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Original contribution

Sporadic oncocytic tumors with features intermediate between oncocytoma and chromophobe renal cell carcinoma: comprehensive clinicopathological and genomic profiling☆,☆☆,☆☆☆

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Summary Morphology, clinical behavior, and genomic profiles of renal oncocytoma (RO) and its malignant counterpart chromophobe renal cell carcinoma (ChRCC) are distinctly different. However, there is a substantial group of sporadic oncocytic tumors with peculiar hybrid phenotypes as well as a perplexing degree of morphologic and immunohistochemical overlap between classic RO and ChRCC with eosinophilic cytoplasm. The aim of this study is to provide detailed characterization of these hybrid tumors.Thirty-eight sporadic oncocytic neoplasms with ambiguous morphology from two institutions were reviewed by 4 pathologists. CKIT positivity was used as a selection criterion. We correlated CK7 and S100A1 immunostaining and detailed morphologic features with cytogenetic profiles. DNA from the formalin-fixed paraffin-embedded tissues was extracted and analyzed using cytogenomic microarray analysis (CMA) to evaluate copy number alterations (CNA) and ploidy. CMA categorized cases into 3 groups: RO (N = 21), RO variant (N = 7), and ChRCC (N = 10). Cytogenetic RO had either no CNA (48%) or loss of chromosome 1p, X, or Y (52%). RO variant had additional chromosomal losses [-9q, −14 (n = 2), −13] and chromosomal gains

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[+1q (n = 2), +4, +7 (n = 2), +13, +19, +20, and +22]. ChRCCs were either hypodiploid with numerous monosomies (40%) or hypotetraploid with multiple relative losses (60%). RO, RO variant, and ChRCC groups differed significantly in tumor architecture ($p < 0.01$), stroma ($p = 0.013$), presence of nuclear wrinkling, perinuclear halos, and well-defined cell borders in >5% of cells ($p < 0.01$), focal cell clearing ($p = 0.048$) and CK7 expression ($p < 0.02$). Pathologic prediction of the cytogenetic subtype using only two categories (benign RO or malignant ChRCC) would overcall or undercall up to 40% of tumors that were ChRCC based on cytogenetics. This finding provides the rationale for an intermediate diagnostic category of the so-called hybrid tumors (hybrid oncocytic/chromophobe tumor [HOCT]). HOCT was a heterogeneous group enriched for cytogenetic RO variant. Other HOCTs have a profile of either RO or ChRCC. The genomic profile allows classification of oncocytic tumors with ambiguous morphology into RO, RO variant, and ChRCC. Several architectural and cytologic features combined with CK7 expression are significantly associated with cytogenetic RO, RO variant, or ChRCC tumors. Doubled hypodiploidy by whole-genome endoduplication is a common phenomenon in eosinophilic ChRCC.

1. Introduction

Distinguishing renal oncocytoma (RO) from eosinophilic chromophobe renal cell carcinoma (ChRCC) is often difficult even among expert urologic pathologists, especially in needle biopsies [1–4]. Yet the distinction is important because RO is benign and ChRCC can not only be locally aggressive but also metastasize. Sporadic hybrid oncocytic/chromophobe tumors (HOCTs) represent a poorly understood controversial entity with gross, architectural, and cellular features that overlap with both RO and ChRCC that have prominent eosinophilic features [3–6]. Although selected immunostains help address this differential diagnosis, there remain cases incompletely resolved by immunohistochemistry. ChRCC is characterized by multiple chromosomal losses (most frequently chromosomes Y, 1, 2, 6, 10, 13, 17, and 21) [7]. Conversely, RO usually has a normal number of chromosomes, occasionally exhibiting genetic abnormalities that include loss of whole chromosome 1 or part of its short or long arm and partial or complete loss of chromosomes 14, 21, and Y [7–11]. We hypothesize that molecular characterization by cytogenomic microarray analysis (CMA) of problematic oncocytic tumors with a noniconic morphology and perplexing immunohistochemical profile that overlap with RO and/or ChRCC can provide a basis for categorizing the majority of these oncocytic tumors.

2. Materials and methods

2.1. Case selection

This retrospective study used cases retrieved from the pathology archives of the University of Washington and the University of Chicago Medical Centers, with approval from Institutional Review Boards. We interrogated the pathology databases of our two institutions, searching for the following categories: “Oncocytic tumor, NOS” or “Oncocytic cell neoplasm” with varying modifiers including “low-grade,” “borderline features,” “unclassified,” “low malignant potential,” “with hybrid features” as well as “Oncocytoma with atypical features,” and “Hybrid oncocytic and chromophobe tumor.” Additional selection criteria included CKIT expression by tumor cells and availability of material for further immunohistochemical and molecular studies. We identified 35 cases of sporadic, unifocal, oncocytic neoplasms that met all selection criteria and had an ambiguous morphology, at least focally, preventing making a definitive diagnosis of either classic RO or ChRCC. Three cases of ChRCC with prominent eosinophilia, suggestive of hybrid morphology, were added from the Cancer Genome Atlas (TCGA) cohort [12]. We excluded cases with Birt-Hogg-Dube (BHD) syndrome, succinate dehydrogenase–deficient syndrome, renal oncocytosis, and angiomyolipomas and cases with a history of tuberous sclerosis complex. None of the selected cases had morphologic features and immunohistochemical profiles of such recently described oncocytic tumor entities as eosinophilic, solid, and cystic renal cell carcinoma, high-grade oncocytic tumor, or low-grade oncocytic tumor [13,14].

Cases were reviewed by 4 pathologists (M.S.T., T.A., C.U., and L.T.) to document architectural patterns (nested/organoid, tubulocystic, solid/confluent), quality of stroma (scant, edematous, fibromyxoid, calcified or with osseous metaplasia), the presence of hemorrhage, necrosis, fat or vascular invasion, frequent binucleation/multinucleation, and cellular pleomorphism. Among atypical features worrisome for malignancy, we recorded cell clearing when present in >5% of tumor cells, raisinoid nuclei, perinuclear halos, *vegetable-like* cell membranes, and >1 mitoses [2,6,7,15]. For comparison purposes, we recorded frequency of various morphologic parameters including tumor architecture, stromal component, cytologic appearance, and atypical features, which were previously shown to be useful in distinguishing RO, HOCT, and ChRCC [2,4,6,16]. Pathologic review was blinded to cytogenetic data.

