2) to understand tumors with “hybrid” features and “collision” tumors, (3) to determine appropriate staging in questions of bilateral tumors and/or metastases, (4) to identify chromosomal aberrations in metastatic CCRCCs associated with a worse prognosis, and (5) to identify new entities.

Of 23 cases of unclassified RCC, a total of 19 (83%) were reclassified with incorporation of cytogenetic and histologic features, including 10 (43% of total) as CCRCC. Clear cell RCC makes up approximately 75% of RCCs1 and is characterized by inactivation of the von Hippel–Lindau gene (VHL) in up to 90% of cases.33 This occurs through 3
Figure 4. Four renal cell carcinomas (RCCs) that remained unclassified after chromosome genomic array testing. Case No. 3 (A and B) showed very heterogeneous architecture, including papillary formation with psammoma bodies transitioning into alveolar growth, solid growth, and areas with single-cell infiltrating pattern with cell spindling. Representative images of case No. 1 shows papillary (C) and tubular (D) architecture with large eosinophilic cells containing finely granular cytoplasm and small nucleoli (International Society of Urologic Pathologists grade 2). This tumor also had solid areas with foamy macrophages and more discohesive cells, reminiscent of epithelioid angiomylipoma (AML). Immunohistochemical panel of 11 markers ruled out AML but did not allow RCC subtyping. Case No. 10 (E) exhibited papillary architecture with solid alveolar nests composed of clear cells with high-grade cytology. Immunohistochemical staining with TFE3 was negative, and CAIX was focally positive, favoring clear cell RCC. Cytogenetic findings were inconsistent with clear cell RCC, showing a few nonspecific segmental gains and losses. Fluorescence in situ hybridization was performed based on high suspicion for microphthalmia-associated transcription factor family RCC and showed TFE3 gene rearrangements at Xp11. Case No. 21 (F) was a 1.2-cm tumor with solid architecture with tightly clustered nests of clear to eosinophilic cells with vacuolated cytoplasm and “spiderlike” flocculent cytoplasmic inclusions. Immunohistochemical analysis with 8 markers, including CAIX, cytokeratin 7, CKIT, vimentin, E-cadherin, TFE3, HMB-45, and Melan-A, was inconclusive. The possibility of succinate dehydrogenase (SDH)–deficient RCC was also considered, but results of SDH genomic studies were negative (hematoxylin-eosin original magnification ×200).
main mechanisms: VHL gene sequence alteration, promoter hypermethylation, and LOH/copy number of the 3p25–26 locus. Loss of function of the VHL protein results in excess hypoxia-inducible factor (HIF) protein, which facilitates neoplastic adaption to oxygen deprivation and angiogenesis. Chromosome 14q losses (HIF1A on 14q23.2 and PHD1-EGLN on 14q13.2) and 5q gains are frequently seen in CCRCC.5,2,9,32,33 5q gains were present almost all cases reclassified as CCRCC in this study. Of these genomic abnormalities, CGAT can directly identify 3p loss and/or LOH, but not epigenetic silencing of VHL (seen in approximately 7% of CCRCCs) or VHL sequence alterations that are not also associated with chromosomal gains or losses, and as such, we may not have identified all cases with VHL pathway abnormalities and/or all cases of CCRCC in this study. Although commonly considered pathognomonic for CCRCC, alterations in the VHL pathway have rarely been described in other settings.33 Supporting this, not all cases with 3p abnormalities in this study represented CCRCC. The CGAT findings were meticulously correlated with morphology for classification, and histologic features remain paramount.

There are significant potential effects of reclassifying unclassified RCC as CCRCC on prognosis, surveillance, and treatment decisions. In patients with metastatic CCRCC, first-line therapy includes high-dose IL2 and targeted therapy. Interleukin 2 is currently the only therapy with the possibility of complete response in metastatic CCRCC.3,33,34,37 Up to 90% of patients receiving targeted therapy (multitargeted tyrosine kinase inhibitors, PDGFR, mTOR, VEGFR inhibitors) will respond and have longer overall survival, but most will become refractory to treatment.33 In this cohort, 2 patients whose tumors were initially unclassifiable and subsequently classified as CCRCC after characteristic cytogenetic changes were identified by CGAT were enrolled into a clinical trial for CCRCC. Reclassification of an unclassified RCC as CCRCC opens different, targeted, and potentially superior therapeutic options to patients.

Chromosome genomic array testing proved particularly useful in discriminating the eosinophilic variant of chromophobe RCC and oncocytoma, which were identified in 3 cases and 1 case, respectively, of eosinophilic tumors with “hybrid” histologic and immunohistochemical features shared between oncocytoma and chromophobe RCC. Classic chromophobe RCCs have frequent loss of chromosomes 1, 2, 6, 8, 19, 13, 17, and 21.13,36 The mTOR inhibitors appear to be effective for chromophobe RCC—perhaps because of their alterations in TSC1, TSC2, and/or MTOR—but not for other entities, like PRCC.33,34 Reclassification of 3 previously unclassifiable cases as chromophobe RCC can help direct targeted therapy and raises the potential clinical utility of an mTOR inhibitor. A definitive diagnosis of benign oncocytoma has obvious clinical ramifications.

