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► **To cite this version:**

Marjorie Gournay, Frederic Dugay, Marc-Antoine Belaud-Rotureau, Benoit Peyronnet, Romain Mathieu, et al.. Renal cell carcinoma with leiomyomatous stroma in tuberous sclerosis complex: a distinct entity. *Virchows Archiv*, 2021, 478 (4), pp.793-799. 10.1007/s00428-020-02910-9 . hal-03039966

HAL Id: hal-03039966

<https://hal.science/hal-03039966>

Submitted on 10 Dec 2020

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**Renal cell carcinoma with leiomyomatous stroma in Tuberous Sclerosis Complex:
a distinct entity.**

Renal cell carcinoma with leiomyomatous stroma in TSC

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Conflict of interest Statement

Disclosure of potential conflicts of interest: The authors of this article have no relevant financial relationships with commercial interests to disclose and no funding to declare.

Words: 1479 (excluding abstract and references)

Abstract:

1
2 Renal cell carcinoma with leiomyomatous stroma (RCCLS) is an emerging entity frequently
3 associated with tuberous sclerosis complex (TSC). We described herein a series of RCCLS in
4 TSC patients at pathological and cytogenetic levels. Three male patients with TSC and
5 RCCLS were identified between 2000 and 2019 at the University Hospital of Rennes.
6
7 Histologically, the architecture was tubulo-papillary with thick bundles of smooth muscle
8 cells. The tumor cells showed clear cytoplasm with eosinophilic globules. The
9 immunohistochemical profile was identical with an intense positivity of CK7, CAIX, CD10
10 and a heterogeneous positivity of CK20. SDHB was low but positive and TFE3 was not
11 expressed. Comparative genomic hybridization (CGH) did not show any quantitative
12 chromosome abnormality. No recurrence was observed with a median follow-up of 4-years.
13
14 RCCLS in TSC patients has morphological, immunohistochemical and cytogenetic distinct
15 features that could constitute a distinct entity and a sentinel manifestation for the diagnosis of
16 TSC.
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27 Keywords :

28 Renal cell carcinoma, leiomyomatous stroma, Tuberous Sclerosis Complex, TSC1, TSC2.
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1. Introduction

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Tuberous Sclerosis Complex (TSC) is an autosomal dominant disease characterized by multisystemic tumors (brain, skin, heart, lungs and kidneys) and hamartomas. This disease is caused by alterations in *TSC1* or *TSC2* genes, which encode hamartin and tuberlin respectively, and form the TSC protein complex that regulate mTOR pathway, and thus increase protein translation, cell growth, decreased autophagy, and metabolic adaptations favoring the emergence of tumors [1].

Renal involvement in TSC is largely represented by angiomyolipoma with a minority of renal cell carcinoma (RCC) (2-5%). Yang *et al.* reported 46 renal epithelial tumors from 19 TSC patients distributed in TSC-associated papillary RCC (n=24), hybrid oncocyctic /chromophobe tumors (n=15) and unclassified tumors (n=7) [2]. The same year Guo *et al.* described a cohort of 57 RCC from 18 TSC patients distributed in RCC with smooth muscle stroma (n=17), chromophobe RCC (n=34) and eosinophilic-macrocytic RCC (n=6) [3]. Even if the taxonomy is different, the latter category shared morphological characteristics with the unclassified tumors by Yang. Moreover, TSC-associated papillary RCC and RCC with smooth muscle stroma could constitute the same entity. Unfortunately, these studies did not explore the cytogenetic profile of these tumors.

Leiomyomatous stroma is present in a variety of RCC mainly including clear cell RCC (ccRCC), papillary RCC (pRCC), clear cell papillary RCC (cpRCC), MiTF translocated RCC (tRCC) and TCEB1-mutated RCC [4–7]. However, it remains unclear whether the presence of leiomyomatous stroma should classify the tumor as a RCCLS.

The aim of this study was to describe a series of RCCLS in TSC patients in order to characterize this rare entity at pathological and cytogenetic level.