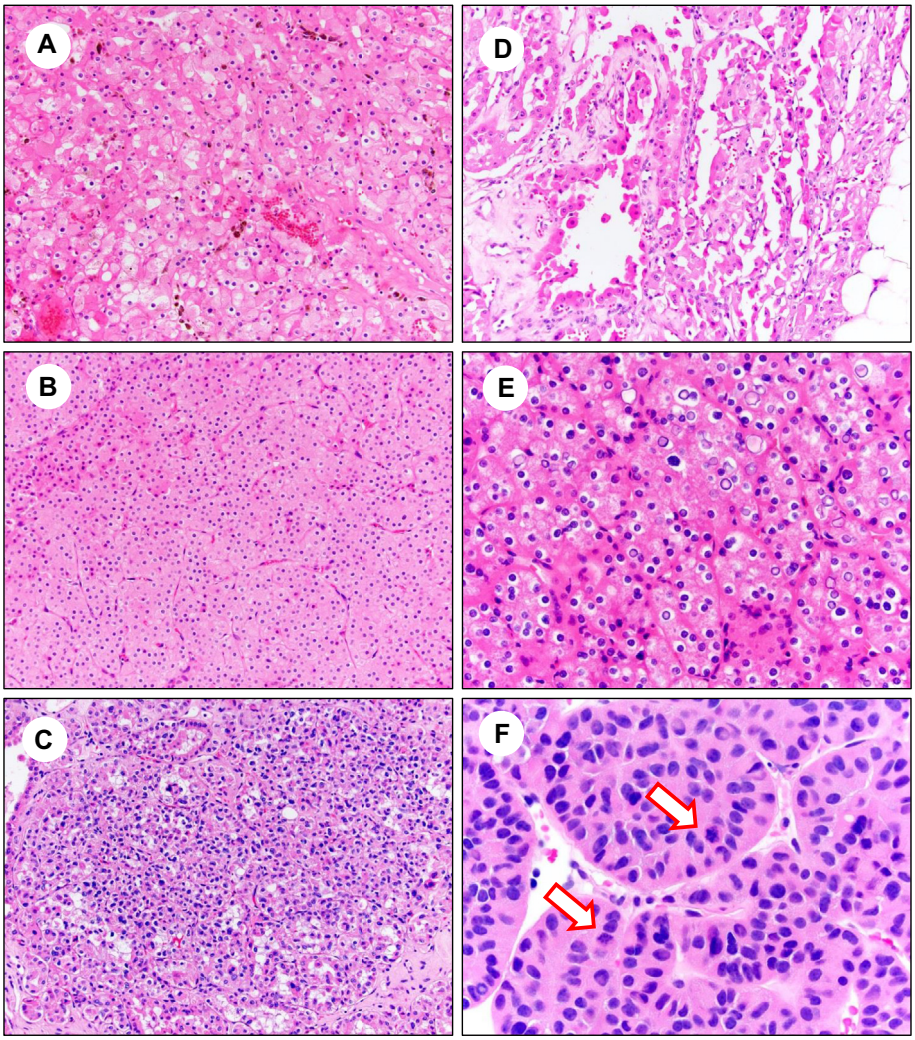

Fig. 1 Representative examples of morphologic features of oncocytic renal cell tumors worrisome for malignancy. A, Focal cell clearing and perinuclear halos in case #1 (×20). B, Solid/confluent growth pattern and well-defined cell borders in case #4 (×20). C, Nuclear wrinkling (irregular nuclear membranes), pleomorphism, and multinucleation in case #29 (×20). D, Tubulocystic architecture and fat invasion in case #12 (×20). E, Diffuse perinuclear clearing and frequent binucleation in case #22 (×40). F, Increased mitotic activity (arrows) in case #35 (×60).

2.2. Immunohistochemistry

The unstained sections were deparaffinized by two xylene rinses, followed by two rinses with 100% ethanol. Immunostaining was performed in a Clinical Laboratory Improvements Amendments (CLIA) (regulates all laboratory testing in U.S.)-certified diagnostic immunohistochemistry laboratory as per standardized protocol. In brief, antigen retrieval was performed using an automated immunostainer (BondTM, Leica, Germany) using H2 buffer (pH 8.0) for 20 min. After rinsing and endogenous peroxidase blocking, the slides were incubated for 25 min with mouse antibodies against CKIT (1:250, CD117, polyclonal, cat A4502, Dako/Agilent, Santa Clara, CA, USA), CK7 (1:200, clone OV-TL, cat M7018, DAKO/Agilent), and S100A1 (1:25, cat NCL-CD10-270, clone 56C6, Novocastra/Leica Biosystems, Buffalo Grove, IL, USA). The slides were then rinsed multiple times and incubated for 30 min with antimouse polymer detection reagent (Refine Kit, Leica, Germany). This was followed by multiple rinses, incubation with the diaminobenzidine chromogen, and hematoxylin counterstaining. In negative controls, the primary antibody was replaced with nonimmune mouse serum. The immunostaining result was interpreted as previously described in the study addressing optimization of immunohistochemical profiles in the differential diagnosis of RO from ChRCC [17]. The neoplasm was considered positive when around >50% of tumor cells stained diffusely and was considered negative when only single cells and small cell clusters stained.

