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Low-grade oncocytic tumour of the kidney is characterised by genetic alterations of *TSC1*, *TSC2*, *MTOR* or *PIK3CA* and consistent GATA3 positivity

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Low-grade oncocytic tumour of the kidney is characterised by genetic alterations of *TSC1*, *TSC2*, *MTOR* or *PIK3CA* and consistent GATA3 positivity

Low-grade oncocytic tumour (LOT) of the kidney has recently emerged as a potential novel tumour type. Despite similarity to oncocytoma or eosinophilic chromophobe renal cell carcinoma, it shows diffuse keratin 7 immunohistochemistry (IHC) and negative KIT (CD117), which differs from both. We aimed to identify the molecular characteristics of these tumours. Seventeen tumours (one male, 16 female, nine previously published) fitting the original description of this entity (solid eosinophilic cell morphology, often with areas of tumour cells loosely stretched in oedematous stroma, and the above IHC features) were analysed with a next-generation sequencing panel of 324 cancer-associated genes from formalin-fixed, paraffin-embedded tissue. All tumours harboured at least one alteration in either *TSC1* (n = 7, 41%), *TSC2* (n = 2, 12%), *MTOR* (n = 5, 29%) or *PIK3CA* (n = 4, 24%). Four tumours harboured a second alteration, including two *NF2*, one each in conjunction with *MTOR* and *TSC2* alterations, one *PTEN* with *TSC1* alteration and one tumour with both *MTOR* and *TSC1* alterations. No other renal cancer-

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related or recurring gene alterations were identified. In addition to the previously described IHC findings, 16 of 16 were positive for GATA3. Eleven patients with follow-up had no metastases or recurrent tumours. Recurrent tuberous sclerosis/MTOR pathway gene alterations in LOT support its consideration as a distinct morphological, immunohistochemical and genetic entity. *PIK3CA* is another pathway member that may be altered in these tumours. Further study will be necessary to determine whether tumour behaviour or syndromic associations differ from those of oncocytoma and chromophobe carcinoma, warranting different clinical consideration.

Keywords: low-grade oncocytic tumour, MTOR, oncocytoma, *PIK3CA*, TSC1, TSC2

Introduction

Low-grade oncocytic tumour (LOT) of the kidney has been recently recognised as a potential distinct entity in the classification of renal neoplasms.[1–7] Despite bland cytology potentially mimicking oncocytoma several features are distinctive, including oedematous areas with loosely distributed cells (rather than round nests of cells), diffuse keratin 7 immunohistochemistry (IHC) and negative IHC for KIT (CD117).[1] Until recently, the only molecular characterisation has been predominantly copy number analysis, showing a few copy number alterations that are not prototypical of oncocytoma or chromophobe renal cell carcinoma.[1] However, emerging data now suggest a role for alterations in the tuberous sclerosis genes or mammalian target of rapamycin (*MTOR*) pathway. A subset of tumours was identified in tuberous sclerosis complex (TSC) patients in one study.[5] In another study, although the nomenclature 'LOT' was not used, alterations of these genes were found in eosinophilic renal cell tumours with diffuse keratin 7 IHC (group 2 in the study).[8] While the current study was in progress, a handful of other publications have also appeared in early online release, finding alterations of this pathway in LOT.[9–12] One study from some of us also found similar gene alterations in oncocytic tumours with diffuse keratin 7 labelling, even when morphology is more typical of oncocytoma.[13] We therefore sought to molecularly characterise low-grade oncocytic tumour of the kidney to attempt to identify defining genetic alterations.