2. Material and Methods

2.1. Patient selection

From January 2000 to January 2019, in the University Hospital of Rennes, we identified 3 patients with TSC who underwent partial nephrectomy for RCC in the Department of Urology.

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2 2.2. Pathological analysis

3 Fresh surgical specimens were received at the Department Pathology. Partial nephrectomy
4 were weighed and measured. Tumors were described at gross examination and samples were
5 formalin fixed and paraffin embedded with hematoxylin, eosin and safran staining.
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10 2.3. Immunohistochemistry

11 Four-µm thick whole tissue sections were cut and mounted on glass slides (Superfrost+,
12 Menzel Glazer). The preparations were dried for 1 hour at 58°C, and then overnight at 37°C.
13 The sections were deparaffinized with toluene and rehydrated with ethanol. The preparations
14 were pretreated and immunostained using Ventana Benchmark XT. Immunohistochemical
15 analyses were carried out using the following antibodies: CK07 (mouse monoclonal antibody,
16 clone OV-TL 12/30, dilution 1/150, Dako), CK20 (mouse monoclonal antibody, clone
17 Ks20,8, dilution 1/25, Dako), CAIX (rabbit polyclonal antibody, ab15086, dilution 1/1500,
18 Abcam), CD10 (mouse monoclonal antibody 56C6, dilution 1/75, Leica), AMACR (rabbit
19 monoclonal antibody, clone 13H4-Dako, prediluted), SDHB (clone 21A11AE7, dilution
20 1/200, Abcam) and TFE3 (rabbit monoclonal antibody, clone MRQ-37-Cell, Ventana,
21 prediluted). The detection was performed using horseradish peroxidase-labeled polymer
22 conjugated secondary antibodies using diaminobenzidine as chromogen (Sigma-Aldrich,
23 France).
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38 2.4. Comparative genomic hybridization (CGH) analysis

39 Genomic DNA was extracted from formol fixed paraffin embedded (FFPE) tissue using the
40 Generead DNA FFPE kit (Qiagen, Hilden, Germany). Oligonucleotide array-comparative
41 genomic hybridization (CGH) was performed using the standard version of the Agilent
42 Human Genome CGH microarray 180K (Agilent Technologies, Santa Clara, CA, USA).
43 Microarrays were scanned using the Agilent scanner G2565BA. Images were extracted using
44 Agilent Feature Extraction software and data were analyzed with Agilent Cytogenomics
45 v.2.5.8.11 software.
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54 **3. Results**

55 3.1. Clinical Features

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1 All patients were male, with a median age of 31 years (22-42 years) at diagnosis (Table 1).
2 Two patients appeared to be first cousins. Patient 1 reported concomitant angiomyolipoma
3 and patient 2 presented aortic bicuspid valve, patient 3 was asymptomatic. Patient 1 harbored
4 a mutation of TSC1 (c.2585insT) whereas the first cousins patients 2 and 3 showed the same
5 TSC2 mutation (c.440C>G). With a median follow-up of 4 years the patients were all alive
6 without any signs of local recurrence or metastasis.
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10 11 12 3.2. Pathological findings

13 The three tumors showed similar gross examination. Macroscopically, they were well
14 circumscribed, brownish with an irregular pseudo-capsule. The cut surface was solid with
15 fibrotic changes. Neither hemorrhagic nor necrotic areas were found. The average tumor size
16 was 3.3 cm (1-6 cm) and was limited to the kidney and staged pT1. Microscopically, they
17 shared a tubulo-papillary architecture (Figure 1). The tumor cells had either clear cytoplasm
18 with the presence of eosinophilic globules or an eosinophilic and granular cytoplasm. Some
19 tumor cells exhibit cytoplasm vacuolization. The cytoplasmic membrane was well defined
20 with a plant-cell like appearance. The nucleus was round, with conspicuous, large nucleoli
21 equivalent to grade 3 according to WHO/ISUP histologic grading system for clear cell and
22 papillary renal cell carcinoma. The stromal component consisted of thick bundles of well
23 differentiated smooth muscle cells. In one case, bone metaplasia was identified. There were
24 no sinusoidal vessels with branching vessels seen in ccRCC. There was no prominent atypia,
25 mitoses, rhabdoid or sarcomatoid component or necrotic area.
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40 3.3. Immunohistochemical features