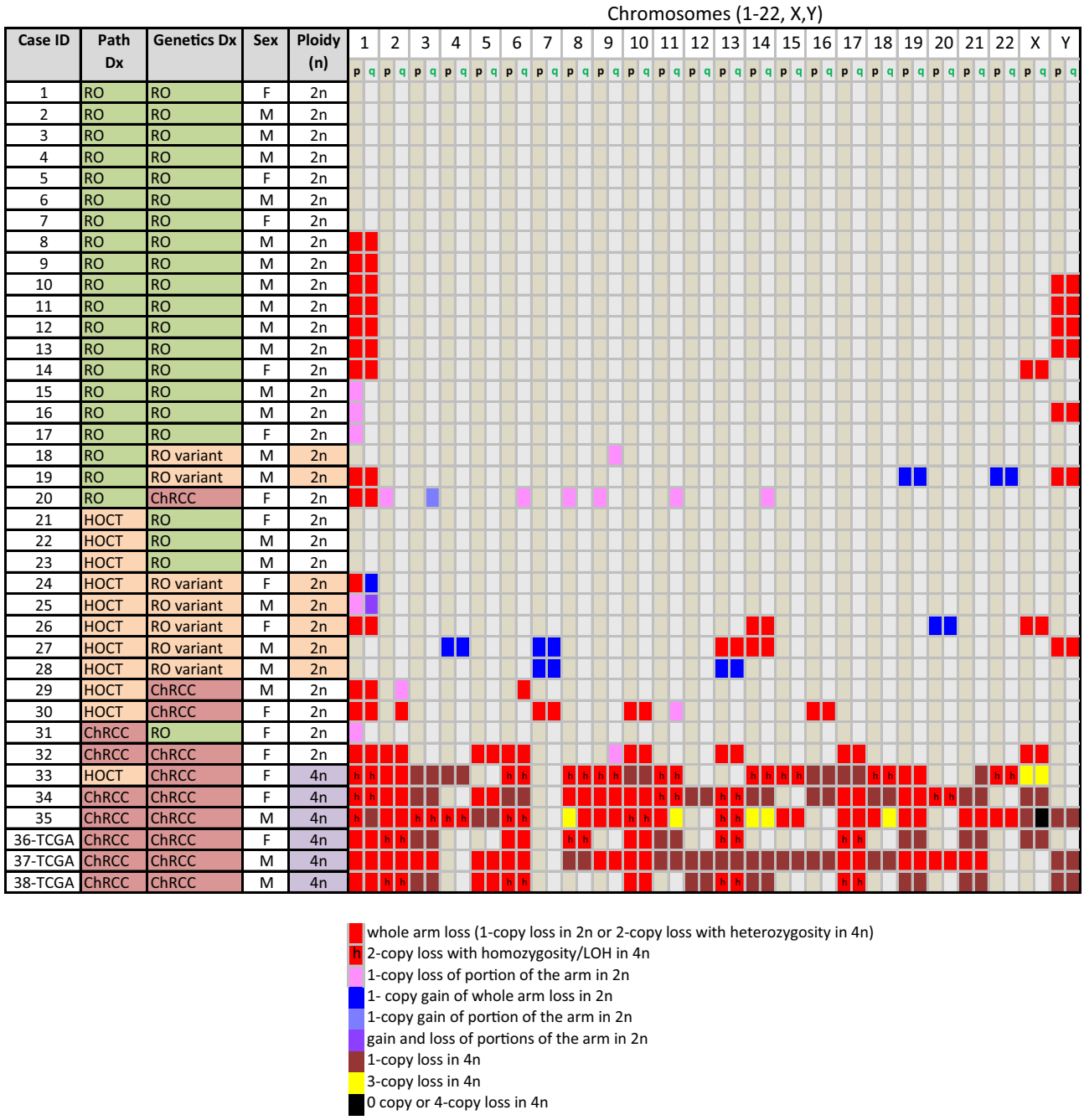

Fig. 2 Cytogenetic results (actual) classifying cases as RO, RO variant, or ChRCC compared with favored pathology diagnosis (predicted). RO, HOCT, and ChRCC shows match in 28 cases (74%) and mismatch or reclassification in 10 cases (26%). RO, renal oncocytoma; HOCT, hybrid oncocytic/chromophobe tumor; ChRCC, chromophobe renal cell carcinoma; TCGA, The Cancer Genome Atlas; LOH, loss of heterozygosity.

2.3. Cytogenomic microarray analysis

All 35 specimens used in this study were archived formalin-fixed paraffin-embedded (FFPE) tissue. The methods for DNA isolation from FFPE specimens, genomic microarray analysis using Agilent Technologies, Santa Clara, CA, USA, SurePrint G3 Cancer CGH+SNP 4x180K Array (http://www.chem-agilent.com/pdf/5990-9183en_lo_CGH+SNP_Cancer.pdf), and copy number evaluation have been described in the study by Liu et al [18].

Six cases with complex copy number aberrations (Fig. 2, cases 1, 2, 3, 4, 5, 34) were also analyzed using the Illumina, Inc, San Diego, CA, USA, Infinium CytoSNP-850K BeadChip to confirm the ploidy status. Genomic DNA extracted from the FFPE specimen was end repaired, amplified, and hybridized using Illumina, Inc, San Diego,