Materials and methods

Following institutional review board approval, 17 renal tumours meeting the criteria previously described for LOT were retrieved from the authors' files.[1] The required features included predominantly solid eosinophilic cell architecture with areas of oedematous stroma containing loosely dispersed cells extending lengthwise, contrasting with the round nest architecture typical of oncocytoma. By inclusion criteria, tumours showed diffusely positive keratin 7 and negative KIT IHC. Of these, nine tumours were previously published in the original study describing this entity,[1] and the remaining eight had not been previously published; however, neither cohort had been previously studied for mutation status with next-generation sequencing. Thus, we aimed to focus upon the molecular alterations in the current study. More extensive IHC staining was not performed, as more than half were already comprehensively evaluated in the prior study.[1] However, based on anecdotal experience of GATA3 positivity in these tumours, we also performed IHC for GATA3 on 16 tumours (one did not have remaining tissue after molecular testing). GATA3 staining was performed with the GATA3 Biocare (Concord, CA, USA) clone L50-823 (mouse monoclonal) at 1:600 dilution with onboard heat-induced epitope retrieval and high pH CC1 buffer, using Ventana Ultraview diaminobenzidine (DAB) detection on the Ventana Benchmark Ultra (Tucson, AZ, USA) instrument. Follow-up information was reassessed in the patients from the previously published cohort and collected for the additional patients, whose data had not been previously published. Molecular analysis was performed on formalin-fixed, paraffin-embedded tumour tissue blocks from the tumours, using a panel that detects small nucleotide variants/substitutions, small insertions or deletions and copy number variations in 324 cancer-associated genes (including the tuberous sclerosis genes and members of the MTOR pathway). The panel was based on the Illumina® HiSeq 4000 platform, and used the same methods recently reported in another study.[13]

Results

Patient ages ranged from 41 to 80 years. One patient was male; the remaining 16 were female. All 17 tumours (Figure 1) were solitary/unifocal and showed diffuse positive immunohistochemistry for keratin 7 and a negative result for KIT immunohistochemistry. All tumours (16 of 16) showed IHC positivity for GATA3 Figure 2, most often diffusely distributed with strong intensity (n = 11) and not less than multifocal moderate positivity (Figure 2). From the genes tested in the sequencing panel, all tumours harboured at least one alteration in either *TSC1* (n = 7, 41%), *TSC2* (n = 2, 12%), *MTOR* (n = 5, 29%) or *PIK3CA* (n = 4, 24%, Table 1, Figures 3 and 4). In addition, four tumours harboured a second alteration, including two *NF2*, one each in conjunction with *MTOR* and *TSC2* alterations, one *PTEN* with *TSC1* alteration and one tumour with both *MTOR* and *TSC1* alterations. No recurrent alterations were identified in the remainder of the genes studied in the panel. Although we did not test normal tissue, blood or saliva to assess for germline status there were fewer than 50% variant allelic fractions, suggesting that these mutations were less likely to be germline. An additional length of follow-up was available in a subset of the previously published patients, and a subset of the new patients had available information. Eight patients from the previous cohort had updated follow-up, now ranging from 38 to 169 months, with none