41 Immunohistochemically, all 3 tumors displayed similar immunochemical profile (Figure 2).
42 The tumor cells were strongly and diffusely positive for CK7 with no expression of AMACR.
43 CD10, CAIX were diffusely expressed whereas CK20 was more heterogeneous. The
44 expression of SDHB was very weak and TFE3 was negative (Supplementary Data S3, S4 and
45 S5). The smooth muscle cells expressed muscle specific-actin and smooth muscle actin.
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51 3.4. Cytogenetic data

52 A neutral profile was observed for each case without any gene copy number abnormalities. In
53 particular, neither deletion of 3p and monosomy 8 nor trisomy of chromosomes 7 and 17 were
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4. Discussion

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4 In this study, we identified in TSC patients, RCCLS sharing the same morphology: they
5 showed nested, papillary or trabecular architecture, an epithelial component with clear and
6 eosinophilic granular cytoplasm and a stromal component with thick bundles of smooth muscle
7 cells. They expressed CK7, CK20, CAIX, and CD10 and their cytogenetic profile showed no
8 alteration.
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14 Yang *et al.* described TSC-associated papillary RCC whose morphology is similar to the RCC
15 we described herein [2]. They had a papillary architecture with clear and eosinophilic cells
16 and eosinophilic cytoplasmic globules. The tumor was surrounded by thick fibrous stroma.
17
18 The immunophenotype was the same: CK7+, CAIX+, CD10+, AMACR- and TFE3- except
19 that SDHB was misinterpreted as negative[8]. Guo *et al.* also described similar RCC with
20 smooth muscle stroma in TSC patients [3]. The epithelial component had a branching
21 architecture with clear tumor cells. The immunohistochemical profile was also CK7+ and
22 CAIX+. Bah *et al.* reported a family case of RCCLS with the same morphology and
23 immunohistochemical profile [9]. It is very likely that these previous cases belong to the same
24 entity.
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34 Few studies analyzed the cytogenetic profile of these RCCLS in TSC patients. Parilla *et al.*
35 confirmed the absence of chromosomal alteration and Tyburczy *et al.* performed whole
36 exome sequencing and did not find any copy number changes either [10]. The absence of
37 chromosomal abnormality is very rare in RCC and consistent with the lack of aggressiveness
38 of these RCCLS [5,11,12].
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45 Leiomyomatous stroma is encountered in a wide variety of RCC; mainly in ccRCC, pRCC,
46 cpRCC, TCEB1-mutated RCC and tRCC (Supplementary data S1). RCCLS remains a
47 diagnosis of exclusion and the diagnosis should be based on the epithelial component. First of
48 all, ccRCC could be excluded by the presence of VHL deletion frequently observed in this
49 entity (Supplementary data S2) [5,12,13]. The absence of AMACR IHC staining and the lack
50 of trisomy 7 and 17 do not favor a pRCC [12,13]. In addition, the apical position of nuclei and
51 basal positivity of CAIX is not encountered in RCCLS [14]. Furthermore, a translocation of
52 *TFE3* or *TFEB* should be excluded by IHC and/or FISH analysis [7]. *TCEB1* mutated RCC
53 display a similar morphology to RCCLS but typically harbor an 8 monosomy [6]. Thus, we
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1 hypothesize that RCCLS in TSC patients is a distinct entity and the absence of chromosomal
2 alterations could help the differential diagnosis.
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5 Leiomyomatous stroma was demonstrated to be polyclonal and reactive contrary to the
6 epithelial component [4]. As *TSC1/2* is a tumor suppressor gene, a second hit is pivotal in
7 oncogenesis. Indeed, Tyburczy *et al.* and Bah *et al.* identified biallelic inactivation of TSC2
8 in RCCLS of TSC patients [9,10]. Interestingly, very small number of additional somatic
9 genetic events has been found, with no association with *VHL* mutation confirming the driver
10 impact of TSC inactivation. As for *VHL* in ccRCC, a biallelic inactivation of TSC could be
11 the driver of both hereditary and sporadic RCCLS. This sporadic counterpart was recently
12 demonstrated by Shah *et al* with somatic mutations of *TSC1* and *TSC2* identified in RCC with
13 the same morphology and immunohistochemical profile [5,15].
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23 In conclusion, even if our study is limited by the very low frequency of this entity, we
24 described, along with previous cases, a distinct subgroup of RCCLS in TSC patients. This
25 entity could be considered as a sentinel manifestation of TSC and thus added as a diagnostic
26 criterion. In consequence, an oncogenetic counselling should be advised to identify a TSC
27 disease.
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33 **Compliance with ethical standards**

