Sangoi Ankur R (Orcid ID: 0000-0003-0874-8964)
Al-Obaidy Khaleel (Orcid ID: 0000-0001-9890-8751)
Chan Emily (Orcid ID: 0000-0002-7290-4004)
Mehra Rohit (Orcid ID: 0000-0002-6955-8884)
Tretiakova Maria (Orcid ID: 0000-0002-0819-9638)
Smith Steven Christopher (Orcid ID: 0000-0003-0982-4607)
Williamson Sean R (Orcid ID: 0000-0002-3898-1460)

Clear Cell Renal Cell Carcinoma with Focal Psammomatous Calcifications: A Rare Occurrence Mimicking Translocation Carcinoma

Ankur R. Sangoi[1], Khaleel I. Al-Obaidy[2], Liang Cheng[3], Chia-Sui Kao[4], Emily Chan[5], Sudha Sadasivan[6], Albert M. Levin[6], Isabel Alvarado-Cabrero[6], L. Priya Kunju[7], Rohit Mehra[7], Rahul Mannan[7], Xioming Wang[7], Jasreman Dhillon[8], Maria Tretiakova[9], Steven C. Smith[10], Ondrej Hes[11*], Sean R. Williamson[12]

[1]El Camino Hospital, Mountain View, CA, [2]Henry Ford Health System, Detroit, MI; [3]Brown University Warren Albert Medical School, Providence RI, [4]Stanford Medicine/Stanford University, Stanford, CA, [5]University of California, San Francisco, San Francisco, CA, [2]Henry Ford Health System, Detroit, MI, [6]Mexican Oncology Hospital SXXI, IMSS, Mexico City, Mexico, [7]University of Michigan, Ann Arbor, MI, [8]H. Lee Moffitt Cancer Center & Research Institute, Tampa, FL, [9]University of Washington, Seattle, WA, [10]VCU School of Medicine, Richmond, VA, [11]Biopticka laboratory., Plzen, Czech Republic, [12]Cleveland Clinic, Cleveland, OH

*Deceased

Corresponding Author: Ankur Sangoi, MD

asangoi2@yahoo.com, 650-964-4732

El Camino Hospital, Department of Pathology, 2500 Grant Road GC33

Mountain View, CA 94040

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ARS established the study design and wrote the original draft of the manuscript. SRW collected specimens, analyzed immunohistochemistry and histopathology, and provided critical revision and final approval of the manuscript. SS and AML contributed to study and database design. All other authors contributed study specimens and/or interpreted testing and provided critical revision and final approval of the manuscript.

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DECLARATION OF CONFLICTING INTERESTS

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ABSTRACT

Aims: Renal cell carcinoma (RCC) with clear cells and psammoma-like calcifications would often raise suspicion for MITF family translocation RCC. However, we have rarely encountered tumors consistent with clear cell RCC that contain focal psammomatous calcifications.

Methods & Results: We identified clear cell RCCs with psammomatous calcifications from multiple institutions and performed immunohistochemistry and fluorescence and RNA in situ hybridization (FISH and RNA ISH). Twenty-one tumors were identified: 12 men, 9 women, ages 45 to 83 years. Tumor size was 2.3 to 14.0 cm (median 6.75 cm). Nucleolar grade was 3 (n=14), 2 (n=4), or 4 (n=3). In addition to clear cell pattern, morphology included eosinophilic (n=12), syncytial giant cell (n=4), rhabdoid (n=2), branched glandular (n=1), early spindle cell (n=1), and poorly differentiated components (n=1). Labeling for CA9 was usually 80-100% of the tumor cells (n=17/21) but was sometimes decreased in areas of eosinophilic cells (n=4). All (19/19) were positive for CD10. Most (19/20) were positive for AMACR (variable staining, 20-100%). Staining was negative for keratin 7, although 4 showed rare positive cells (4/20). Results were negative for cathepsin K (0/19), melan A (0/17), HMB45 (0/17), TFE3 (0/5), TRIM63 RNA-ISH (0/13), and *TFE3* (0/19) and *TFEB* rearrangements (0/12). Seven of 19 (37%) showed chromosome 3p deletion. One (1/19) showed trisomy 7 and 17 without papillary features.

Conclusions: Psammomatous calcifications in RCC with a clear cell pattern suggests a diagnosis of MITF family translocation RCC; however, psammomatous calcifications can rarely be found in true clear cell RCC.

