Clinical Utility of Concurrent Single-Nucleotide Polymorphism Microarray on Fresh Tissue as a Supplementary Test in the Diagnosis of Renal Epithelial Neoplasms

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ABSTRACT

Objectives: The histologic and immunohistochemical variability of renal epithelial tumors makes classification difficult; with significant clinical implications, efforts to make the proper diagnoses are necessary. Single-nucleotide polymorphism (SNP) microarray analysis has been proposed as a supplementary study for the classification of renal epithelial neoplasms; however, its practical use in the routine clinical setting has not been explored.

Methods: Surgical pathology cases that were classified histologically as renal epithelial tumor subtypes and had concurrent SNP microarray were retrospectively reviewed to correlate tumor morphology and SNP microarray results.

Results: Of the 99 cases reviewed, 88 (89%) had concordant histologic and microarray results. Four (4%) cases were unclassifiable by microarray due to uncharacteristic chromosomal abnormalities. Seven (7%) of the 99 cases had discordant microarray and histologic diagnoses, and following review of the histology, the diagnoses in two of these cases were subsequently changed.

Conclusions: For most cases, concurrent SNP microarray confirmed the histologic diagnosis. However, discrepant microarray results prompted review of morphology and further ancillary studies, resulting in amendment of the final diagnosis in 29% of discrepant cases. SNP microarray analysis can be used to assist with the diagnosis of renal epithelial tumors, particularly those with atypical morphologic features.

The four most common subtypes of adult renal epithelial tumors are clear cell renal cell carcinoma (RCC), chromophobe RCC, papillary RCC, and oncocytoma. In the past 20 years, a classification system has emerged, which is based on the morphology, immunohistochemistry (IHC), and, more recently, the specific cytogenetic characteristics of these adult renal epithelial tumors. Although this system has aided in standardization of the pathologist’s diagnosis and, in turn, the patient’s prognosis, renal epithelial tumors are a heterogeneous group of neoplasms, and the histologic overlap and IHC variability make classification and diagnosis difficult.1,2 With significant prognostic and treatment implications, assigning the proper diagnosis is essential. Although an assortment of prognostic nomograms using a variety of clinical parameters have been proposed, they remain relatively imprecise.3,4

Each of the RCC subtypes is characterized by specific genetic abnormalities.5 Almost all clear cell RCCs have deletions in the short arm of chromosome 3 (including the VHL gene); papillary RCC typically has trisomies for chromosomes 7 and 17 and loss of the Y chromosome; and chromophobe RCC shows characteristic loss of multiple chromosomes, including 1, 2, 6, 10, 13, 17, and 21, and the X or Y chromosome. Benign oncocytomas usually have a normal genetic profile but may have loss of 1p and/or Y. Metaphase chromosomal analysis has been used to detect characteristic genetic aberrations6; however, this method is labor intensive and has the potential to produce false-negative results if normal stromal cells are cultured and analyzed. More recently, copy number microarray analysis has been shown to be useful for defining the genetic subtypes.7-10

Single-nucleotide polymorphism (SNP) microarray analysis
has been proposed as an ancillary study for the classification of renal epithelial tumors based on the speed and amount of detailed and specific genetic information it provides; however, its real-time, practical use in the day-to-day clinical setting as a supplementary tool has not been explored.\textsuperscript{7,11}

For this study, we sought to review data on the utility of SNP microarray during routine clinical testing.

**Materials and Methods**

Ninety-nine surgical pathology cases from the Medical University of South Carolina (MUSC) were retrospectively reviewed, with approval from the institutional review board, to correlate tumor morphology, IHC characteristics, and SNP microarray results. All cases consisted of complete or partial nephrectomy specimens that were collected during a 20-month time period (November 2011 to June 2013). Fresh kidney tissue was sent directly to pathology where the specimens were grossed according to standard protocols. Fresh tissue was sent for routine histology, and a fragment of grossly identified tumor (typically about 1 $\times$ 1 cm) was excised and forwarded to the cytogenetics laboratory for concurrent SNP microarray.

For the SNP microarray analysis, genomic DNA from the kidney tissue that had been subjected to collagenase and trypsin degradation was extracted using the QIAamp Genomic DNA Purification kit from Qiagen (Valencia, CA). If there were multiple fragments of tissue submitted or the tissue had grossly different areas, a portion of each representative fragment/area was used for processing. Microarray-based chromosome analysis was performed using the Infinium HD Human Omni 1 BeadChip (Illumina, San Diego, CA). Copy number and genotype data were analyzed using GenomeStudio (Illumina), KaryoStudio 1.2 (CNV Plugin V2.4.4.0; Illumina), and Nexus 5.0 (BioDiscovery, El Segundo, CA). Two parameters, signal intensity as assessed by the log\textsubscript{2}R ratio (LogR) and the specific allele (B allele) frequency, provided information regarding copy number and genotype, respectively, and were determined by visual inspection of KaryoStudio and Nexus files. General criteria for designating a reportable aberration included deletions larger than 200 kb and duplications larger than 500 kb with a minimum of 20 consecutive markers that were deemed mosaic (present in a percentage of cells less than 100%). Aberrations present in 100% of cells were considered constitutional and were not used for diagnosis. Genomic linear positions were given relative to National Center for Biotechnology Information Build 37 (http://genome.ucsc.edu/).

