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## **RENAL ONCOCYTOSIS. A CLINICO-PATHOLOGIC AND CYTOGENETIC STUDY OF 42 TUMORS OCCURRING IN 11 PATIENTS**

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## **Introduction**

Oncocytoma of the Kidney (OK) is an epithelial lesion that accounts for 3-7% of all adult renal epithelial neoplasm and most commonly affects males in the seventh decade of life. OK is by definition a benign tumor according to the literature, with no patients died of this disease and only two patients reported to bear possible metastases [1]. OKs are typically single, well-defined, non-encapsulated lesions. In rare instances, OKs may present as multifocal and bilateral and in this setting the disease is named Renal Oncocytosis. [2] Renal oncocytosis is an infrequent pathological condition characterized by the presence of multiple and/or bilateral oncocytic tumors with a spectrum of histological changes ranging from renal OK to chromophobe renal cell carcinoma (CHRCC). In addition, the association of OK with other histotypes of renal cell cancer can be found in 10-32% of renal oncocytosis with unilateral or bilateral tumor localization. [2]

A common finding in renal oncocytosis is the occurrence of “hybrid” oncocytic tumors with intermediate morphological features between OK and CHRCC. Many hypotheses have been offered in past years about the relationships among OK, CHRCC and “Hybrid Oncocytic Tumor” (HOT). Since the three lesions frequently occur in the context of renal oncocytosis, this pathological condition was often used as a model for understanding the biological background of oncocytic tumors and their possible interconnections. Some authors suggested that a spectrum of oncocytic lesions may exist, evolving from OK through HOT and finally to CHRCC. This hypothesis is supported by data on specific chromosome losses shared by HOT and CHRCC such as loss of chromosomes 14 and 21. [3] Other authors suggested that HOT is not related to CHRCC, but rather represents the evolution of OK, following the occurrence of additional chromosome aberrations and that HOT and CHRCC actually derive from OK, as a common precursor lesion. [3] Finally, according to another hypothesis all these tumors represent independent entities, both phenotypically and genotypically. [4] The most recent classification of renal tumors proposed by the International Society of Urologic Pathology (ISUP) in 2013 supports this latter hypothesis and introduced hybrid oncocytic tumors as a separate entity. Therefore, according to this recent classification of renal tumors, renal oncocytosis-related HOTs should be considered distinct tumors and not intermediate steps of the morphologic progression from renal oncocytosis to CHRCC. [5]

From the clinical standpoint, HOTs may occur in three different clinico-pathological settings: sporadic, in association with renal oncocytosis, or in carriers of the Birt-Hogg-Dube’s syndrome (BHD), a rare autosomal inherited dominant disease characterized by skin lesions (fibrofolliculomas of the face and head and neck), pulmonary cysts and renal neoplasms. [6] Tumors occurring in the three above conditions share similar morphological features also common to OK and CHRCC. [4,7,8,9] However, the different molecular characteristics of each lesion are still not completely defined.

Here, we provide an histological, immunohistochemical (IHC) and cytogenetical analysis of 42 different lesions from 11 patients with renal oncocytosis not associated with BHD syndrome.

## Materials and Method

We retrospectively evaluated 42 kidney lesions from 11 patients with renal oncocytosis diagnosed from 2009 to 2013 in the pathology divisions at the S.Orsola-Malpighi Hospital (Bologna) and the San Luigi Hospital (Orbassano, Turin). We aimed to analyze any lesion that was removed from each patient to confirm their histological, immunohistochemical and cytogenetical features. Renal oncocytosis was defined as the presence of at least 2 oncocytomas either in the same kidney or bilaterally.

The mean age of the patients at nephrectomy was  $63.8 \pm 15.97$  years (range 41-85), 6 (54.5%) patients were males and 5 (45.5%) females. Twenty-five (60.9%) lesions were in the left kidney and 16 (39%) in the right kidney. Five patients (45.5%) underwent laparoscopic radical or partial nephrectomy.

The majority (10/11) of the patients were asymptomatic at the time of the diagnosis and the detection of renal masses has been incidental during abdominal ultrasound performed for other clinical reasons or routine check-up. One case presented with haematuria. (Table 1)

All lesions were histologically reviewed by three dedicated uro-pathologists, blinded to the original pathology report, and classified according to the 2013 ISUP classification. [5] Surgical specimens were formalin-fixed, paraffin embedded and routinely processed for histological diagnosis. Three- $\mu$ m-thick sections were cut from paraffin blocks and stained with Haematoxylin-Eosin and four- $\mu$ m-thick sections were prepared from blocks comprehensive of normal renal tissue and neoplastic tissue for immunohistochemical and Fluorescent In Situ Hybridization (FISH) analyses.