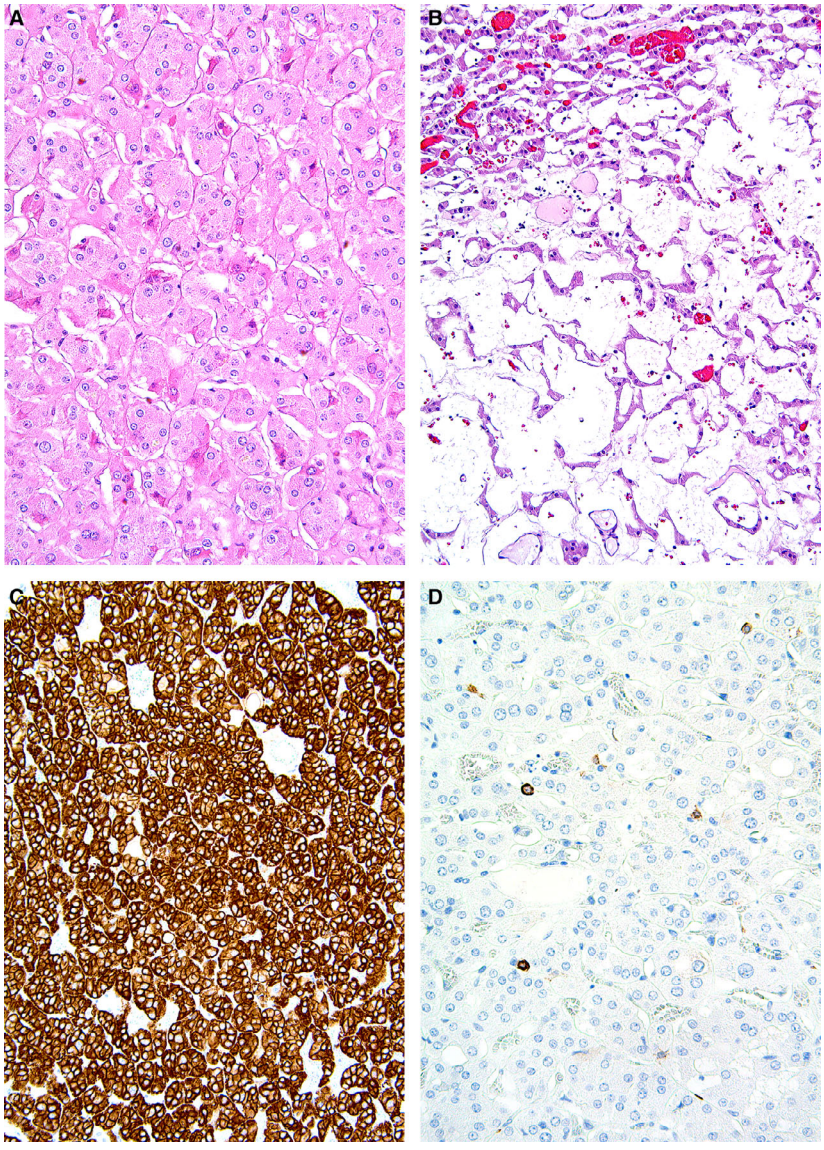

Figure 1. Tumours showed predominantly compact eosinophilic cell morphology (**A**), often with oedematous stroma containing tumour cells loosely stretched in this oedema (**B**). By inclusion criteria and fitting the original description of this entity, all were positive for keratin 7 (**C**) and showed negative KIT staining (**D**), often with scattered mast cells being positive.

