A Genomic Algorithm for the Molecular Classification of Common Renal Cortical Neoplasms: Development and Validation

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Purpose: Accurate discrimination of benign oncocytoma and malignant renal cell carcinoma is useful for planning appropriate treatment strategies for patients with renal masses. Classification of renal neoplasms solely based on histopathology can be challenging, especially the distinction between chromophobe renal cell carcinoma and oncocytoma. In this study we develop and validate an algorithm based on genomic alterations for the classification of common renal neoplasms.

Materials and Methods: Using TCGA renal cell carcinoma copy number profiles and the published literature, a classification algorithm was developed and scoring criteria were established for the presence of each genomic marker. As validation, 191 surgically resected formalin fixed paraffin embedded renal neoplasms were blindly submitted to targeted array comparative genomic hybridization and classified according to the algorithm. *CCND1* rearrangement was assessed by fluorescence in situ hybridization.

Results: The optimal classification algorithm comprised 15 genomic markers, and involved loss of *VHL*, 3p21 and 8p, and chromosomes 1, 2, 6, 10 and 17, and gain of 5qter, 16p, 17q and 20q, and chromosomes 3, 7 and 12. On histological rereview (leading to the exclusion of 3 specimens) and using histology as the gold standard, 58 of 62 (93%) clear cell, 51 of 56 (91%) papillary and 33 of 34 (97%) chromophobe renal cell carcinomas were classified correctly. Of the 36 oncocytoma specimens 33 were classified as oncocytoma (17 by array comparative genomic hybridization and 10 by array comparative genomic hybridization plus fluorescence in situ hybridization) or benign (6). Overall 93% diagnostic sensitivity and 97% specificity were achieved.

Abbreviations and Acronyms

aCGH = array comparative genomic hybridization
ccRCC = clear cell renal cell carcinoma
chrRCC = chromophobe renal cell carcinoma
CNA = copy number alteration
FFPE = formalin fixed, paraffin embedded
FISH = fluorescence in situ hybridization
OC = oncocytoma
pRCC = papillary renal cell carcinoma
QPCR = quantitative polymerase chain reaction
RCC = renal cell carcinoma
TCGA = The Cancer Genome Atlas

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For another article on a related topic see page 1660.

Editor's Note: This article is the first of 5 published in this issue for which category 1 CME credits can be earned. Instructions for obtaining credits are given with the questions on pages 1732 and 1733.

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Conclusions: In a clinical diagnostic setting the implementation of genome based molecular classification could serve as an ancillary assay to assist in the histological classification of common renal neoplasms.

Key Words: carcinoma, renal cell; comparative genomic hybridization; DNA copy number variations; classification; oncocytoma, renal

ACCURATE diagnostic discrimination of major renal cortical neoplasm subtypes is not only useful to guide appropriate treatment strategies but also to estimate prognosis. While histology and associated immunohistochemical assays of these neoplasms serve as the gold standard, in the era of precision medicine, classification based on molecular biomarkers is appealing, well suited for use with specimens with limited tissue availability and may reveal novel molecular targets for therapy.[1–3] Common renal cortical neoplasms include malignant renal cell carcinoma (clear cell, papillary and chromophobe) and benign subtypes such as oncocytoma. A clear discrimination between benign OC and the malignant eosinophilic variant of chrRCC is often difficult to achieve solely based on histology due to overlapping morphological features.[4,5]

Another histological challenge involves the up to 6% of cases that do not fit into an established diagnostic category, the so-called unclassified RCC.[6,7] The introduction of an ancillary assay to assist histology in achieving the correct renal tumor classification could impact the overall management and outcome of patients with RCC. Renal neoplasms are characterized by genomic CNAs and chromosomal rearrangements (for instance, *CCND1* rearrangement in OC), which could be used for diagnostic and prognostic purposes, as suggested by several studies.[8–16] Therefore, in this study we develop and validate a robust algorithm for the molecular classification of common renal cortical neoplasms based on genomic imbalance (copy number gain and loss) that could be implemented in a clinical diagnostic setting as an adjunct assay to assist in accurate diagnosis.

MATERIALS AND METHODS

FFPE Specimens

A total of 191 surgically resected renal cortical neoplasm specimens obtained from 191 patients as part of their routine care at the Cleveland Clinic were used in this institutional review board approved study (supplementary table 1, http://jurology.com/). The specimens were selected based on the presence of at least 80% tumor burden (as evaluated by hematoxylin and eosin staining, 1 section per cm tumor diameter) and pathological diagnosis according to WHO classification (as per pathology report), as having approximately equal numbers of ccRCC, pRCC, chrRCC and OC.[7] The specimens ranged from 12 to 150 mm with a median of 42 (median ccRCC 48 mm, median pRCC 34 mm, median chrRCC 54 mm, median OC 35.5 mm).

Targeted and Whole Genome aCGH

Specimen DNA was extracted and when greater than 800 bp in size was subjected to heat fragmentation at 95C until the bulk of the DNA fragments reached 400 to 800 bp. Using similarly fragmented sex-matched normal male/female gDNA (Promega, Madison, Wisconsin), aCGH was performed and analyzed as described in the supplementary material (http://jurology.com/).

FISH and QPCR

FISH was performed on 4 micron sections to assess *CCND1* rearrangement and gain of 17q. TaqMan® based QPCR copy number assays were performed on extracted DNA to assess gain of 17q as described in the supplementary material (http://jurology.com/).

RESULTS

Development of a RCC Classification Algorithm

To construct a robust algorithm by which common renal neoplasms (malignant and benign) could be subtyped based on genomic imbalance, we first identified CNAs diagnostic of each of the 3 malignant subtypes that in a hierarchical manner could be optimized for classification using the copy number profiles for 489 ccRCC, 75 pRCC and 65 chrRCC available in TCGA (http://cancergenome.nih.gov).[17] Since ccRCC is the most predominant RCC subtype and is characterized by loss of *VHL,*[18] all 629 TCGA specimens were initially categorized into the 2 major groups of 450 (71%) with *VHL* loss (3p25 locus, 10.1-10.2 Mb) and 179 without. In the latter group 68 specimens were positive for 5qter gain (169-181 Mb), a CNA also predominantly observed in ccRCC. There were 34 ccRCCs among the 111 samples without *VHL* loss or 5qter gain. Of the 518 cases positive for *VHL* loss or 5qter gain 455 were ccRCC, 15 were pRCC (9 with *VHL* loss) and 48 were chrRCC (15 with *VHL* loss). Thus, while the sensitivity of ccRCC classification was 92% based on these 2 aberrations, the specificity was poor at 55%. Clearly the consideration of other biomarkers was required to optimize the algorithm.

It has been well documented that pRCC exhibits gain, in particular of chromosomes 3, 7, 12, 16, 17 (mostly 17q) and 20, and chrRCC exhibits loss, often involving chromosomes 2, 6, 10 and 17.[16,18–22] Thus, the gain/loss status of chromosome 17 was

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