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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Abstract:

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

Keywords :

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

1. Introduction

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 tuberin 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 oncocytic /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-macrocystic 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.

2.2. Pathological analysis

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

2.3. Immunochemistry

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

2.4. Comparative genomic hybridization (CGH) analysis

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

3. Results

3.1. Clinical Features

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

3.2. Pathological findings

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

3.3. Immunohistochemical features

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

3.4. Cytogenetic data

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

4. **Discussion**

In this study, we identified in TSC patients, RCCLS sharing the same morphology: they showed nested, papillary or trabecular architecture, an epithelial component with clear and eosinophilic granular cytoplasm and a stromal component with thick bunds of smooth muscle cells. They expressed CK7, CK20, CAIX, and CD10 and their cytogenetic profile showed no alteration.

Yang *et al.* described TSC-associated papillary RCC whose morphology is similar to the RCC we described herein [2]. They had a papillary architecture with clear and eosinophilic cells and eosinophilic cytoplasmic globules. The tumor was surrounded by thick fibrous stroma. The immunophenotype was the same: CK7+, CAIX+, CD10+, AMACR- and TFE3- except that SDHB was misinterpreted as negative[8]. Guo *et al.* also described similar RCC with smooth muscle stroma in TSC patients [3]. The epithelial component had a branching architecture with clear tumor cells. The immunohistochemical profile was also CK7+ and CAIX+. Bah et al. reported a family case of RCCLS with the same morphology and immunohistochemical profile [9]. It is very likely that these previous cases belong to the same entity.

Few studies analyzed the cytogenetic profile of these RCCLS in TSC patients. Parilla *et al.* confirmed the absence of chromosomal alteration and Tyburczy *et al.* performed whole exome sequencing and did not find any copy number changes either [10]. The absence of chromosomal abnormality is very rare in RCC and consistent with the lack of aggressiveness of these RCCLS [5,11,12].

Leiomyomatous stroma is encountered in a wide variety of RCC; mainly in ccRCC, pRCC, cpRCC, TCEB1-mutated RCC and tRCC (Supplementary data S1). RCCLS remains a diagnosis of exclusion and the diagnosis should be based on the epithelial component. First of all, ccRCC could be excluded by the presence of VHL deletion frequently observed in this entity (Supplementary data S2) [5,12,13]. The absence of AMACR IHC staining and the lack of trisomy 7 and 17 do not favor a pRCC [12,13]. In addition, the apical position of nuclei and basal positivity of CAIX is not encountered in RCCLS [14]. Furthermore, a translocation of *TFE3* or *TFEB* should be excluded by IHC and/or FISH analysis [7]. *TCEB1* mutated RCC display a similar morphology to RCCLS but typically harbor an 8 monosomy [6]. Thus, we

hypothesize that RCCLS in TSC patients is a distinct entity and the absence of chromosomal alterations could help the differential diagnosis.

Leiomyomatous stroma was demonstrated to be polyclonal and reactive contrary to the epithelial component [4]. As *TSC1/2* is a tumor suppressor gene, a second hit is pivotal in oncogenesis. Indeed, Tyburczy *et al.* and Bah *et al.* identified biallelic inactivation of TSC2 in RCCLS of TSC patients [9,10]. Interestingly, very small number of additional somatic genetic events has been found, with no association with *VHL* mutation confirming the driver impact of TSC inactivation. As for *VHL* in ccRCC, a biallelic inactivation of TSC could be the driver of both hereditary and sporadic RCCLS. This sporadic counterpart was recently demonstrated by Shah et al with somatic mutations of *TSC1* and *TSC2* identified in RCC with the same morphology and immunohistochemical profile [5,15].

In conclusion, even if our study is limited by the very low frequency of this entity, we described, along with previous cases, a distinct subgroup of RCCLS in TSC patients. This entity could be considered as a sentinel manifestation of TSC and thus added as a diagnostic criterion. In consequence, an oncogenetic counselling should be advised to identify a TSC disease.

Compliance with ethical standards

Research involving human participants and/or animals: All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent: Informed consent was obtained from all individual participants included in the study.

