Proliferation of myofibroblasts in the stroma of renal oncocytoma

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Abstract

Objectives: Myofibroblasts are a vital component of stroma of many malignant neoplasms, but it is not yet established whether stromal myofibroblasts also exist in benign tumours such as oncocytoma of the kidney.

Materials and methods: Histomorphological and immunohistochemical analysis of 16 renal oncocytomas diagnosed at Chang Gung Memorial Hospital, Taiwan, has been performed.

Results: Renal oncocytomas were composed of oncocytes, large cells with granular eosinophilic cytoplasm, arranged mostly in sheets, in tubulocystic or combined pattern. Few oncocytes appeared to be undergoing proliferation or apoptosis. MIB-1 and active caspase 3 indices were low, but higher in tumour than in surrounding non-tumour parenchyma (MIB-1: 0.93 ± 0.09 versus 0.46 ± 0.07 , $P < 0.001$ and active caspase 3: 0.76 ± 0.08 versus 0.41 ± 0.09 , $P < 0.001$). Wnt/ β -catenin signalling was not implicated in this neoplasm, as there was no loss of E-cadherin membranous localization or expression of intranuclear β -catenin in the cells. Clumps of oncocytes were stained with periodic acid Schiff and had collagen I-, collagen III- and fibronectin-positive, but desmin- and human caldesmon-negative stromas. Importantly, α -smooth muscle actin (SMA)-immunostaining established the myofibroblastic nature of many of the stromal cells. Some of the myofibroblasts were also positive for MIB-1, indicating a proliferative role for them in the stroma.

Conclusions: Renal oncocytomas were composed of two independent compartments: benign oncocytes and pronounced fibrotic stroma, which consisted of

proliferating myofibroblasts (SMA- and MIB-1-positive) which were associated with excessive deposition of extracellular matrix (periodic acid Schiff-component, collagen I-, collagen III- and fibronectin-positive, and desmin- and human caldesmon-negative).

Introduction

Oncocytoma, a rare benign kidney tumour of collecting duct cell origin, was first described by Zippel in 1942 (1). This tumour has been subsequently identified in thyroid, parathyroid, salivary and adrenal glands. In 1976, Klein and Valensi (2) suggested that renal oncocytoma was a distinct clinicopathological entity and since then, renal oncocytoma has been diagnosed more frequently. Macroscopically, renal oncocytoma is typified by its well-circumscribed, frequently encapsulated appearance, its uniform tan colour and a lack of evidence of necrosis or haemorrhage (3). On cross-sectioning, a central satellite scar is often observed. Microscopically, renal oncocytoma is characterized by predominance of eosinophilic epithelial cells called oncocytes, with prominent mitochondria-rich cytoplasm arranged in a solid trabecular or tubular pattern (3); other organelles are sparse. Mitotic activity is usually absent, and nuclear pleomorphism is rare.

Malignant solid tumours are composed of two interdependent compartments: malignant cells and the stroma that they induce around themselves and in which they are dispersed (4). Tumour stroma is a complex medium in which a variety of interactions takes place between tumour and normal tissue cells. Tumour cells proliferate and invade the stroma whereas immune cells congregate around tumour nests and from where tumour angiogenesis is promoted (5). Dynamic changes in cancer-associated tissue stroma are collectively termed a desmoplastic reaction as they resemble wound-healing (4). The desmoplastic reaction is supported mainly by activation of local fibroblasts as myofibroblast type (6). These myofibroblasts have differentiated from fibroblasts and now express SMA as

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cytoplasmic microfilaments, and desmin to a limited extent, whereas quiescent host-resident fibroblasts express vimentin as intermediate filament proteins (7).

Myofibroblasts are associated with tumour cells at all stages of cancer progression (8). In various malignancies, tumour-dependent differentiation of fibroblasts toward myofibroblasts further promotes neoplastic progression (9). It is known that collagen I, the major extracellular matrix component produced by myofibroblasts, not only functions as a scaffold for the tissue but also regulates expression of genes associated with cell signalling and metabolism and gene transcription and translation, thus affecting fundamental cellular processes that are essential for tumour progression. For example, collagen I expression has been found to be a signal for invasion, and its intratumoral expression level has been associated with tumour invasiveness (10).