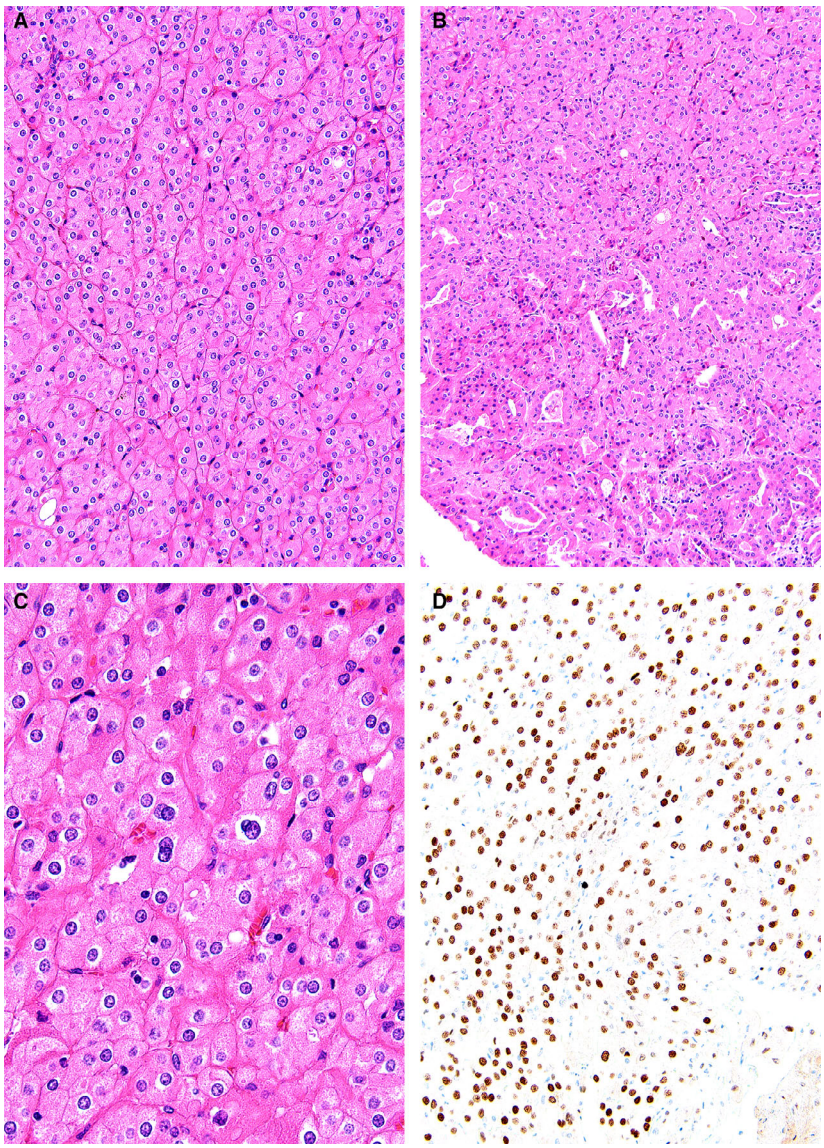

Figure 2. In this low-grade oncocytic tumour (**A**), morphology is predominantly solid, with focal tubular architecture (**B**, bottom). Perinuclear clearing ('halo') is appreciable at high magnification (**C**). GATA3 showed consistent positivity (**D**).

having metastasis or recurrence. One had died of other causes at 16 to 40 months, again with none showing recurrence or metastasis.

Discussion

Low-grade oncocytic tumour of the kidney has emerged recently as a potential diagnostic entity in renal tumour pathology.[1–3,5–7,11,14,15] The morphology of these tumours closely resembles that of oncocytoma and eosinophilic chromophobe renal cell carcinoma; however, some subtle morphological differences are notable, including the predominantly solid growth pattern with strands of tumour cells (rather than nests) in oedematous stroma. Similarly, the immunophenotype differs from typical oncocytoma and chromophobe carcinoma. Keratin 7 positivity in renal tumours is usually minor in tumours with eosinophilic cells, such that oncocytoma shows a predominantly negative pattern with only scattered individual cells positive.[16,17] Classic chromophobe renal cell carcinoma is often keratin 7 positive with membranous accentuation. Eosinophilic chromophobe renal cell carcinoma may have slightly greater keratin 7 staining than oncocytoma; however, a greater extent than that of oncocytoma is not necessarily a requirement.[16] We have also seen occasional classic chromophobe renal cell carcinomas with negative or minimal keratin 7 labelling. Surprisingly, LOT is uniformly eosinophilic, but it shows diffuse keratin 7

positivity. Secondly, positive IHC for KIT is common in both oncocytoma and chromophobe carcinoma; however, LOT is typically negative (often showing only mast cells highlighted by this staining reaction). Despite differences from oncocytoma and chromophobe carcinoma, LOT appears to be non-aggressive with no reports of metastasis to date, in keeping with the idea that renal oncocytic neoplasms typically have a low risk of adverse behaviour.

We also noted consistent GATA3 immunohistochemical positivity in the low-grade oncocytic tumours in the current study. Although GATA3 is widely considered a marker of urothelial and breast carcinoma, positivity has been noted in several renal cell tumour types, probably suggesting a distal tubular phenotype. This includes subsets of chromophobe renal cell carcinomas and oncocytomas (less frequently for the latter),[18] which are typically thought of as having an intercalated cell phenotype of the distal tubule. Other renal cell tumours with GATA3-positive reactions include clear cell papillary renal cell tumour[19] and the recently recognised papillary renal neoplasm with reverse polarity.[20] Some of us have also noted a pattern of tubular proliferation under the proposed designation of distal tubular hyperplasia, which is consistently GATA3-positive.[21] Although these small lesions would probably be most in keeping with papillary adenomas based on prior classification schemes, they seem to exhibit several morphological and immunohistochemical differences from papillary adenoma, suggesting a different tubular phenotype. Therefore, GATA3 is not an entirely specific marker in the context of renal cell neoplasms, but the finding of consistent positivity further elucidates the expected pattern of immunohistochemical markers in low-grade oncocytic tumour.