34 Research involving human participants and/or animals: All procedures performed in studies
35 involving human participants were in accordance with the ethical standards of the institutional
36 and/or national research committee and with the 1964 Helsinki declaration and its later
37 amendments or comparable ethical standards.
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43 Informed consent: Informed consent was obtained from all individual participants included in
44 the study.
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List of abbreviations

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3 AMACR : Alpha-Methylacyl-CoA Racemase
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5 ccRCC : clear cell Renal Cell Carcinoma
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7 CGH : Comparative Genomic Hybridization
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9 cpRCC : clear cell papillary Renal Cell Carcinoma
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11 DNA : DesoxyriboNucleic Acid
12
13 FFPE : Formol Fixed Paraffin Embedded
14
15 FISH : Fluorescence In situ Hybridization
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17 IHC : Immunochemistry
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19 ISUP : International Society of Urological Pathology
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21 mTOR : mechanistic Target Of Rapamycin
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23 pRCC : papillary Renal Cell Carcinoma
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25 RCC : Renal Cell Carcinoma
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27 RCCLS : Renal Cell Carcinoma with Leiomyomatous Stroma
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29 SDHB : Succinate DeHydrogenase complex iron sulfur subunit B
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31 TCEB1 : Transcription Elongation factor B
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33 TFE3 : Transcription Factor binding to IGHM Enhancer 3
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35 tRCC : translocated Renal Cell Carcinoma
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37 TSC : Tuberos Sclérosis Complex
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39 VHL : Von Hippel Lindau
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Cases	Mutation	Age at diagnosis		Size (cm)	TNM	CK7	CK20	CAIX	CD10	AMACR	SDHB	TFE3	CGH	Follow-up (years)
		(years)	Sexe											
1	c.2585insT exon 20 TSC1	22	M	3	pT1a	++	+	++	++	-	+	-	neutral	12
2	c.440C>G exon 4 TSC2	31	M	6	pT1a	++	+	++	++	-	+	-	neutral	2
3	c.440C>G exon 4 TSC2	42	M	1	pT1a	++	+	++	++	-	+	-	neutral	1

TABLE 1. Summary of clinical, Immunohistochemistry, FISH and array-CGH features of the three RCCLS cases.

	Immunohistochemistry					Cytogenetic alteration			
	CK7	AMACR	CAIX	CD10	TFE3	VHL deletion	Monosomy 8	Trisomy 7 or 17	TFE3 or TFEB translocation
ccRCC	+/-	+/-	+	+	-	+	-	-	-
pRCC	+	+	-	-	-	-	-	+	-
ccpRCC	+	-	+	-	-	-	-	-	-
tRCC	-	+	-	+	+	-	-	-	+
TCEB1-mutated RCC	+	na	+	-	na	-	+	-	-
RCCLS in TSC patients	+	+	+	+	-	-	-	-	-

Supplementary Data S2. Immunohistochemistry and cytogenetics features of different RCC with leiomyomatous stroma.