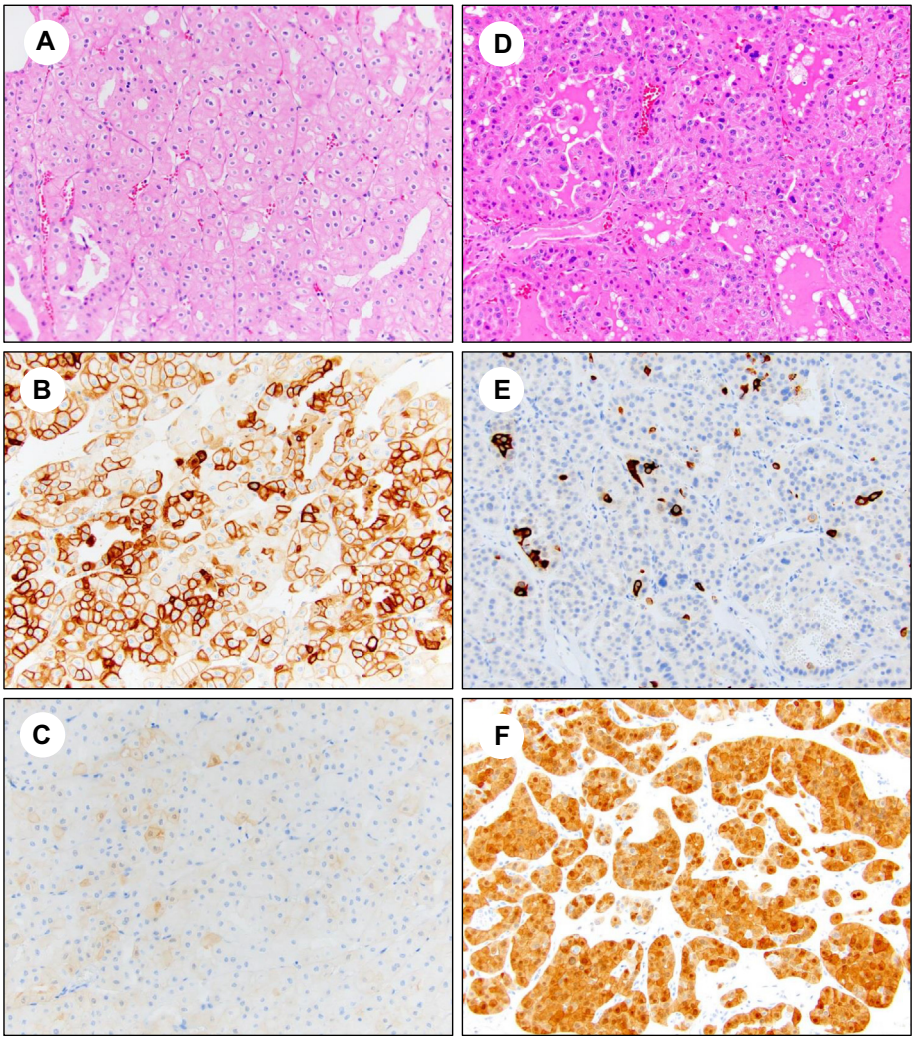

Fig. 3 Two cases with major discrepancy between cytogenetic (actual) and pathologic (predicted) diagnoses. Case #7 with cytogenetic RO profile (loss of 1p), but morphologically (A) most consistent with the eosinophilic variant of ChRCC with diffuse CK7 expression (B) and negative for S100A1 (C). Case #34 with cytogenetic ChRCC profile (loss of 1p, 2p, 6q, 8p, 9p, 11q, 14q), but morphologically (D) most consistent with RO with scattered CK7 positivity (E) and diffuse nuclear and cytoplasmic S100A1 (F); magnification ×20. RO, renal oncocytoma; ChRCC, chromophobe renal cell carcinoma.

CA, USA, Infinium CytoSNP-850K BeadChip version 1.1 (www.illumina.com/content/dam/illumina-marketing/documents/products/datasheets/datasheet_CytoSNP850K_POP.pdf). The microarray was washed, labeled, stained, and scanned using an Illumina iScan as specified by the manufacturer (Illumina Inc, San Diego, CA, USA). Allele and intensity ratio data of the fluorescent signals were generated. Microarray data were analyzed and visualized using Nexus Copy Number 10.1 (BioDiscovery Inc., Hawthorne, CA, USA) to identify chromosomal copy number variants and regions of copy number neutral loss of heterozygosity (LOH). Genome build GRCh37 was used.

2.4. Statistical analysis

Demographic and clinical characteristics were summarized for each subtype of oncocytic tumor and compared across subtypes using the Kruskal-Wallis test for continuous variables (age and tumor size) and the chi-square or Fisher's exact test for categorical variables (stage and morphologic and immunohistochemical features). Median follow-up time was reported for patients who were alive at the end of follow-up. The results were considered statistically significant only if $p \leq 0.05$.

3. Results

3.1. Clinicopathologic and immunohistochemical findings

The mean patient age was 59 years, with slight predominance of men over women (1.6:1 ratio). All tumors were unifocal, with a mean tumor size of 4.8 cm (range =

1.8−20.6 cm). Twenty-five tumors were treated by partial nephrectomy (65.8%), and twelve were treated by radical nephrectomy (31.6%). One patient (2.6%) was not surgically treated. Pathologic stage at surgery was distributed as follows: pT1a (65.8%), pT1b (15.8%), pT2a (5.3%), pT2b (5.3%), and pT3a (7.8%). The median follow-up was 56 months (range = 2−137). None of the patients had metastases or died of disease. However, tumor recurred in 2 patients, and 3 patients died from other causes.

Morphologically, all tumors were composed of round to polygonal tumor cells with densely granular bright eosinophilic cytoplasm and round to oval nuclei with largely inconspicuous nucleoli. Architecturally, 32 of 38 (84%) tumors had a nested/*archipelagenous* or organoid architecture, 21 of 38 (55%) had tubulocystic areas, and 21 of 38 (55%) had a solid/confluent architecture; the combination of 2 architectural patterns was noted in 20 of 38 (53%) tumors, whereas all three architectures were present in 8 of 38 (21%) neoplasms. Edematous paucicellular stroma was present in 19 of 38 (50%), fibromyxoid was present in 23 of 38 (61%), and calcifications and/or osseous metaplasia was observed in 5 of 38 (13%) tumors. Hemorrhage; prominent, pleomorphic nuclei; and multinucleation were seen in 42%, 24%, and 32% of cases, respectively. Of cytologic features present in >5% of tumor cells, we saw (1) nuclear wrinkling in 11 of 38 (29%), (2) perinuclear halos in 15 of 38 (40%), (3) well-defined cell borders in 12 of 38 (32%), (4) cell clearing in 14 of 38 (37%), and (5) increased mitotic activity in 3 of 38 tumors (8%) (Fig. 1). Fat invasion was present in 4 of 38 (11%) cases, whereas vascular invasion was present in 1 of 38 (3%) cases. No tumors in our cohort had areas of necrosis or apoptotic debris.

CD117 (CKIT) immunoreactivity was invariably positive in the majority of tumor cells. Diffuse positivity of CK7 (>50% cells) was documented in 11 of 38 (29%) cases. S100A1 cytoplasmic and nuclear expression was present in the majority of tumors cells, that is, 23 of 32 (72%) cases.

3.2. Prediction of benign versus borderline vs malignant diagnoses

The diagnostic criteria favoring benign RO included one atypical morphologic feature plus a negative CK7/positive S100A1 immunoprofile (N = 20). The eosinophilic variant of ChRCC was favored in cases with >3 atypical features and cases with a positive CK7/negative S100A1 immunoprofile (N = 7). All other cases with borderline/intermediate features were considered HOCTs (N = 11).

3.3. Cytogenetic findings

The criteria of genetic profiles for RO included normal CMA or chromosomal loss −1/1p, −14 (less frequent), -X, and/or -Y [7,10]. RO variant genetic profiles included a few additional copy number aberrations (mostly gains) in addition to a chromosomal loss −1/1p-, −14, -X, and/or -Y in RO. The World Health Organization criteria of genetic profiles for ChRCC was hypodiploidy, including a combination of numerous chromosomal losses of one copy of the entire chromosomes −1, −2, −6, −10, −13, −17, −21, and -Y [7].

Table 1 Karyotypes of six ChRCC cases with hypotetraploidy (doubled hypodiploidy).