List of abbreviations

AMACR : Alpha-Methylacyl-CoA Racemase

ccRCC : clear cell Renal Cell Carcinoma

CGH : Comparative Genomic Hybridization

cpRCC : clear cell papillary Renal Cell Carcinoma

DNA : DesoxyriboNucleic Acid

FFPE : Formol Fixed Paraffin Embedded

FISH : Fluorescence In situ Hybridization

IHC : Immunochemestry

ISUP : International Society of Urological Pathology

mTOR : mechanistic Target Of Rapamycin

pRCC : papillary Renal Cell Carcinoma

RCC : Renal Cell Carcinoma

RCCLS : Renal Cell Carcinoma with Leiomyomatous Stroma

SDHB : Succinate DeHydrogenase complex iron sulfur subunit B

TCEB1 : Transcription Elongation factor B

TFE3 : Transcription Factor binding to IGHM Enhancer 3

tRCC : translocated Renal Cell Carcinoma

TSC : Tuberous Sclérosis Complex

VHL : Von Hippel Lindau

WHO : World Health Organization

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Table + Supp data S2

Cases	Mutation	Age at diagnosis (years)	Sexe	Size (cm)	TNM	CK7	CK20	CAIX	CD10	AMACR	SDHB	TFE3	CGH	Follow-up (years)
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, Immunochemistry, 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. Immunochemistry and cytogenetics features of different RCC with leiomyomatous stroma.

Fig 1 & 2

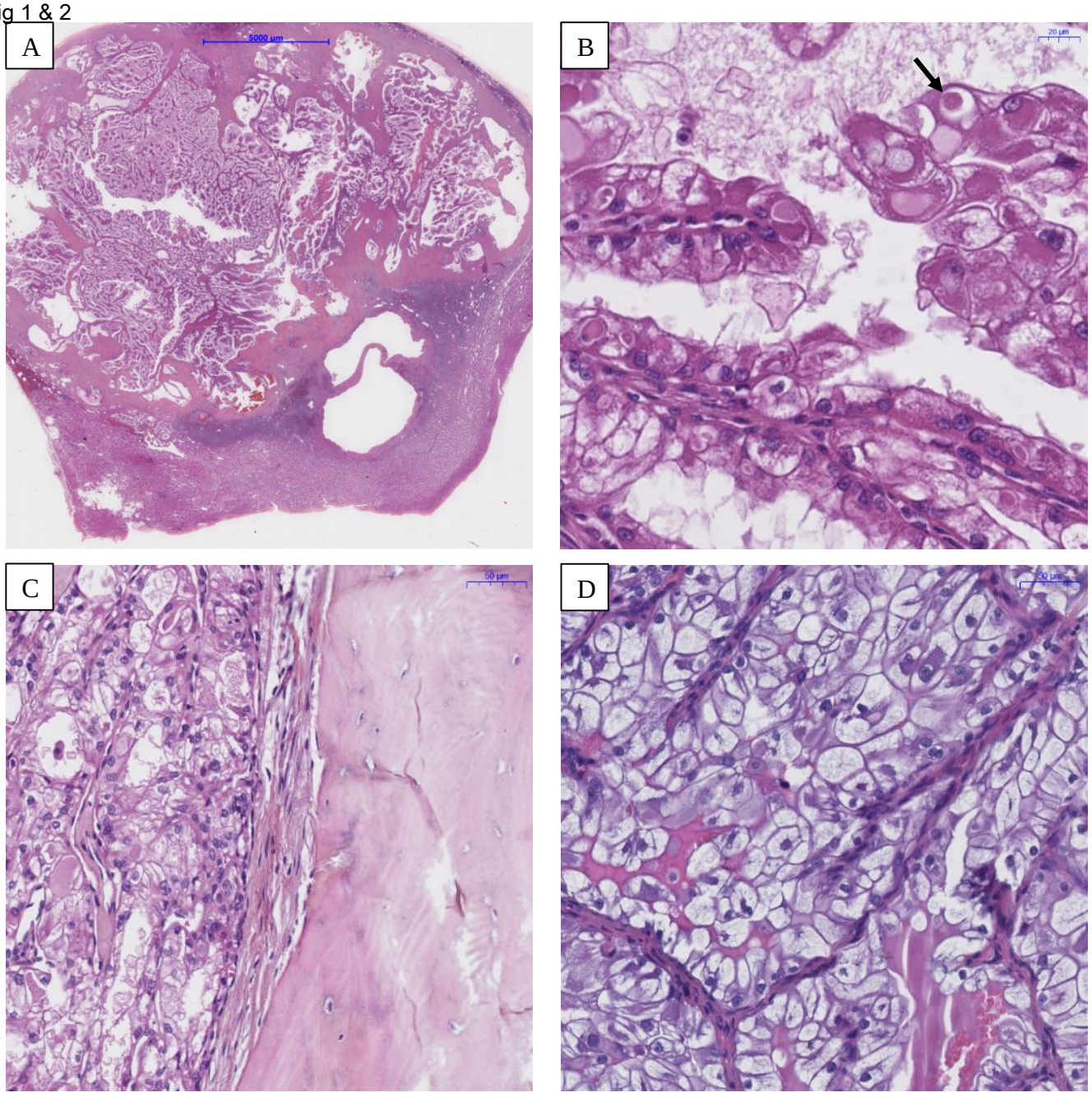


Figure 1: Case 1 showing well-circomscribed tumor (A , HES) and clarified and eosinophilic components with papillary architecture and tumor cells with eosinophilic globules (arrow) (B, HESx40). Case 2 showing osseous metaplasia (C, HESx20). Case 3 with the same papillary architecture (D, HESx20).