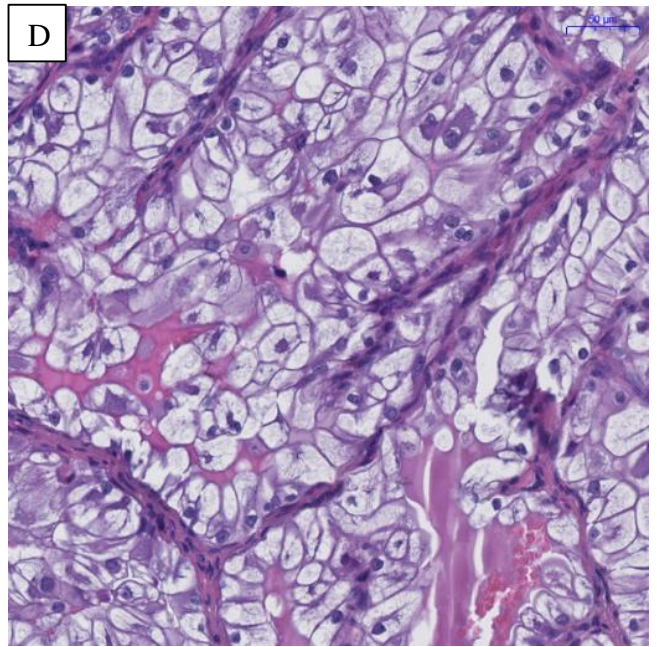
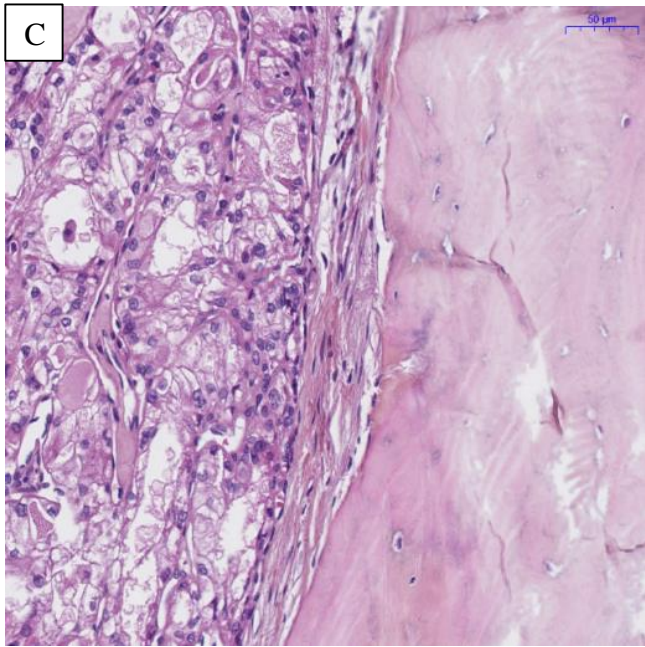
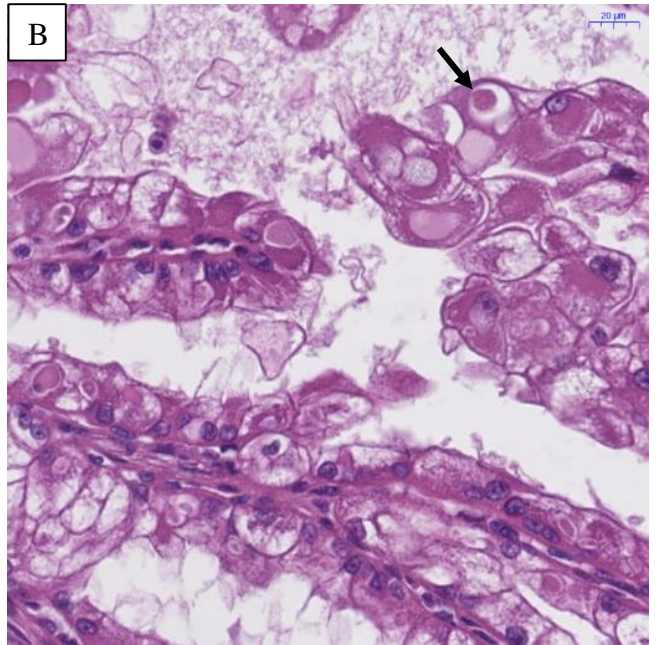
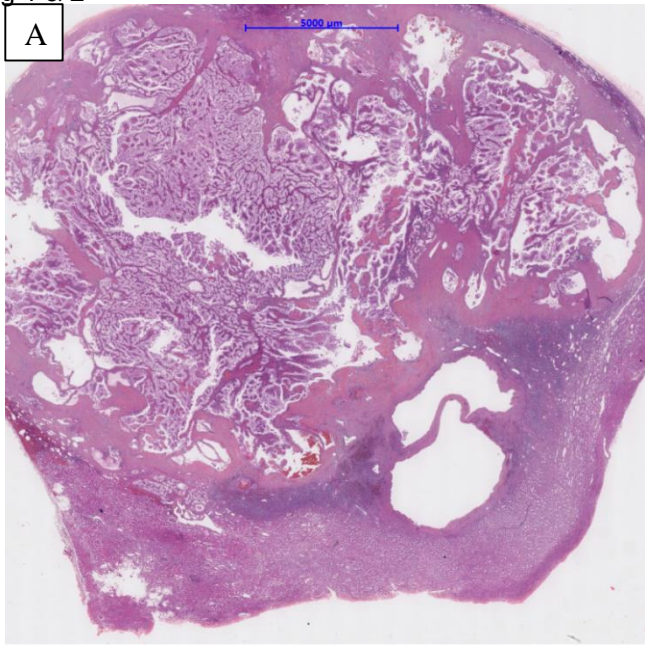