Case ID	Karyotypes based on CMA results
1	<4n>61,X,-X,-X,-X,(-1^{h},-2,-6^{h},-8^{h},-9^{h},-11^{h},-14^{h},-15^{h},-18^{h},-19,-22^{h})x2,-3,-4,-10,-16,-17,-21
2	<4n>62,XXX,-X,(-1^{h},-2,-5,-8,-9,-10,-11^{h},-13^{h},-17,-19,-20^{h})x2,-3,-6,-12,-14,-16,-18,-21
3	<4n>55,del(X) (q13.2)Y,-X,-Y,(-1p^{h},-2^{hp},-3^{h},-4^{h},-6^{h},-8^{hp},-9,-10^{h},-11^{hp},-13^{h},-14^{h},-15,-17,-18^{hp},-19,-21,-22)x2,-1q,-5,-8p,-11q^{p},-14,-18q^{p}
36	<4n>73,XXX,-X,(-1,-2^{h},-6,-8^{h},-10,-13^{h},-17^{h})x2,-3,-11,-19,-21
37	<4n>61,XXY,-Y,(-1,-2,-3,-5,-6,-9,-10,-17,-19,-20,-21)x2,-8,-11,-12,-13,-14,-15,-16,-18
38	<4n>72,XXY,-Y,(-1,-2^{h},-5,-6^{h},-10,-13^{h},-17^{h})x2,-3,-12,-14,-19,-21

Abbreviations: ChRCC, chromophobe renal cell carcinoma; CMA, cytogenomic microarray analysis; ^{h}, homozygosity or loss of heterozygosity; ^{hp}, homozygosity for portion of the chromosomal arm; ^{p}, portion of the chromosomal arm (see Supplemental Table 1 for details).

3.3.1. RO by CMA

Twenty-one cases (55%) had genetic profiles characteristic of RO. These included ten cases with normal CMA and seven cases with loss of chromosome 1, which included an additional loss of the Y chromosome in four cases and of an X chromosome in one case. And there were four cases with loss of portions of the short arm (p) of chromosome 1 (1p-), which included one case that also had loss of the Y chromosome (Fig. 2).

3.3.2. RO variant by CMA

Seven tumors (18.5%) that had aberrations common in ROs, such as chromosomal loss −1/1p-, −14, -X, and −Y, also had one to three additional copy number aberrations, which were mostly gains. We interpreted these as RO variants. These aberrations included two cases with gain of 1q; two cases with gain of 7; and one case each with gain of chromosomes 13, 19, 20 and 22, sole deletion of portion of 9q31.3qter, and gain of chromosome 4 in addition to loss of chromosome 13 (Fig. 2).

3.3.3. Chromophobe RCC by CMA

Ten cases (26.5%) had multiple chromosomal abnormalities including four cases with hypodiploidy, which is characterized as losses of portions or entire chromosomes such as 1, 2, 6, 10, 11, 16, and 17. These changes are

Table 2 Demographic and clinicopathological features of cytogenetically classified oncocytic tumors: classic renal oncocytoma (RO), RO variant, and chromophobe renal cell carcinomas (ChRCCs).

Features	Details	RO	RO variant	ChRCC	*P*-value
Cases	N = 38	N = 21	N = 7	N = 10	
Patient age (mean)	59.1 years	63.2	58.3	50.8	0.09
M:F ratio	1.6:1	1.5:1	2.5:1	0.7:1	0.21
Surgery	Partial nephrectomy	18/21 (86%)	5/7 (71%)	7/10 (70%)	0.52
	Radical nephrectomy	3/21 (14%)	2/7 (29%)	2/10 (20%)	
	Biopsy	–	–	1/10 (10%)	
Tumor size (mean)	4.8 cm	4.3 cm	3.5 cm	6.6 cm	0.21
Stage	pT1a	–	–	4/9 (44%)	–
	pT1b	–	–	2/9 (22%)	
	pT2a	–	–	–	
	pT2b	–	–	2/9 (22%)	
	pT3a	–	–	1/9 (11%)	
Follow-up (median)	56 months	59 months	24 months	67 months	0.14
Outcomes	No evidence of disease	13/21 (62%)	4/7 (57%)	8/10 (80%)	0.53
	Recurrence	1/21 (5%)	–	1/10 (10%)	
	Died of other cause	2/21 (9%)	1/7 (14%)	–	
	Lost to follow-up	5/21 (24%)	2/7 (29%)	1/10 (10%)	
Architecture	Nested/archipelagenous	21/21 (100%)	6/7 (86%)	5/10 (50%)	**< 0.01**
	Tubulocystic	16/21 (76%)	1/7 (14%)	4/10 (40%)	**< 0.01**
	Solid/diffuse growth	7/21 (33%)	5/7 (71%)	9/10 (90%)	**< 0.01**
Stroma	Fibromyxoid	17/21 (81%)	3/7 (43%)	3/10 (30%)	**0.014**
	Edematous	15/21 (71%)	2/7 (29%)	2/10 (20%)	**0.013**
	Calcified/osseous metaplasia	4/21 (19%)	1/7 (14%)	–	
	Hemorrhage	10/21 (48%)	2/7 (29%)	4/10 (40%)	0.67
Cytology	Pleomorphism	7/21 (33%)	1/7 (14%)	1/10 (10%)	0.29
	Multinucleation	7/21 (33%)	2/7 (29%)	3/10 (30%)	0.96
	Nuclear wrinkling [(A)]	1/21 (5%)	4/7 (57%)	6/10 (60%)	**< 0.01**
	Perinuclear halos [(A)]	3/21 (14%)	4/7 (57%)	8/10 (80%)	**< 0.01**
	Well-defined cell borders [(A)]	1/21 (5%)	3/7 (43%)	8/10 (80%)	**< 0.001**
	Cell clearing [(A)]	5/21 (24%)	3/7 (43%)	7/10 (70%)	**0.048**
Aggressive features	>1 mitoses [(A)]	1/21 (5%)	–	2/10 (20%)	0.18
	Apoptosis/necrosis [(A)]	–	–	–	–
	Fat invasion	3/21 (14%)	–	1/10 (10%)	0.74
	Vascular invasion	1/21 (5%)	–	–	–
Immunostaining	CK7 (>50% cells)	3/21 (14%)	2/7 (29%)	6/9 (67%)	**0.016**
	S100A1 (>50% cells)	15/19 (80%)	4/6 (67%)	4/7 (57%)	0.52

Note. – means feature not present/not applicable; [(A)] means atypical features.
Bold font is used to highlight discriminating morphologic features of statistical significance.

genetically consistent with ChRCC. In addition, six cases, including three TCGA cases with eosinophilic ChRCC phenotype, had multiple chromosomal losses due to hypotetraploidy (doubled hypodiploidy). The karyotypes inferred from the CMA results are summarized in Table 1 and Fig. 2. The status of chromosomes with 2 copy numbers and LOH indicated that one copy of these chromosomes was most likely lost initially in the diploid state (2n) before whole-genome endoduplication, whereas for chromosomes with 2 or 3 copy numbers without LOH, chromosome loss took place in the tetraploid state (4n) after the whole-genome endopduplication event. The most frequently affected chromosomes with two-copy loss in these cases with hypotetraploidy (doubled hypodiploidy) included chromosomes 1 (100%), 2 (100%), 5 (50%), 6 (83%), 8 (67%), 9 (67%), 10 (83%), 11 (50%), 13 (67%), 17 (83%), 19 (67%), X (100% in women), and Y (100% in men). These patterns of chromosomal losses are consistent with ChRCC genetic profiles. The chromosomes with three (one-copy loss in 4n) or four copies (no loss in 4n) in these six hypotetraploid ChRCC cases were most frequently chromosomes 3 (67%), 4 (83%), 7 (100%), 12 (100%), 14 (67%), 15 (67%), 16 (100%), 18 (67%), 20 (67%), 21 (67%), and 22 (67%) (Table 1, Supplemental Table 1).