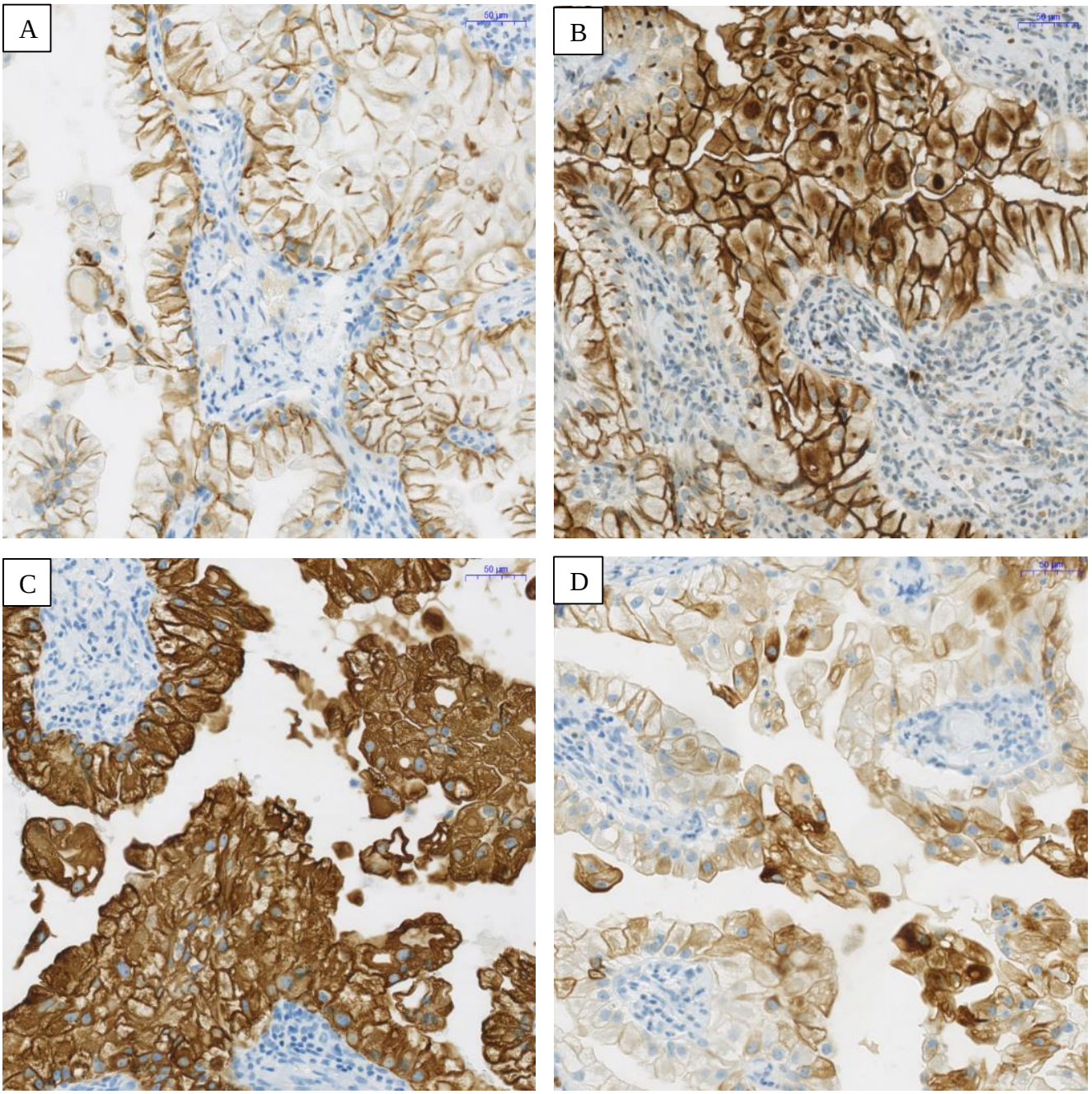


Figure 2: Immunohistochemistry x20, the immunohistochemistry profile was identical with membranous CAIX (A) and cytoplasmic CD10 (B), CK07 (C) and CK20 positivity (D).