Figure 1: Case 1 showing well-circumscribed tumor (A , HES) and clarified and eosinophilic components with papillary architecture and tumor cells with eosinophilic globules (arrow) (B, HESx40). Case 2 showing osseous metaplasia (C, HESx20). Case 3 with the same papillary architecture (D, HESx20).

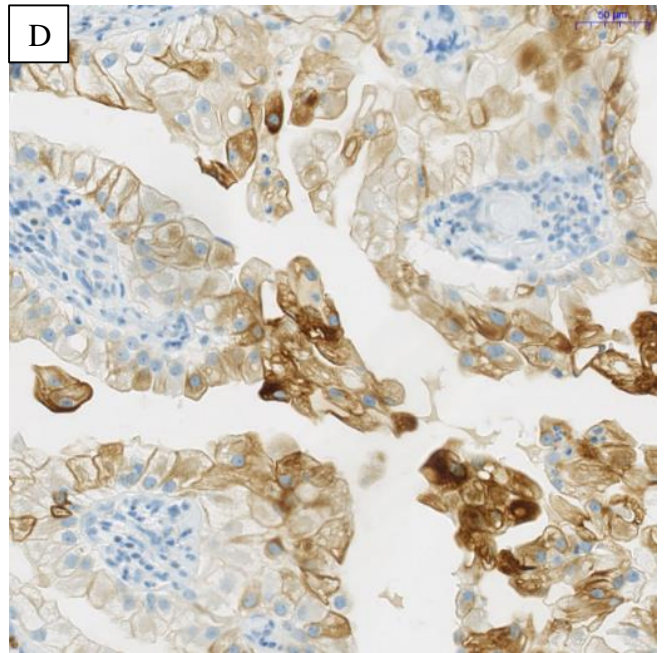
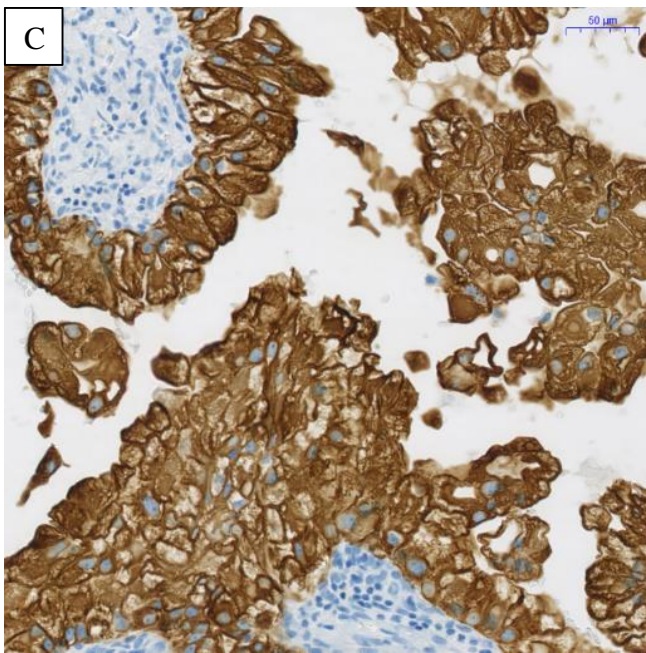
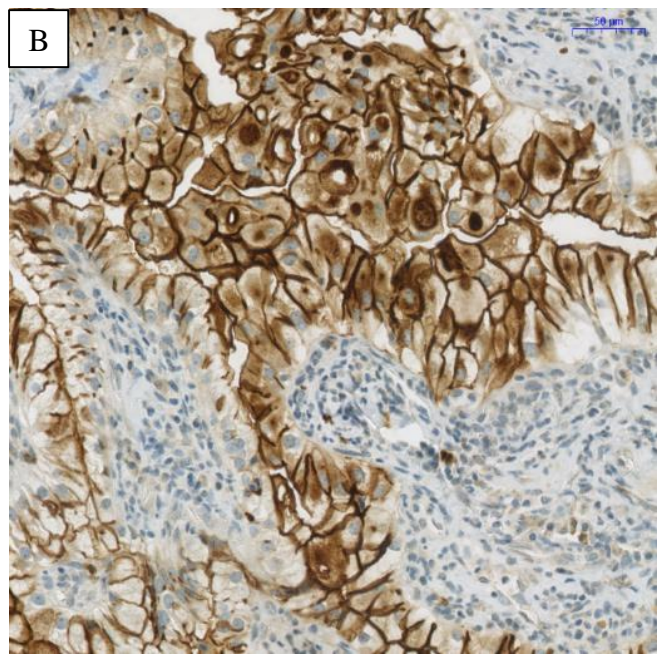
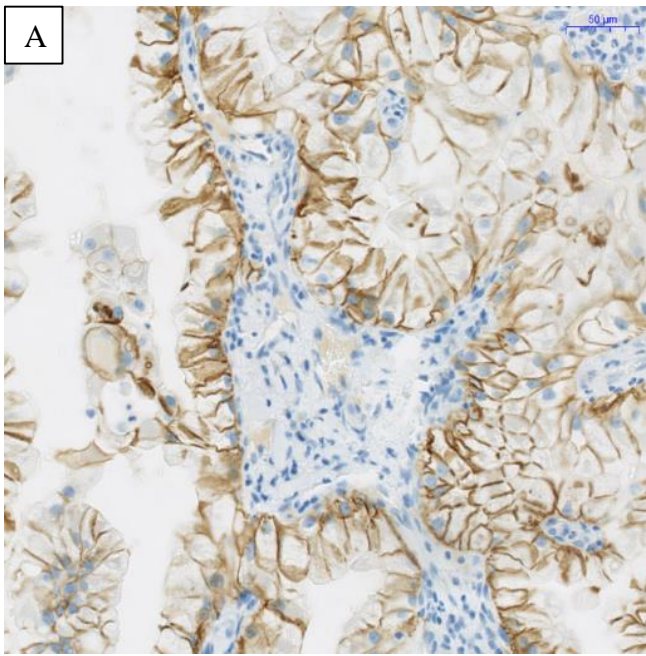


Figure 2: Immunohistochemistry x20, the immunohistochemistry profile was identical with membranous CAIX (A) and cytoplasmic CD10 (B), CK07 (C) and CK20 positivity (D).