3.4. Clinicopathological features of cytogenetically classified oncocytic tumors

There were no statistically significant differences between the three subtypes (RO, RO variant, and ChRCC) in

age, gender, type of surgery, tumor size, stage, or outcomes (Table 2). Comparison of architectural patterns showed higher frequency of nested/organoid or the so-called *archipelagenous* architecture in RO and RO variant than in ChRCC ($p < 0.01$), whereas solid/confluent architecture was more common in ChRCC and RO variant, but not in RO ($p < 0.01$). Tubulocystic morphology was more frequent in RO and ChRCC and less common in RO variant ($p < 0.01$). Fibromyxoid and edematous stroma was characteristic for the majority of RO cases and much less common in RO variant and ChRCC ($p = 0.014$ and $p = 0.013$, respectively). Presence of hemorrhage, stromal calcification, or osseous metaplasia was not discriminatory between three tumor types. From cytologic features, nuclear wrinkling, perinuclear halos, and well-defined borders in >5% of cells were typical for the majority of ChRCC cases and common in RO variant tumors, but not in RO ($p < 0.01$). Focal cell clearing was present in 70% of ChRCC cases, 43% of RO variant tumors, and 24% of RO cases, marginally reaching statistical significance ($p = 0.048$). Presence of cellular pleomorphism and binucleation/multinucleation was not discriminatory between tumor types ($p = 0.29$ and $p = 0.96$, respectively). Among atypical features, very few cases showed increased mitotic activity, fat invasion, or vascular invasion, and no cases had tumor necrosis/apoptosis, thus not showing statistically significant difference between the study groups.

Expression of CK7 in more than 50% of tumor cells occurred in 9% of RO cases, 43% of RO variant cases, and 67% of ChRCC cases, yielding statistically significant difference ($p = 0.016$). Diffuse nuclear and cytoplasmic S100A1 positivity was more frequent in the RO group and RO variant group (80% and 67%, respectively) than in ChRCC cases (57%), but not significantly ($p = 0.52$).

Table 3 Distribution of actual and predicted by pathology diagnoses.

Tumor subtype		Actual (cytogenetic)			Total
		Positive		Negative	
		ChRCC	RO variant	RO	
Predicted by pathology	ChRCC	6	0	1	7
	HOCT	3	5	3	11
	RO	1	2	17	20
Total		10	7	21	38

Note. Sensitivity, specificity, and predictive values of morphologic assessment in discriminating oncocytic tumors into benign, borderline, and malignant categories.
Abbreviations: ChRCC, chromophobe renal cell carcinoma; HOCT, hybrid oncocytic/chromophobe tumor; RO, renal oncocytoma.

3.5. Correlation between immunomorphologic and cytogenetic diagnoses

From seven immunomorphologically predicted ChRCC cases ($n = 7$), six were confirmed cytogenetically (86% match), whereas one case had RO cytogenetics (Fig. 3, case 34). From twenty predicted RO cases ($n = 20$), seventeen were cytogenetically RO (85% match), whereas two were RO variants with 9q loss and gain of 19 and 22 (Fig. 2, cases 11 and 28), and one tumor had ChRCC cytogenetics (Fig. 3, case 7). And finally, in morphologically borderline tumors (HOCT, $n = 11$), three cases (27%) were classified as ChRCC, five were classified as RO variants (46%), and three were classified as RO (27%) by cytogenetic profiling (Fig. 2, Tables 3, 4 and 5).

We performed detailed analysis of diagnostic test characteristics of immunomorphologic assessment by compiling 2×2 tables with actual (cytogenetic) and predicted (pathologic) results. If RO considered the only true benign cytogenetic diagnosis (ChRCC plus RO variant lumped as nonbenign), pathologic prediction had a sensitivity of 82%, a specificity of 81%, a positive predictive value (PPV) of 78%, a negative predictive value (NPV) of

Table 4 Distribution of actual and predicted by pathology diagnoses in which only RO is considered benign diagnosis (ChRCC and RO variant lumped as nonbenign).

Diagnostic test characteristics[a]		Actual (Cytogenetic)			Total
		Positive		Negative	
		ChRCC	RO-variant	RO	
Predicted by pathology	ChRCC	TP (14)		FP (4)	18
	HOCT				
	RO	FN (3)		TN (17)	20
Total		17		21	38

Note. Sensitivity, specificity, and predictive values of morphologic assessment in discriminating oncocytic tumors into benign, borderline, and malignant categories.
Abbreviations: ChRCC, chromophobe renal cell carcinoma; HOCT, hybrid oncocytic/chromophobe tumor; RO, renal oncocytoma; PPV, positive predictive value; NPV, negative predictive value; FN, false negative; FP, false positive; TP, true positive; TN, true negative.
[a] Sensitivity 82%, Specificity 81%, PPV 78%, NPV 85%, Accuracy 82%

Table 5 Distribution of actual and predicted by pathology diagnoses in which only ChRCC is considered malignant diagnosis (RO and RO variant lumped as benign).

Diagnostic test characteristics[a]		Actual (Cytogenetic)			Total
		Positive	Negative		
		ChRCC	RO-variant	RO	
Predicted by pathology	ChRCC	TP (6)	FP (1)		7
	HOCT	FN (4)	TN (27)		31
	RO				
Total		10	28		38

Note. Sensitivity, specificity, and predictive values of morphologic assessment in discriminating oncocytic tumors into benign, borderline, and malignant categories.

Abbreviations: ChRCC, chromophobe renal cell carcinoma; HOCT, hybrid oncocytic/chromophobe tumor; RO, renal oncocytoma; NPV, negative predictive value; PPV, positive predictive value.