### *Immunohistochemical analysis*

Immunohistochemistry for cytokeratin 7 (SP52 Ventana Roche, Ventana Medical Systems, Inc, Tucson, USA Prediluted) and for Alpha Methyl CoA Racemase (P504S, Ventana Medical Systems; 1:100 was performed in all cases using an automated Benchmark Ultra instrument (Ventana Roche, Ventana Medical Systems, Inc, Tucson, USA)

We performed immunohistochemistry for CK 7 for the differential diagnosis among oncocytic lesions according to the recommendations of the ISUP. [5]

Immunoreactivity for CK7 was scored as negative when only single scattered tumor cells or entrapped native renal tubules were stained; intermediate when the immunoreactivity was limited to clusters of tumor cells; and positive when the tumors were strongly and diffusely positive for CK7.

### *FISH analysis*

FISH analyses were performed by using: i) the ZytoLight SPEC VHL/CEN3 Dual Color Probe (ZytoVision GmbH, Germany) to detect chromosome 3p status; ii) centromeric DNA probes for chromosome 1 (CEP 1, Spectrum Orange), chromosome 6 (CEP 6, Spectrum Green), chromosome 7 (CEP 7, Spectrum Green), and chromosome 17 (CEP 17, Spectrum Orange; all CEP probes from Abbott Molecular, Downers Grove, USA), to detect chromosomes losses/gains.

Briefly, slides were baked at 60°C overnight, deparaffinized, pretreated at 98°C for 15 minutes in citric acid solution (PT1, ZytoVision GmbH, Germany) and digested with pepsin solution (ES1, ZytoVision) at 37°C for 8 min. Co-denaturation and hybridization were performed on an automated

ThermoBrite (Abbott Molecular) at the following conditions: i) 75°C for 10 min and 37°C overnight, for VHL/CEP3 probes; ii) 85°C for 2 min and 42°C overnight for CEP1/CEP 6 and CEP7/17 probes. After two washes in 2XSSC/0.3%NP40, at room temperature for 2 min and at 73°C for 2 min, slides were air-dried and counterstained with DAPI I (4'-6'-Diamidino-2-phenylindole dihydrochloride hydrate) antifade solution (Abbott Molecular).

Slides were evaluated with an epi-fluorescence microscope (Nikon Eclipse 80, Nikon Corporation, Tokio, Japan) equipped with single band-pass filters. For each sample, 80-100 neoplastic nuclei were analyzed under high-power magnification (1000x), and non-neoplastic kidney parenchyma was scored as well and used as control.

The cut-off values for the definition of chromosomal gains and losses were set at the mean  $\pm 3$  SD of the control values (non-neoplastic cells). Any tumor with a signal score beyond the cut-off value was considered to have gain or loss of that chromosome.

## Results

Clinical, histological and molecular characteristics of all the lesions are described in Tables 1 and 2. Mean tumor size was  $2.8 \pm 2.01$  (range 0.6-9). Histological review of the cases was concordant among the three dedicated genito-urinary pathologists and the 42 lesions were classified as follows: 36/42 (85,7%) oncocytoma, 2 (4.76%) hybrid oncocytic tumor, 1 (2.4%) clear cell carcinoma, 1 (2.4%) papillary renal cell carcinoma (pRCC), 1 typical angiomyolipoma (2,4%), and 1 mixed epithelial/stromal tumor of the kidney (2,4%) (Table 2)

### Oncocytomas

All the OKs in the series had typical gross features: well circumscribed nodules with a mahogany-brown cut surface. Histologically, they displayed a solid growth pattern, small clusters of tubule-like structures and nests of large round to polygonal cells with granular eosinophilic cytoplasm, round nuclei and central single nucleoli. No necrosis or atypical mitoses were observed. Two cases presented with an additional infiltrative pattern of the peri-nephric adipose tissue.