Cases were assigned to a renal epithelial subtype based on the detection of typical genetic aberrations: loss of 3p25 for designation as clear cell RCC; extra copies of chromosomes 7 and 17 for papillary RCC; hypodiploidy with loss of chromosomes 1, 2, 6, 10, 13, 17, and 21 for chromophobe RCC; and a normal genotype or loss of the short arm of chromosome 1 and/or loss of Y for oncocytoma.

IHC and special stains were performed, as per routine, on cases that were considered morphologically challenging (most often eosinophilic cell tumors) using standard accepted methods according to the College of American Pathologists’ regulations. All IHC stains, with the exception of carbonic anhydrase IX (performed as a send-out test at Clarient Labs, Aliso Viejo, CA), were performed on either the BiocareintelliPATH FLX (Biocare Medical, Concord, CA) or the Ventana BenchMark XT (Ventana Medical Systems, Tucson, AZ) systems at MUSC. The single special stain used (Hale’s colloidal iron) was performed manually by a histotechnologist at MUSC.

**Results**

Of the 99 cases reviewed, 88 (89%) cases had concordant histologic and microarray results Table 1. Of these concordant cases, 63 were classified as clear cell RCC, 14 as papillary RCC, four as chromophobe RCC, and seven as benign oncocytoma.

Four (4%) of the 99 cases were considered unclassifiable by SNP microarray due to unique chromosomal abnormalities not typical of a specific renal epithelial tumor subtype Table 2. Three of these cases (cases 1-3) were diagnosed as clear cell RCC based on histology and IHC.

<table>
<thead>
<tr>
<th>SNP Microarray Result</th>
<th>Clear Cell RCC</th>
<th>Papillary RCC</th>
<th>Chromophobe RCC</th>
<th>Oncocytoma</th>
<th>Other</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clear cell RCC</td>
<td>63</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Papillary RCC</td>
<td></td>
<td>14</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chromophobe RCC</td>
<td>1</td>
<td></td>
<td>4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oncocytoma/normal karyotype</td>
<td>3</td>
<td>1</td>
<td>2</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>Unclassifiable</td>
<td>3</td>
<td></td>
<td></td>
<td></td>
<td>1$^a$</td>
</tr>
</tbody>
</table>

RCC, renal cell carcinoma; SNP, single-nucleotide polymorphism.

$^a$ Case was originally diagnosed morphologically as RCC, with clear cell, papillary, and chromophobe features.
Image 1

Results

Case 2 had aberrations typical for clear cell RCC (deletion of 1q, 4p, 8p, and 8q and loss of 13), indicating that this was most likely a rare clear cell RCC without deletion of 3p. Case 4 showed features of clear cell RCC, chromophobe RCC, and papillary RCC and was ultimately diagnosed as unclassifiable RCC after receipt of the atypical microarray results.

Seven (7%) of the 99 cases had discordant microarray and histologic diagnoses Table 3. Five of the discordant cases (cases 5-8, and 10) were considered morphologically challenging, and IHC stains were performed to assist with diagnosis. Two cases (cases 8 and 10) were originally diagnosed as clear cell RCC and papillary RCC and was ultimately diagnosed as unclassifiable RCC after receipt of the atypical microarray results.

Seven (7%) of the 99 cases had discordant microarray and histologic diagnoses Table 3. Five of the discordant cases (cases 5-8, and 10) were considered morphologically challenging, and IHC stains were performed to assist with diagnosis. Two cases (cases 8 and 10) were originally diagnosed as clear cell RCC and papillary RCC, respectively, based solely on morphology; however, following receipt of the microarray results, IHC stains were performed, histologic sections were reevaluated, and the final diagnoses were changed to chromophobe RCC for case 8 and oncocytoic neoplasm for case 10.