INTRODUCTION

Speaking at a local pathology meeting in San Francisco, CA in 2018 on the topic of tumors in the morphologic differential diagnosis of clear renal cell carcinoma (RCC), the late Dr. David Grignon (to whom this manuscript is dedicated) proclaimed that "finding psammoma bodies is a clue that that you are probably dealing with a translocation RCC; if you have a picture of a psammoma body in a clear cell RCC please email it to me as these are excruciatingly rare!" After finding such an index case ironically a few months after this meeting by ARS and following discussion with SRW, a former trainee of Dr. Grignon who had collected a few such tumors, a multi-institutional search was conducted to gather specimens for a clinicopathologic review of psammomatous calcifications (a well-described morphologic feature of *TFE3* rearranged RCC and *TFEB* altered RCC[1-9]) identified in clear cell RCC.

METHODS AND MATERIALS

The pathology archives of multiple institutions were searched for tumors in which a diagnosis of clear cell RCC was rendered, containing any admixed psammoma-like or punctate calcifications (excluding dystrophic calcification in fibrous stroma, Gamna-Gandy like structures, or metaplastic bone formation). All diagnoses were confirmed by at least 2 genitourinary pathologists. Clinicopathologic features including notable tumoral histology and pattern/extent

of calcification were recorded on all cases. Immunohistochemistry performed for CA9, CD10, AMACR, keratin 7, cathepsin K, melan A, HMB45, and TFE3 at the various institutions were noted with staining pattern semi-quantitatively scored. Fluorescence in situ hybridization (FISH) testing for *TFE3* rearrangement, *TFEB* rearrangement / amplification, chromosome 3p deletion, trisomy chromosome 7, and trisomy chromosome 17 was performed on a subset of cases as described elsewhere[8,10-12]. Additionally, RNA in situ hybridization against target probe *TRIM63* (TRIM63 RNA-ISH) was performed on a subset of cases as described elsewhere[7].

RESULTS

A total of 21 tumors were retrieved from 12 institutions. All specimens were radical nephrectomies (11 left-sided, 10 right-sided). Patients were 12 men and 9 women, with ages ranging from 49 years to 83 years (mean=63.1 years). Tumor sizes ranged from 2.3 cm to 14.0 cm (mean=6.5 cm) and showed WHO/ISUP nucleolar grades of 3 (n=14), 2 (n=4), and 4 (n=3). Most tumors were stage category pT3a (n=11) followed by pT1b (n=5), pT2a (n=3), and pT1a (n=2). The pattern of calcification was psammomatous in all tumors (100%), with 6 also showing another pattern of calcification (5 with punctate calcification and 1 with ring-shaped calcification). These calcifications were not associated with necrosis. In all tumors, the extent of calcification was focal (located only in 1-2 slides / blocks). Regarding histomorphology, all tumors showed areas of typical clear cell RCC (Figures 1, 2), with some other notable features as listed on Table 1. Of the immunohistochemistry recorded for this study (Table 1), CA9 showed positive membranous labeling in all tumors (21/21, complete membranous pattern), typically within 80-100% of the tumor cells (n=17/21). Tumors with less than 100% CA9 reactivity all contained eosinophilic cells and had a minimum of 50% staining, with the higher-grade

component showing decreased staining compared to the well-differentiated component. All tumors studied (19/19) were also positive for CD10 and most (18/19) were also positive for AMACR (notably variable staining with AMACR, 20-100%). Tested tumors were typically negative for keratin 7 (16/20); however, 4 had rare cells positive, estimated only 1% of cells. There was no staining for cathepsin K (0/19), melan A (0/17), HMB45 (0/17), and TFE3 protein (0/5). TRIM63 RNA-ISH was negative in 11 tested specimens, with 1 showing focal positivity with H-score of 85, interpreted as negative / nonspecific. *TFE3* rearrangement was negative in all tested specimens (0/19). Seven of 19 tumors (37%) showed chromosome 3p deletion. One tumor (1/19) showed trisomy chromosomes 7 and 17; however, this lacked papillary morphology and showed a typical clear RCC immunoprofile (diffusely CA9 positive, CD10 positive, keratin 7 negative). *TFEB* showed no rearrangement or amplification all tested tumors (0/12).

DISCUSSION

In evaluation of RCC nephrectomies, psammomatous calcifications are not uncommon, prototypically seen in papillary RCC[13]. These calcifications can also be seen in some classical RCC subtypes (chromophobe RCC[14]) as well as in some more recently-characterized entities (eosinophilic solid and cystic RCC[15], biphasic hyalinizing psammomatous RCC[16], NF-mutated RCC[17], and RCC with sex-cord/gonadoblastoma-like features[18]). However, when encountering a renal cell carcinoma showing clear cell morphology and admixed psammomatous calcifications, the archetypal tumor type that comes to mind is the MiTF family translocation RCC (includes *TFE3* rearranged RCC and *TFEB* altered RCC[1-9]). In fact, this is often considered a morphologic clue to suspect a diagnosis of translocation RCC. To date, psammomatous calcifications

encountered in clear cell RCC have not (to our knowledge) been well-described or reported, thus being the focus of the current study.