Immunohistochemistry for CK7 turned out negative in all these lesions (FIG. 1 A, B). FISH analysis confirmed the histological diagnosis of OK with a diploid status for chromosomes 6 in all the lesions and loss of chromosome 1 in just 5 of 29 tumors. (FIG. 2 B, C)

Two oncocytomas in patient 6 were otherwise histologically typical except for the presence along the central fibrous scars of tubulo-papillary structures with an infiltrating pattern and apparently interconnected with the main oncocytic lesion. Unlike the oncocytic counterpart these cells arranged in the tubulo-papillary structures were strongly immunoreactive for AMACR and CK7 (FIG. 1 E, F). The FISH analysis confirmed that the oncocytic lesion was diploid for chromosomes 1,6,7 and 17 while the tubulo-papillary structures showed the gain of chromosome 17, typical of pRCC. (FIG.2 C, D). Other four lesions of same patient were conventional "pure" OK at histological, IHC and FISH analysis.

### Hibrid Oncocytic tumors

The two HOTs found in patients 8 and 11 were grossly well circumscribed, with a brown- greyish color. Histological examination showed a mixture of areas with oncocytic features and others resembling CHRCC, with eosinophilic, granular cytoplasm and a clear halo around raisin-like nuclei. Immunohistochemistry for CK 7 showed intermediate immunoreactivity in one nodule of

patient 8 and positive staining in a lesion of patient 11. (FIG. 1 C, D). FISH analysis showed loss of chromosome 1 in patient 8 and a diploid status for chromosomes 1 and 6 in patient 11. (FIG. 2 B)

#### Non-Oncocytic encountered lesions

Patient 2 also harboured a 5.5 cm pRCC, type I with sparse foamy macrophages. The lesion was positive for CK 7 and the FISH analyses showed the gain of chromosome 7. A small (1.5 cm) ccRCC with typical polygonal cells with clear cytoplasm and fine vascular network, negative for CK 7 was found in patient 5. FISH analysis showed the typical loss of chromosome 3p in this tumor nodule. A typical angiomyolipoma with prevalent vascular component was found in patient 4. Patient 11 presented with one HOTAIR, two OKs and a cystic lesion with septa filled by endometrial-like stroma that was diagnosed as a mixed epithelial/stromal tumor of the kidney (MEST), based on the immunoreactivity for estrogen and progesterone receptors and CD10 in the stromal cells.

#### Follow-up

After a mean follow-up of  $21.5 \pm 10.12$  months, all patients were alive. One patient (patient 3), originally diagnosed with three oncocytomas, showed a local recurrence at imaging after 17 months from surgery, and underwent close clinical follow up.

### **Discussion**

Renal Oncocytosis is a recently established clinical entity characterized by multiple oncocytic tumors and diffuse oncocytic changes in the tubules of the surrounding parenchyma.[10]. Clinically, renal oncocytosis occurs with subtle and not specific symptoms. Rarely it presents with more aggressive clinical course such as renal failure due to the severe bilateral involvement of both kidneys by multiple lesions. Histologically, renal oncocytosis is characterized by multiple oncocytic tumors with a spectrum of different histological patterns, often with overlapping benign and malignant features. The differential diagnosis between benign and malignant oncocytic lesions is made possible by the association of histology and immunohistochemistry using well standardized criteria.

In the present study we provide the largest available series of patients with renal oncocytosis with complete immunohistochemical and molecular characterization of all the incident nodules. We demonstrated that three experienced genito-urinary pathologists with the sole help of CK7 immunohistochemistry obtained a complete diagnostic concordance in all the 42 oncocytic lesions. The immunoreactivity for CK7 has been widely proposed as a reliable tool for differentiating CHRCC from oncocytoma, based on the strong and diffuse staining in the former and the almost negative staining in the latter. [11] Our data, although obtained on a limited number of cases, confirm the robustness of the CK7 test and also its applicability to the differential diagnosis between HOTAIR and OK. In fact all the HOTAIR nodules in our series stained for CK7 in small clusters of neoplastic cells compared to the rare and isolated cells that turned out immunoreactive in the OK lesions (FIG. 1 B e D). Therefore, it seems reasonable to propose that the spectrum of immunoreactivity for CK7 is a reliable tool for the differential diagnosis of OK, HOTAIR and CHRCC. Cytogenetic analyses are needed for diagnostic confirmation only in a very small subset of renal cell tumors only, in which histology and IHC are not conclusive. In the present series of renal