Papillary RCC comprises approximately 10% to 15% of RCCs, and in our series of 23 unclassified tumors, PRCC made up 2 cases (9%) that could be reclassified with CGAT. Type 1 PRCC is characterized by gains of 3, 7, and 17.41 Using next-generation sequencing and single-nucleotide polymorphism–based copy number analysis, 1 group of investigators1,32 found that PRCC had the greatest amount of protein-altering somatic mutations and significant enrichment for pathways involving MET (most of which affected the kinase domain of MET). In our study, 2 cases were reclassified as solid variant of PRCC, which has direct clinical implications because MET inhibitors show promise in trials for PRCC.42,43 Papillary type 2 has reproducible gains in chromosomes 1 and 8 and losses in 3, 6, and 9, although various other cytogenetic abnormalities have also been reported.33,41 The relatively low number of cases reclassified as PRCC may be due to a combination of relatively easily recognized type 1 PRCC (hence, fewer were initially unclassified), and the diverse morphology and cytogenetic landscape described in type 2 PRCC, which make the diagnostic boundaries and criteria of the latter less clear. Multiple distinct entities with papillary architecture and eosinophilic cells which may otherwise have been considered type 2 PRCC have been discovered in recent years, and as currently defined, type 2 PRCC likely encompasses more than 1 entity.41 Similar to these trends in the better understanding of unclassified and difficult to classify RCCs with papillary architecture, a recently described entity with TFEB amplification was also identified in this cohort.30,43

The CGAT workup also provided evidence that tumors with “collision” morphology characterized by the coexistence of phenotypically distinct tumors at the same site were genetically distinct, although they may have had a shared oncologic origin. Appropriate staging in 2 patients was determined by identifying distinct, nonshared cytogenetic profiles.

Finally, CGAT provided additional cytogenetic information in patients with CCRCC. For patients with localized CCRCC, there is no consensus on the optimal frequency of surveillance body imaging. In patients with metastatic CCRCC, IL2 is currently the only therapy with possibility of complete response,33,33,36,37 but not all patients are candidates for this therapy. Patients with rapidly progressive disease are at risk of experiencing disease complications waiting for IL2 therapy to be coordinated. In our study, 63% of patients with metastatic CCRCC had cytogenetic features associated with a poor prognosis.2,32 Although there are currently no predictive markers to guide the optimal choice between follow-up and treatment, knowledge of prognostic cytogenetic findings can potentially help tailor the frequency of follow-up imaging surveillance in the setting of localized RCC, and better inform the decision between targeted therapies and IL2 in the metastatic setting. Further studies are needed to refine the role of CGAT in these clinical decisions.

Weaknesses in this study include the limitations of CGAT, which cannot identify balanced translocations or nonstructural changes in chromosomes. Thus, translocation–associated carcinoma, gene sequence alterations, and promoter hypermethylation—including that of 3p that occurs in a subset of CCRCC—are not detected by this technology. The gold standard for classification of RCCs is based on morphology and appropriate ancillary studies. In general, the information gained from CGAT alone was not sufficient to independently classify carcinomas, but it was valuable when taken in combination with morphology and other immunohistochemical and/or genomic studies. In addition, the cost of CGAT (approximately $200,000 in equipment and $600 in reagent per sample) and expertise needed for interpretation currently provide some barriers for use of this technology.

In summary, CGAT has practical value in our institution, where a molecular profile diagnostically separating morphologically difficult to classify clear cell, papillary, chromophobe, and unclassified RCC influences treatment recommendations and/or clinical trial eligibility. Analysis
Figure 5. Oncocytic tumors classified as oncocytoma or chromophobe renal cell carcinoma (RCC) after chromosome genomic array testing (CGAT). Case No. 20 classified as oncocytoma (A), with compact nested and microcystic architecture with very scant stroma and abundant foamy macrophages. Immunostaining profile was not consistent with either oncocytoma or chromophobe RCC due to lack of CKIT and cytokeratin 7 expression. Three cases were classified after CGAT as chromophobe RCC, oncocytic variant (B through F). Case No. 19 exhibited solid growth pattern, with variably sized oncocytic cells with prominent vacuoles and inclusions, imparting a succinate dehydrogenase–deficient RCC-like appearance (B). Case No. 22 showed predominantly compact sheets and tubulopapillary architecture with discohesive eosinophilic cells (C). Case No. 23 demonstrated solid and trabecular architecture with monotonous eosinophilic small cells (D). Both case Nos. 22 and 23 had been unclassified because of diffuse expression of CAIX (E) and AMACR (F) (hematoxylin-eosin, original magnification ×200 [A through D]; original magnification ×200 [E and F]).
of underlying cytogenetic abnormalities can also determine staging in cases of bilateral carcinomas or in other settings in which a neoplasm may represent a metastasis or separate primary.

References