Three cases (5, 9, and 11) showed an absence of genetic aberrations or loss of the Y chromosome as a sole abnormality, which would be most consistent with a diagnosis of oncocytoia; however, inadequate tumor sampling could not be excluded for these cases. Case 5 was diagnosed as a chromophobe RCC primarily due to the ambiguous histology and focal positive IHC result for CK7. For case 9, which was diagnosed as a clear cell RCC, review of the SNP microarray results detected a small percentage (below the validated level of detection) of DNA with a loss of chromosome 3p, which is characteristic of clear cell RCC. This loss of 3p was not reported in the final microarray results because of the low percentage of cells affected by the chromosomal change. For an unknown reason, case 11 had very little tissue submitted for microarray. Therefore, the discordant results of the latter two cases could be attributed to tumor heterogeneity but more likely reflected a sampling error.

Cases 6 and 7 were considered morphologically challenging cases due to the presence of eosinophilic cytoplasm. Case 6 had ambiguous morphology with features suggestive of either an oncocytoia or chromophobe RCC. The microarray results were typical for oncocytoia (loss of chromosomes 1, 22, and Y), but the tumor had negative staining for PAX-2, positive staining for AE1/AE3 and CD10, and partial positive staining for CK7, which favored a diagnosis of chromophobe RCC. Case 7 was diagnosed as clear cell RCC, eosinophilic cell type, based on histology and IHC results Image 2. Following receipt of the microarray results (loss of chromosomes 1, 14, and Y) that were most consistent with oncocytoia, repeat and additional IHC stains were performed, which were still most consistent with clear cell RCC. The final diagnosis of clear cell RCC, eosinophilic cell type was made with a comment regarding the discordant microarray results and the need for clinical correlation. For these cases, the higher grade tumor was used for the final diagnosis due to the potential impact on follow-up and prognosis for the patient.

Discussion

The increased frequency of incidentally found renal masses has resulted in a change in the paradigm of the
approach to the disease. Treatment has evolved from radical nephrectomy, to nephron-sparing partial nephrectomy and tumor ablation, to observation of renal masses. During this time, clinicians have recognized that while some of these tumors can grow and metastasize aggressively, others behave in a very indolent fashion. As a result, an increasing premium has been placed on efforts to risk-stratify these tumors so that clinicians can determine which patients require treatment and how aggressive one needs to be with the treatment.

Chromosomal analysis and, as an extension, SNP microarray analysis offer a potential addition to our current methods to identify and characterize renal tumors. The purpose of this study was to assess the diagnostic utility of concurrent SNP microarray on fresh tissue from renal epithelial neoplasms compared with standard-of-care morphologic diagnosis with or without immunohistochemical stains. Our study showed that the concordance rate of histology, IHC (when used), and SNP microarray was 89%. This high concordance rate indicated that SNP microarray can reliably be used to confirm a diagnosis of RCC.

Microarray genetic analysis is most applicable for those cases with uncertain diagnoses based on morphology and/ or cases with eosinophilic cytoplasm. Approximately 5% of cases in our study were considered morphologically challenging, and the microarray results prompted reevaluation of histologic sections and additional IHC stains. For two of these five cases, IHC could not exclude the diagnosis of chromophobe RCC, even though the microarray diagnosis
was consistent with oncocytoma. Given that patients with oncocytoma and chromophobe RCC have excellent survival, changing the final diagnosis for these cases would not have significantly changed patient management. However, for two cases, the final diagnosis was changed from a high-risk clear cell RCC to a low-risk renal tumor (chromophobe or oncocytic tumor), significantly affecting clinical management and potentially sparing the patients unnecessary therapy. Thus, while most routine cases showed concordant results, microarray analysis significantly affected patient care in approximately half of the challenging cases.

Four cases had unclassifiable microarray results that may define an unusual variant of renal neoplasms with uncharacteristic genetic abnormalities or may represent clear cell RCCs with an atypical initiating genetic event.12,13 Cases 1 and 3 both showed clear cell RCC morphology along with loss of heterozygosity (LOH) on chromosome 16 by microarray. The significance of this finding is unknown, and further studies are required to determine if this genetic aberration may be associated with a new subtype of RCC. Case 2 had genetic abnormalities that are recurrent in clear cell RCC but lacked allelic loss of 3p, which was used as the criterion to designate clear cell RCC. Approximately 4% of clear cell RCC cases do not exhibit loss or LOH for 3p12; thus, this case likely represented a clear cell RCC with a mutation in the \textit{VHL} gene sequence and not a loss of genetic material, or possibly loss of the \textit{TCEB1} gene on 8p that is complexed with \textit{VHL}.14 Our results suggest that genomic analysis using microarrays can assist in identifying new subclassifications of renal tumors or new genetic mechanisms associated with known tumor types.