Herein, we report our findings from 21 clear cell RCC tumors containing admixed psammoma-like or punctate calcifications. While the true incidence of this finding is unclear, based on our own experiences in high-volume nephrectomy practices of several of the co-authors, finding psammomatous calcifications in clear cell RCC is uncommon. . Specifically, of the co-authors that had tabulated total clear cell RCC cases reviewed to identify clear cell RCC with admixed psammomatous calcifications, the incidence ranged from 1 study case per 75 clear cell RCC to 1 study case per 823 clear cell RCC (cumulative incidence of approximately 1%). Notably, one co-author who also had recorded the number of MiTF family RCC discovered during retrospective case review (mostly by observing unusual tumor morphology coupled with concomitant psammomatous calcifications) noted 40 MiTF RCC identified among 843 RCC reviewed (incidence of approximately 5%). This more than 5-fold estimated incidence reiterates the notion that although psammomatous calcifications can occur in clear cell RCC, more often than not, it clues into the possibility of MiTF RCC. Nonetheless, based on the somewhat more extensive immunohistochemical panel employed by most co-authors than might be used for a typical clear cell RCC (see Table 1), it appears that MiTF family translocation RCC was a serious diagnostic considered based on the admixed psammomatous calcifications. Moreover, expression of AMACR (often positive in MiTF family translocation RCC[19]) in our cohort exemplifies this as a potential immunohistochemical pitfall[21], as variable AMACR positivity has been described clear cell RCC[20,22]. Given the well-known issues with TFE3 immunohistochemistry as a diagnostic marker for MiTF family translocation RCC,[12,23-26] only a minority of laboratories offer this marker in a clinical (non-research) setting[27]. Therefore, it may

not be surprising that TFE3 immunohistochemistry was infrequently utilized among the coauthors of this study (24%, only 5/21 specimens; which showed a negative TFE3 stain).

While we acknowledge that testing for *TFE3* rearranged RCC and *TFEB* altered RCC by FISH methodology, as we performed in this study, has its own diagnostic issues[4,9,23,28] particularly compared to next-generation sequence (NGS) testing, for this reason we tested all cases with available tissue blocks/unstained slides for TRIM63 RNA-ISH staining. This novel biomarker has been shown to be both highly sensitive (90%) and specific (100%) for MiTF family translocation RCC[7], and in the context of our study serves as a reliable "screening tool" to exclude *TFE3* rearranged RCC and *TFEB* altered RCC, the key morphologic differential diagnostic entities of clear cell RCC containing psammomatous calcifications.

Although we acknowledge that the rate of identifying chromosome 3p deletions in our study cohort (7/19, 37%) is less than what might be expected for clear cell RCC, in some instances variability in diagnostic cut-off values can contribute to wide rates of detection (38% to 100%[29]). Moreover, while we did not test for *VHL* gene abnormalities (mutations, deletions, or methylations) which could represent the molecular oncogenesis in our cohort, we felt confident in our diagnoses based the fact that all our tumors showed at least some areas with typical clear RCC morphology coupled with strong positivity for CA9 immunohistochemical staining.

In summary, when encountering psammomatous calcifications in a RCC showing areas of clear cell morphology that is not overt papillary RCC, it may still be prudent to consider MiTF family translocation RCC and perform a diagnostic workup. However, in this study we have shown that although finding psammoma bodies in a clear cell RCC may be uncommon, it is not incompatible with the diagnosis in rare instances.

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FIGURE LEGENDS

Figure 1. (A) This clear cell renal cell carcinoma shows more eosinophilic cells around the psammomatous calcifications; however, other areas of the same tumor (B) have typical clear cell morphology. Labeling for CA9 was typically diffuse, but sometimes decreased in the areas corresponding to eosinophilic cells or high grade cells (C, lower right). Another tumor (D) shows larger, granular calcifications in addition to psammomatous calcifications.

Figure 2. Other morphologies encountered in the clear cell renal cell carcinomas with psammomatous calcifications (not shown here) included syncytial-type giant tumor cells (A, lower right), or rhabdoid cells (B). Fluorescence in situ hybridization shows two copies of the chromosome 3 centromere (red) per cell, but only one copy of 3p (green, C). RNA in situ hybridization demonstrates negative TRIM63 expression (D), arguing against translocation-associated renal cell carcinoma.