Emerging molecular data now suggest a role for alterations of the tuberous sclerosis genes and MTOR pathway in these tumours. Until recently, only scattered suggestions of molecular data for these tumours could be gleaned from studies that did not focus specifically on this entity or use this terminology.[8,22–24] However, concurrently with this study, a few different groups have found similar results to those reported in this series.[9,10,23] Although they largely did not use the LOT terminology, Tjota and colleagues found that eosinophilic renal tumours with the keratin 7-positive, keratin 20-negative, vimentin-negative phenotype had mutations in *TSC1*, *TSC2* and *MTOR*.[23] They noted that these tumours lacked the typical copy number alterations of a comparison group of chromophobe renal cell carcinoma. In their Discussion section, they noted that this cohort probably corresponds to LOT, as would another cohort designated 'group 2' in a previous study by several of the same authors.[8] At the same time, Morini and colleagues found similar alterations (predominantly *MTOR* and occasionally *TSC1*) in eight of 10 tumours, with consistently negative immunohistochemistry for forkhead box I1 (FOXI1), contrasting with normal distal tubular intercalated cells.[10] Tong and Hu also noted that FOXI1 was typically positive in intercalated cells, chromophobe renal cell carcinoma and oncocytoma. An outlier group with negative FOXI1 also harboured frequent *MTOR* mutations, probably corresponding to LOT.[25] This is interesting, as GATA3 positivity is shared with a subset of chromophobe renal cell carcinomas and oncocytomas which are thought to be of intercalated cell phenotype, but FOXI1 differs in low-grade oncocytic tumour. Again concurrently with the study by Morini, Kapur and colleagues found alterations in *MTOR*, *TSC1* and *RHEB* in seven patients with LOT. Some of these patients had multiple tumours, including one who was found to have a probably pathogenic germline *TSC1* mutation.[9] As such, like the other emerging entity eosinophilic solid and cystic renal cell carcinoma, it appears that there are probably both hereditary (TSC-associated) and sporadic forms of this tumour. For example, a subset of tumours in patients with tuberous sclerosis has been noted to be chromophobe-like,[26] at least some of which probably correspond to LOT. Another study by some of us also recently found that oncocytic renal tumours with diffuse keratin 7 reactivity harbour recurrent *MTOR*, *TSC1*, *TSC2*, *STK11* and *PI3KCA* alterations.[13] Finally, Zhang *et al.* also recently noted *TSC1*, *TSC2* and *MTOR* alterations in LOT.[11]

It is notable now that several emerging subtypes of eosinophilic renal neoplasm are characterised by TSC1/TSC2/MTOR pathway alterations, including low-grade oncocytic tumour, eosinophilic vacuolated tumour (formerly known as high-grade oncocytic tumour or renal cell carcinoma with eosinophilic vacuolated cytoplasm) and eosinophilic solid and cystic renal cell carcinoma. However, despite these tumours sharing the features of eosinophilic cells and MTOR/TSC pathway alterations, there are consistent morphological and immunohistochemical differences between these three entities, suggesting that they can be recognised as distinct in practice.[6,15,27–31] However, there are probably also tumours with overlapping features between two or more of these entities.[32] Similarities and differences between these neoplasms are shown in Table 2.