In addition to its contribution to the final diagnosis, SNP microarray also provided additional genetic information that may be useful as prognostic indicators for the clinician.15 RCC subtypes have different prognoses that may be related to the underlying genetic aberrations. For clear cell RCC, loss of chromosome 14 and loss of 9p have been associated with histologic markers for an adverse prognosis and clinical outcome.16 In addition, genetic analyses are predicted to be used as markers for targeted therapy.17,18 The more detailed genetic

### Table 3

<table>
<thead>
<tr>
<th>Case No.</th>
<th>Histologic Diagnosis</th>
<th>Immunohistochemical and Special Stain Results</th>
<th>SNP Microarray Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>8</td>
<td>Original: Clear cell RCC; final: chromophobe RCC</td>
<td>CD10: Positive CD117: Positive Hale’s colloidal iron: Positive PAX-2: Positive, partial RCC marker: Positive, focal Vimentin: Negative</td>
<td>Consistent with chromophobe RCC: loss of 1, 3, 5, 6, 9, 10, 13, 15, 17, 18, 21, and X</td>
</tr>
<tr>
<td>9</td>
<td>Clear cell RCC</td>
<td>None performed</td>
<td>No genetic aberrations</td>
</tr>
<tr>
<td>10</td>
<td>Original: Papillary RCC; final: oncocytic neoplasm</td>
<td>CD117: Positive</td>
<td>Most consistent with oncocytoma: loss of 1 and Y (60% of cells); loss of distal 9q, 14, and 22q (40% of cells)</td>
</tr>
<tr>
<td>11</td>
<td>Clear cell RCC</td>
<td>None performed</td>
<td>Most consistent with oncocytoma: loss of Y</td>
</tr>
</tbody>
</table>

RCC, renal cell carcinoma; SNP, single-nucleotide polymorphism.

a Low-level loss of 3p below reportable limit.
information that SNP microarray provides, especially in the cases of clear cell RCC, may help guide the clinicians’ decisions regarding patient prognosis, follow-up, and management as well as potentially aid in genetic-specific treatment options, which has become an area of increased focus.

One limitation of the study of fresh renal tissue from the gross specimen was the lack of histologic confirmation of the sampled tumor. Sampling error or tumor heterogeneity, which has recently been documented by molecular analyses, was suspected in several cases with clear cell morphology and normal microarray results. Although fresh tissue provides the best nucleic acid quality for microarray testing, the use of DNA extracted from macrodissected formalin-fixed, paraffin-embedded renal tumor tissue is technically acceptable, and assessment of histologically characterized samples would provide added confidence in the microarray results.

While there are many reasons that SNP microarray could be considered a helpful ancillary tool to aid in the diagnosis of renal epithelial tumors, there are also multiple challenges. Most facilities do not have the equipment, resources, or staff available to perform SNP microarray as an in-house test, although testing is available as a send-out. In addition, microarray analysis is a relatively expensive test; however, compared with conventional cytogenetics, the laboratory costs are similar, yet microarray provides more information and can be performed on both fresh and fixed tissue samples. In addition,
when one considers the impact on patient care, follow-up, and health care costs (repeated computed tomography scans, medical appointments, etc) when a more benign tumor is misdiagnosed as a more malignant tumor, the use of microarray as a confirmatory test and its associated cost could be justified. Last, one might argue that IHC stains have a faster turnaround time than microarray, making IHC stains the superior choice. In our experience, although the turnaround time for microarray results was approximately 1 week due to test batching, microarray provided more information and often in a more objective fashion compared with IHC. Because these common renal epithelial tumors are rarely, if ever, a medical emergency requiring immediate clinical action, during this study, the delay in the microarray report was considered acceptable to our clinicians.

The results from this study have influenced the procedure that we use at our institution to diagnose renal epithelial tumors. Because only two of 99 diagnoses were ultimately changed, and the study may have been complicated by gross sampling errors, concurrent SNP microarray on fresh tissue from all renal tumors is no longer performed. Our new diagnostic algorithm uses a retrospective approach. All cases diagnosed histologically as conventional clear cell RCC as well as all tumors with ambiguous histology and/or IHC (such as tumors with extensive eosinophilic cell change) now have SNP microarray performed on confirmed tumor from the formalin-fixed, paraffin-embedded block. This practice eliminates multiple issues, including the need for fresh tissue, the likelihood of gross sampling error, and the use of SNP microarray on cases when histology alone will suffice. The microarray results provide diagnostic information for the ambiguous tumors and potential prognostic information for the clear cell RCCs. Continued longitudinal follow-up will allow potential implementation of risk stratification and resultant modification of imaging follow-up. In addition, understanding what genetic changes may have occurred in the primary tumor may help to dictate types of systemic therapy if there is recurrence in the future. Ultimately, the use of SNP microarray as an ancillary tool in diagnosing renal epithelial tumors provides information to the clinicians that can be used to ensure that the best patient care can be offered and achieved.

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References