[a] Sensitivity 60%, Specificity 96%, PPV 86%, NPV 87%, Accuracy 87%

85%, and an accuracy of 85%. If ChRCC considered the only true malignant cytogenetic diagnosis (RO plus RO variant lumped as benign), pathologic prediction showed much a lower sensitivity of 60% combined with a much higher specificity of 96% and improved PPV (86%), NPV (87%), and accuracy (87%). Using only two immunomorphologic diagnostic categories (RO and ChRCC) will result in either overcalling or undercalling actual malignant tumors in up to 40% cases. On the other hand, tumors with intermediate/borderline features could be reliably classified by cytogenetic profiling into ChRCC, RO, or RO variant subtypes.

4. Discussion

Distinguishing ROs from eosinophilic ChRCC using only histology and immunostaining is challenging. Herein, we studied 38 oncocytic tumors with features intermediate between classic RO and the eosinophilic variant of ChRCC. Genomic profiling allowed classification of all cases into RO, RO variant, or ChRCC categories. Cytogenetic RO consisted of two categories: 48% with normal numeric chromosomal status and 52% with loss of chromosome(s) 1p, X, or Y. The cytogenetic RO variant group had additional chromosomal losses (-9q, −14 [2 cases], −13) and chromosomal gains (+1q [2 cases], +4, +7 [2 cases], +13, +19, +20, and +22). Cytogenetic ChRCC could be divided into two distinct subtypes: 40% had hypodiploidy due to numerous losses of portions or entire chromosomes 1, 2, 6, 10, 11, 14, 16, and 17 and 60% had multiple relative chromosomal losses due to hypotetraploidy.

Cytogenetic RO, RO variant, and ChRCC cases had similar clinical features, with no statistically significant differences in patient demographics, tumor size, stage, or outcomes, although the number of events and length of follow-up were modest. Interestingly, we found significant differences in tumor architectures in the three cytogenetic subtypes. The architectural differences were in histological patterns (organoid/nested, tubulocystic, and solid/confluent), the quality of stroma (fibromyxoid and edematous), nuclear wrinkling (in >5% of cells), perinuclear halos, and well-defined cell borders with clear cytoplasm ($p < 0.05$). The frequency of these features was more pronounced in cytogenetic ROs and ChRCCs, whereas the RO variant group positioned in between, except for tubulocystic architecture, which was least uncommon in RO variant compared with ChRCC or RO cases. Features such as calcifications/osseous metaplasia, hemorrhage, cellular pleomorphism, binucleation/multinucleation, presence of mitoses, fat invasion, or vascular invasion were nondiscriminatory (Table 2). A recent large survey also suggested that in oncocytic tumors, certain morphologic features are more frequently associated with malignancy and should be used for triaging cases for cytogenetic testing to rule out ChRCC, especially in biopsies [4].

Only CK7 immunostaining distinguished study groups ($p < 0.05$), a finding similar to previous studies [4,17,19,20]. S100A1 was diffusely expressed in the vast majority of RO and RO variant cases, but did not reach statistical significance, arguing against using this analyte for the differential diagnoses of the eosinophilic variant of ChRCC.

Morphologic features correlated with the cytogenetic subtype in 85% of ROs. One case (5%) had a genetic profile of ChRCC, and two cases had cytogenetic profiles of RO variants (10%). Morphologic prediction of ChRCC was accurate in 86% of cases having the ChRCC cytogenetic profile, whereas one case had a genetic profile consistent with RO (14%). Similarly, in the TCGA study, four of nineteen eosinophilic ChRCC cases (21%) had no copy number alterations. These should be reclassified as RO [12]. However, our morphologic prediction of cytogenetic ChRCC or RO would miss up to 40% cases if we did not have a third group with mixed morphologic features (Tables 3, 4 and 5). This third group, designated *HOCT*, was cytogenetically very heterogeneous and classified into

cytogenetic RO, RO variant, or ChRCC in 27%, 46%, and 27% of cases, respectively. Therefore, our study strongly argues in favor of using a borderline or intermediate diagnostic category, which is a conclusion similar to that of two recent studies [4,15]. We made a flowchart to enable pathologists to immunopathologically identify candidate HOCT cases for copy number analyses and consequent definitive diagnosis (Fig. 4).

In the past, HOCT may have been diagnosed as oncocytoma based on similarities in morphology, ultrastructure, and molecular features [21]. Although loss of chromosome 14 has been reported in RO [8,22], monosomy 14 was found more frequently with additional chromosomal losses and gains in RO variant than in RO cases in our study (Fig. 2). Some recent studies also showed that HOCT is a genetically heterogeneous group of tumors for which genomic profiling can help in the classification. Fourteen sporadic HOCT cases analyzed by fluorescence in-situ hybridization (FISH) showed recurrent monosomy 20 in 50% of cases and random multiple chromosomal gains and losses of chromosomes 1, 2, 6, 9, 10, 13, 17, 21, and 22 [23]. These should be reclassified as ChRCC. Twelve HOCT cases studied by a French group using array CGH showed no CNA in 58% of cases and a small number of random chromosomal losses and gains in the remaining 42% of cases involving 1p, 3q, 5p, 7p, 10, and 18 [24]. This study suggested that HOCTs are not true hybrid tumors and might be more appropriately classified as ROs. A recent molecular study of 27 HOCT cases by the MD Anderson group [15] reported 40% of tumors without CNA that should be reclassified to RO and remaining 60% with either loss of chromosome 1 or sex chromosome and segmental losses of 1p, 2q, 5p, 6q, and 22q and polysomy 7. They concluded that HOCT occupies an intermediate cytogenetic position between RO and ChRCC, clustering closer to RO than to ChRCC based on RNA transcript data. Similarly, our study showed that RO variant was enriched in the HOCT cohort (46%) and had genetic profiles similar to RO, which was characterized by typical RO chromosomal losses (−1/1p-, −14, -X, and −Y) with a few additional copy number aberrations and gains in 1q and 7 (Fig. 2). Our study showed that the genomic profile of RO variant was quite different from the ChRCC genetic profile of hypodiploidy with chromosomal losses −1, −2, −6, −10, −13, −17, −21, and -Y in combination [7]. To summarize, tumors with intermediate/borderline features (HOCT) could be reliably classified by cytogenetic profiling into ChRCC, RO, or RO variant subtypes.