Table 1. Patient characteristics and genetic alterations in the studied tumours

Patient number	Included in prior series	Age	Gender	Size (mm)	Gene symbol	Variant annotation (p.)	Variant annotation (c.DNA)	Pathogenic role	Variant type	Molecular consequence
1		61	F	80	MTOR	p.Ile2500Met	c.7500 T > G	Pathogenic	Single nucleotide variant	Missense
2		73	F	69	MTOR	p.Leu2427Gln	c.7280 T > A	Pathogenic	Single nucleotide variant	Missense
3		80	F	66	NF2	p.Glu463Lys	c.1387G > A	Probably benign/ uncertain significance	Single nucleotide variant	Missense
3*					TSC2	p.Arg120Ter	c.358A > T	Pathogenic	Single nucleotide variant	Nonsense
4	Y	77	M	60	PIK3CA	p.Glu545Lys	c.1633G > A	Pathogenic/ probably pathogenic	Single nucleotide variant	Missense
5	Y	63	F	52	MTOR	p.Ser2215Phe	c.6644C > T	Pathogenic/ probably pathogenic	Single nucleotide variant	Missense
5*					TSC1	p.Arg786Ter	c.2356C > T	Pathogenic	Single nucleotide variant	Nonsense
6	Y	68	F	50	TSC1	p.Ser403Ter	c.1208C > A	Pathogenic	Single nucleotide variant	Nonsense
7		70	F	38	TSC1	p.Glu876Ter	c.2626G > T	Pathogenic	Single nucleotide variant	Nonsense
8	Y	61	F	30	NF2	p.Glu463Lys	c.1387G > A	Pathogenic	Single nucleotide variant	Missense
8*					MTOR	p.Cys1483Phe	c.4448G > T	Pathogenic	Single nucleotide variant	Missense
9	Y	53	F	25	PIK3CA	p.Glu81Lys	c.241G > A	Pathogenic	Single nucleotide variant	Missense
10	Y	66	F	23	TSC1	p.Gln956Ter	c.2866C > T	Pathogenic	Single nucleotide variant	Nonsense
11	Y	68	F	22	TSC1	p.Glu876Ter	c.2626G > T	Pathogenic	Single nucleotide variant	Nonsense
12		64	F	22	PIK3CA	p.Glu545Lys	c.1633G > A	Pathogenic	Single nucleotide variant	Missense
13	Y	68	F	20	PIK3CA	p.Phe83Ser	c.248 T > C	Pathogenic	Single nucleotide variant	Missense
14		65	F	20	TSC1	Allelic loss 9q34.13	Allelic loss 9q34.13	Pathogenic	Allelic loss 9q34.13	Deletion
14*					PTEN	Bi-allelic loss del(1p36.33)	Bi-allelic loss del(1p36.33)	Pathogenic	Bi-allelic loss	Deletion
15		41	F	20	MTOR	p.Ile2500Phe	c.7498A > T	Probably pathogenic	Single nucleotide variant	Missense

Table 1. (*Continued*)

Patient number	Included in prior series	Age	Gender	Size (mm)	Gene symbol	Variant annotation (p.)	Variant annotation (c.DNA)	Pathogenic role	Variant type	Molecular consequence
16		77	F	16	TSC1	p.Gln956Ter	c.2866C > T	Pathogenic	Single nucleotide variant	Nonsense
17	Y	56	F	11	TSC2	p.Val534Leu	c.1600G > T	Benign/ uncertain significance	Single nucleotide variant	Missense

*Indicates a second genetic alteration in the same patient.