Myofibroblasts have been described in fibrocontractive disease (11) and in tumours such as cancers of the colon (12), liver (13), lung (14), prostate (15), pancreas (16) and breast (17). It has been proposed that appearance of myofibroblasts precedes onset of metastatic cell invasion and contributes to tumour growth and progression (18). Their pro-invasive activity is dependent on capacity to secrete extracellular matrix degrading proteases (19) and to participate in synthesis of extracellular matrix components, which alter adhesive and migratory properties of epithelial cancer cells (20).

The aim of this study was to examine whether stromal myofibroblasts also exist in benign neoplasms such as oncocytoma of the kidney.

Materials and methods

Clinical data

This analysis included 16 consecutive oncocytomas, from 1667 patients with renal neoplasms, diagnosed between 1987 and 2002 at the Chang Gung Memorial Hospital, Taipei, Taiwan. Table 1 shows the summary of clinical courses of the patients, eight men and eight women, age range 47–79 years (mean, 54.5 years). Tumours varied in size from 3–15 cm diameter (mean, 6.6 cm), ten were in the right kidney, six in the left, none found bilaterally. Approximately 38% (6 of 16) were discovered incidentally during diagnostic evaluation for complaints unrelated to kidney disease or to renal neoplasia. In addition, three patients (or 18.8%) reported abdominal pain, four (25%) reported flank pain, two (12.5%) reported gross haematuria and one (6.2%) had recent-onset hypertension. Neither weight loss nor fever was a symptom in any patient; all were examined thoroughly before surgery, by a range of radiological exam-

inations. However, pre-operatively, all patients were tentatively diagnosed as having renal cell carcinoma. Perioperatively, frozen sections from three patients revealed renal oncocytoma in two of them and renal cell carcinoma in one. Accordingly, surgeons were uncertain about accuracy of diagnosis after frozen section examination. The preliminary diagnosis had been renal cell carcinoma; thus, they performed only unilateral nephrectomy. Hence, 10 patients received right radical nephrectomy and six patients received left radical nephrectomy. Post-operatively, all 16 patients were followed up from 12 to 189 months, mean 58.7 months. All patients survived with no evidence of tumour recurrence.

Immunostaining

Sections cut at 4 µm were dewaxed and incubated in hydrogen peroxide (2.4 ml, 30%) in methanol (400 ml) to block endogenous peroxidases, and rehydrated through graded alcohols with water, then with phosphate-buffered saline (PBS) (21–23) (Table 2). Sections were also incubated for 2 min in acetic acid (20%) in methanol to block endogenous alkaline phosphatase. For antigen retrieval of MIB-1, active caspase 3, β -catenin and E-cadherin, collagen I, collagen III, fibronectin, desmin, human-caldesmon immunostaining, sections were subjected to microwaving (700 W) in 0.01 M citrate buffer at pH 6 for 20 min. For SMA immunostaining, antigen retrieval was performed initially by incubation with trypsin at pH 7.8 for 15 min, but it was found later that the immunostaining also worked well without trypsin pre-treatment (24). To reduce non-specific background staining, sections were pre-incubated with either (i) normal rabbit (X0902; Dako, Glostrup, Denmark) or swine serum (X0901; Dako, Glostrup, Denmark) at dilution of 1:25 for 15 min, or (ii) 3% bovine serum albumin (Sigma, St Louis, MO, USA) at dilution of 1:25 for 15 min. After washing in PBS, sections were incubated for 35 min with primary antibodies against: (i) MIB-1 (M7240; Dako, Glostrup, Denmark) at 1:100 dilution, (ii) active caspase 3 (AF835; R&D, Minneapolis, MN, USA) at 1:100 dilution, (iii) SMA (A2547; Sigma) 1:2000 dilution, (iv) β -catenin (610154; BD, San Jose, CA, USA) 1:100 dilution, (v) E-cadherin (SC8426; Santa-Cruz, CA, USA) 1:30 dilution, (vi) collagen I (1310-01; Southern Biotech, Birmingham, AL, USA) 1:300 dilution, (vii) collagen III (ab6310; Abcam, Cambridge, UK) 1:600 dilution, (viii) fibronectin (ab2413; Abcam, Cambridge, UK) 1:300 dilution, (ix) desmin (M0760; Dako, Glostrup, Denmark) 1:50 dilution and (x) human caldesmon (M3557; Dako, Glostrup, Denmark) 1:200 dilution. For the second layer following PBS washing, sections were incubated with either: (i) biotinylated swine anti-rabbit (E353, Dako) 1:500 dilution, 35 min for rabbit anti-