Although HOCTs are commonly associated with BHD syndrome [5,25], whether sporadic HOCTs are a pathologically and genetically distinct entity remains a point of debate [3,6,9,23,26]. Some believe that HOCT may represent a morphologic variant of RO with excellent outcomes [24,27], an intermediate stage of stepwise progression from

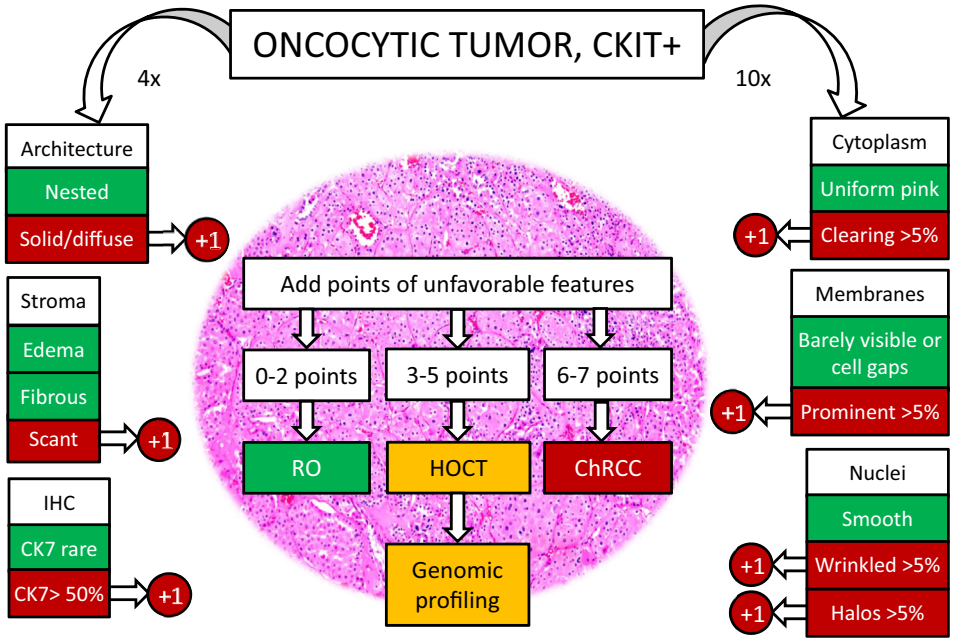

Fig. 4 Diagram for selecting CKIT+ oncocytic tumors for genomic profiling. Review the neoplasm at ×4 to characterize its predominant architecture, the quality and amount of stroma, and CK7 expression. View unfavorable cytologic features at ×10, ie, cytoplasm, prominence of cell membranes, nuclear irregularity, and perinuclear halos. If the sum of unfavorable immunomorphologic features is between 3 and 5, genomic profiling should be carried out. Color coding of immunomorphologic characteristics: red, unfavorable; green, favorable. RO, renal oncocytoma; HOCT, hybrid oncocytic/chromophobe tumor; ChRCC, chromophobe renal cell carcinoma; IHC, immunohistochemistry.

RO to ChRCC [21,28]. Others regard HOCT as a unique entity with metastatic potential based on chromosomal and molecular alterations that are not seen in typical ROs or ChRCCs [15]. Interestingly, syndromic HOCTs are multifocal with mosaic patterns of RO- and ChRCC-like zones, whereas sporadic HOCTs mostly have an ambiguous intermediate morphology, similar to our findings [15,23−25,29]. Future study with a larger sample size and longer follow-up data will be helpful to sort out the association between the genetic profiles and patient outcomes.

Hypodiploidy with multiple chromosomal losses of Y, 1, 2, 6, 10, 13, 17, and 21 is a hallmark of ChRCC [7]. However, some studies reported ChRCC cases with gains of chromosomes 4, 7, 12, 14, 15, 18q, 19, and 20 at lower frequencies of ChRCC cases [12,30−34]. Although these cases were interpreted as hyperdiploid with multiple chromosomal gains, they most likely resemble hypotetraploid ChRCC (4n) described in this study because the chromosomes with three or four copies in the six hypotetraploid ChRCC cases of this study were also most frequently chromosomes 4, 7, 12, 14, 15, 16, 18, and 20 (Supplemental Table 1, Fig. 2). Given the single-nucleotide polymorphism (SNP) pattern and copy number status for each chromosome examined as described in the Results section, these ChRCC cases had in fact doubled hypodiploid genomes with numerous chromosomal losses relative to tumor polyploidy status (4n) including most frequently two-copy loss of chromosomes Y, 1, 2, 6, 10, 13, and 17 (Table 1, Supplemental Table 1, Fig. 2). Our intriguing observation of doubled hypodiploidy (4n) with relative chromosomal losses explained the discrepancy found in the previous studies that some ChRCC cases had gains of chromosomes 4, 7, 12, 14, 15, 18q, 19, and 20 instead of multiple chromosomal losses. Although the majority of ChRCC cases had hypodiploid genomes, some had doubled hypodiploid genomes (4n, hypodetraploidy), and both had the same set of multiple chromosomal losses of Y, 1, 2, 6, 10, 13, 17, and 21. Doubled hypodiploidy was also supported by several earlier studies. A study using flow cytometry and quantitative image cell analyses of a series of ChRCC cases showed a portion of doubled hypodiploid nuclei in ChRCC with their combined DNA content essentially similar to that of single hyperdiploid nuclei, suggesting polyploidy resulting from fusion/doubling of these nuclei [35]. Several cytogenetic studies revealed nine ChRCC cases having hypotetraploid karyotypes with multiple chromosome losses, and four of nine cases also had a hypodiploid stem line [19,36,37]. A more recent study of ChRCC showed imbalanced chromosome duplication (duplication of $\geq$3 chromosomes) in 25% of metastatic ChRCC cases [32] and in one case of HOCT with liver metastasis [15]. These findings demonstrated clonal evolution and polyploidization and were associated with a more aggressive behavior. This phenomenon may indeed be the basis for tumor cell heterogeneity in ChRCC with eosinophilic features, such as separate coexisting clones within the same tumor, and polyploidization as a compensatory mechanism to maintain the genetic balance in nearby haploid/hypodiploid cells. Our study also shows that hypotetraploidy (doubled hypodiploidy) with multiple relative losses of this same set of chromosomes is a common phenomenon that is enriched in eosinophilic ChRCC (60%).

In summary, genomic profiling should be used to reliably categorize oncocytic tumors with ambiguous morphology and immunoprofiles into RO, RO variant, and ChRCC. Specific architectural features and the status of CK7 expression correlate with cytogenetic categories: RO, RO variant, and ChRCC tumors. The histological entity HOCT is a heterogeneous group enriched for cytogenetic RO variant, with an equal chance for cytogenetic RO or ChRCC.

We also found that chromosome ploidy status has a strong correlation with the histologic subtype. Doubled hypodiploidy (by whole-genome endoduplication) is a common phenomenon in eosinophilic ChRCC.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.humpath.2020.07.003.

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