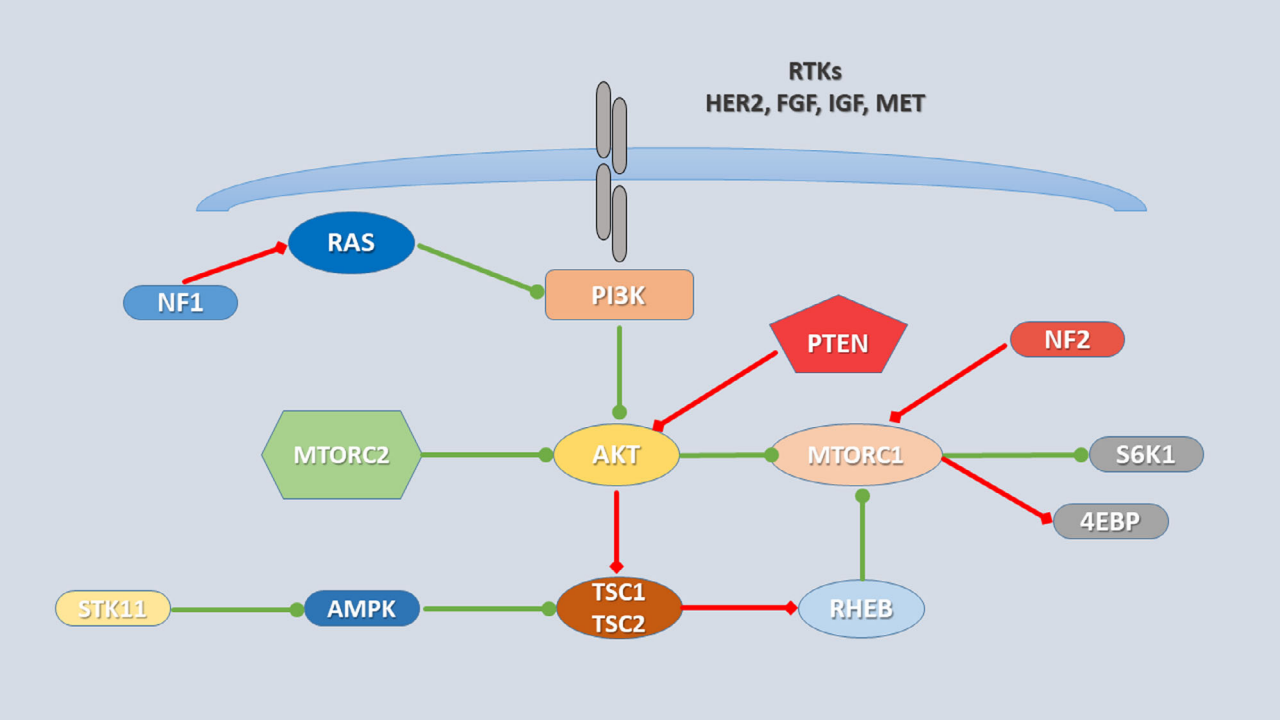

Figure 3. MTORC1 complex is one of the master regulators of cell growth and metabolism. Its activity is regulated by the tuberous sclerosis complex (TSC). TSC is a GTPase activating protein. The GTPase protein RHEB regulates MTOR by increasing its activity. TSC inactivates RHEB and in turn downregulates MTOR activity. AMPK activates TSC complex which, in turn, regulates the activity of MTOR. AKT signalling cascade is activated by receptor tyrosine kinases which induce production of phosphatidylinositol[3–5] triphosphates (PIP3) by phosphoinositide 3-kinase (PI3K). NF1 protein, neurofibromin 1, negatively regulates RAS proteins through GTPase activity. RAS is an activator of the phosphatidylinositol-3-kinase (PI3K)-AKT pathway. The tumour suppressor phosphatase and tensin homologue (PTEN) inhibits AKT activity by dephosphorylating PIP3. AKT regulates cell growth through its effects on the TSC1/TSC2 complex and MTORC signalling. Green arrows indicate positive signals and red arrows indicate negative/inhibitory signals.