oncocytosis, FISH analysis further corroborated and confirmed all the diagnoses made by histology and CK7 immunohistochemistry. The most challenging differential diagnosis for the pathologist in cases of renal oncocytosis is represented by the eosinophilic variant of CHRCC, which can closely mimic OK and the mixed tumors with components of ccRCC and pRCC. Our data demonstrate that FISH analysis for losses of chromosomes 1 and 6 in CHRCC, gain of chromosomes 7 and 17 in pRCC and deletion of chromosome 3p in ccRCC can lead to conclusive diagnosis in these challenging cases. The cytogenetic status of OK is generally diploid with few tumors harboring losses of chromosomes 1 and Y. Therefore in the case of challenging differential diagnosis with OK, the finding of diploidy for chromosomes 6, 7 and 17 can be considered conclusive. [12, 5] The cytogenetic profile of HOT lesions is less defined and may change according to the occurrence in the sporadic, renal oncocytosis or BHD clinical setting. [3, 7, 13] In the two HOT lesions of the current series of renal oncocytosis, the unique detectable alteration was the deletion of chromosome 1 in one case (while the others were diploid for chromosomes 1 and 6).[5]

We have also encountered two lesions in the same patients with otherwise conventional feature of OK but with tubulo-papillary structures immunoreactive for CK7 and AMACR growing with an infiltrative pattern along the fibrous septa of the central scar. The cells of the tubules also showed gain of chromosome 17. These tubulo-papillary CK7+ and AMACR + structures in the scars of OK have been described but it is probably less known that these cells also show a gain of chromosomes 7 and 17, that is typical of pRCC. The recognition of the biological significance of these papillary proliferations found in a small subset of OK is outside of the scope of the present paper and deserves a separate study on a larger series of cases.

The knowledge of the biological nature of the lesions before surgery is critical due to the frequent multifocality and bilaterality of the oncocytic lesions in the setting of renal oncocytosis. Given the infrequency of the renal oncocytosis, the surgical treatment of these patients is not standardized. Surgical decision making is generally based on a case-by-case decision, according to the size and the location of the nodules and the clinical evaluation of renal function. Therefore, pre-operative histological diagnosis of renal oncocytosis nodules could be of relative help in the guidance of surgical strategy. In fact, in case of renal oncocytosis, it is difficult to perform fine needle biopsies (FNAB) of all the lesions. In cases where FNAB is done, the histological diagnosis of oncocytic lesion may be even more challenging just on the basis of morphology and IHC features and FISH analysis could represent the only adjunct for a better histotype definition. Since FNAB is not widely applicable, a nephron-sparing intent should always guide surgical treatment in renal oncocytosis. Patients with a final histological diagnosis of OK and HOT should be just followed. In fact, HOTs seem to behave indolently or exhibit a low malignant potential, although this will require longer follow-up and larger series for confirmation. [5] In our series, although with a limited follow-up, none of the patients with HOT recurred. The unique recurrence that we observed was in a patient with diagnosis of multiple OK. This recurrence was documented only by imaging and the event of a new KO nodule rather than a recurrence of a previous lesion cannot be definitely ruled out. Finally, patients with renal oncocytosis and a diagnosis of any malignant RCC type at nephron sparing surgery should be followed in accordance to the sporadic malignant counterpart.

The general low-malignant potential of these lesions suggests that conservative surgery is still indicated in order to preserve renal function. Radical nephrectomy in renal oncocytosis should be limited to the cases with confirmed malignant RCC with positive margins at partial nephrectomy and to those cases with multiple nodules where nephron-sparing surgery is not applicable.

### **Disclosure/conflict of interest**

The authors declares no conflict of interest.

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Figure Legends:

**FIG. 1.** Oncocytoma (A) with negative immunoreactivity for CK7 (B). Hybrid oncocytic tumor with perinuclear halo (C) and focal immunoreactivity for CK7 (D). Mixed oncocytoma and papillary renal cell carcinoma (E) with immunoreactivity for CK 7 in the papillary component (F). Magnification 10x

**FIG. 2.** Fluorescence in situ hybridization with centromeric probes for chromosomes 1 (Orange) and 6 (Green). Normal kidney parenchyma (A); loss of Chromosome 1 (B); normal cytogenic profile of chromosome 1 and 6 of oncocytic tumor (C). Fluorescence in situ hybridization with centromeric probes for chromosomes 7 (Green) and 17 (Orange). Peripheral papillary component of the mixed tumors with components of oncocytoma and papillary renal cell carcinoma (D).

Table Legends:

**TABLE 1:** Clinical features.

**TABLE 2:** Pathological and cytogenetical features.