In this series, we report molecular features of an additional 17 tumours fitting the reported criteria for LOT. The concurrent finding of alterations involving the TSC1/TSC2/MTOR pathway by several independent groups provides further support for this tumour type as having a distinct pathogenesis that leads to its subtly distinct morphological and immunohistochemical phenotype. Although we believe this warrants consideration of low-grade oncocytic tumour as a distinct morphological, immunohistochemical and molecular entity, it remains to be determined whether it has clinical significance, mainly whether behaviour differs from that of oncocytoma/chromophobe renal cell carcinoma. At the minimum, it is probably relevant that at least a subset of these patients has multiple tumours, some of

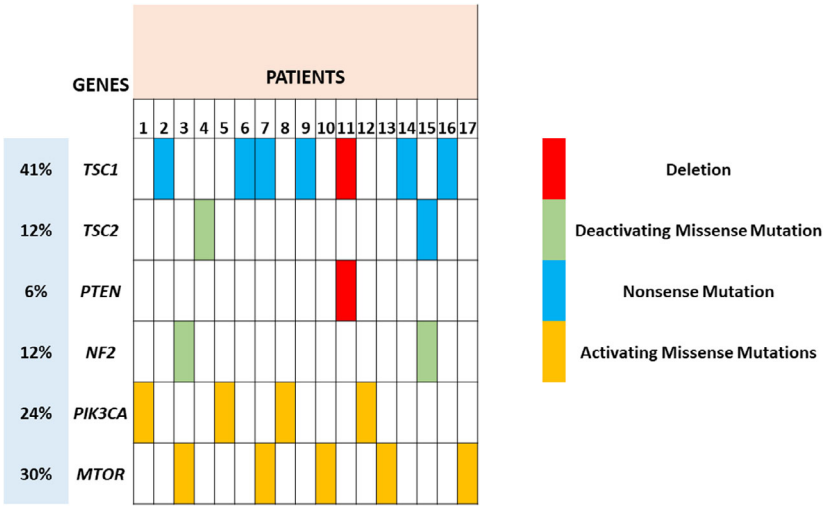

Figure 4. All 17 tumours harboured genomic abnormalities, and all (100%) primarily involved the MTOR pathway. TSC1 inactivating mutation[6] and deletion[1] was identified in seven tumours. TSC2 mutation (one missense and one nonsense) was seen in two tumours. Other mutations observed were bi-allelic loss of PTEN[2] and activating mutations of PI3KCA gene.[4] One tumour had both NF2 and TSC2 mutations and the other tumour had both NF2 and MTOR mutations. One tumour had bi-allelic loss of PTEN and TSC1 deletion, and other had MTOR activating mutation along with chain termination (nonsense) mutation of TSC1.

Table 2. Low-grade oncocytic tumour, eosinophilic solid and cystic renal cell carcinoma and eosinophilic vacuolated tumour are emerging subtypes of renal neoplasm that share genetic findings of alterations in the TSC/MTOR pathways

	Low-grade oncocytic tumour	Eosinophilic solid and cystic renal cell carcinoma	Eosinophilic vacuolated tumour
Morphology	Solid, oncocytoma-like, with loose cells in oedema rather than nests; possible perinuclear clearing (halo)	Solid and frequently cystic growth of eosinophilic cells with prominent nucleoli, hobnail-shaped configuration, and cytoplasmic stippling	Solid or trabecular architecture with variable collagenous stroma and thick blood vessels; cells with prominent nucleoli and cytoplasmic vacuoles
Keratin 7	Diffuse positive	Usually minimal	Usually minimal
KIT (CD117)	Negative, but highlights mast cells	Usually negative	Often positive
Cathepsin K	Negative	Sometimes positive	Often positive
Vimentin	Negative	Often positive	Negative
Keratin 20	Negative	Frequently positive, sometimes focal	Sometimes focal

Despite having similar genetic alterations and eosinophilic cells, there are some recurring differences in morphology and immunohistochemistry.

which probably have tuberous sclerosis complex. Additional points of novelty in this study include the presence of *PIKC3A* alterations in a subset of tumours (Table 1 and Figure 3), which contributes to the same pathways as *TSC1*, *TSC2* and *MTOR*. This probably widens the spectrum of potential genetic alterations in LOT. Secondly, we note consistent GATA3 positivity in LOT, which may serve as another potentially useful diagnostic marker in surgical pathology practice.

Conflicts of interest

Funded in part by Henry Ford Health System internal funding to SRW (A20063).

Data availability statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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