Table 1. Course and outcome of 16 oncocytomas, among 1667 cases of renal neoplasms diagnosed between 1987 and 2002 at Chang Gung Memorial Hospital. (*N* = 16)

Case	Age/ sex	Tumour size (cm)/location/ presentation	Pre-operative diagnosis	Perioperative frozen section	Post-operative diagnosis	Surgery	Months after surgery/ tumour recurrence
1	59/M	12 × 7 × 4/left/incidental	Renal cell carcinoma	Not performed	Renal oncocytoma	Left nephrectomy	20/no
2	56/M	11 × 10 × 7/right/abdominal pain	Renal cell carcinoma	Not performed	Renal oncocytoma	Right nephrectomy	51/no
3	66/F	5 × 4 × 4/left/incidental	Renal cell carcinoma	Not performed	Renal oncocytoma	Left nephrectomy	40/no
4	51/F	11 × 9 × 6/right/flank pain	Renal cell carcinoma	Not performed	Renal oncocytoma	Right nephrectomy	80/no
5	54/F	4 × 4 × 3/right/abdominal pain	Renal cell carcinoma	Not performed	Renal oncocytoma	Right nephrectomy	39/no
6	65/F	11 × 8 × 5/right/flank pain	Renal cell carcinoma	Not performed	Renal oncocytoma	Right nephrectomy	121/no
7	70/M	4 × 3 × 3/right/flank pain	Renal cell carcinoma	Renal oncocytoma	Renal oncocytoma	Right nephrectomy	13/no
8	52/F	5 × 4 × 4/left/high blood pressure	Renal cell carcinoma	Not performed	Renal oncocytoma	Left nephrectomy	13/no
9	79/M	5 × 4 × 3/right/incidental	Renal cell carcinoma	Not performed	Renal oncocytoma	Right nephrectomy	68/no
10	47/M	3 × 3 × 2/left/incidental	Renal cell carcinoma	Renal cell carcinoma	Renal oncocytoma	Left nephrectomy	12/no
11	54/F	4 × 4 × 3/left/abdominal pain	Renal cell carcinoma	Renal oncocytoma	Renal oncocytoma	Left nephrectomy	16/no
12	69/M	4 × 4 × 3/right/incidental	Renal cell carcinoma	Not performed	Renal oncocytoma	Right nephrectomy	44/no
13	52/F	8 × 7 × 6/right/gross haematuria	Renal cell carcinoma	Not performed	Renal oncocytoma	Right nephrectomy	70/no
14	49/M	4 × 3 × 3/right/gross haematuria	Renal cell carcinoma	Not performed	Renal oncocytoma	Right nephrectomy	88/no
15	49/F	15 × 8 × 8/left/incidental	Renal cell carcinoma	Not performed	Renal oncocytoma	Left nephrectomy	189/no
16	49/M	4 × 3.5 × 2/right/flank pain	Renal cell carcinoma	Not performed	Renal oncocytoma	Right nephrectomy	75/no

bodies, (ii) biotinylated rabbit anti-mouse (E354, Dako) 1:300 dilution for 35 min for mouse antibodies, (iii) biotinylated rabbit anti-goat (E466, Dako) 1:500 dilution, 35 min for goat antibodies. For the third layer following PBS washing, streptavidin-peroxidase (P397; Dako) at 1:500 dilution or streptavidin-alkaline phosphatase (D396, Dako) at 1:50 dilution was applied to sections for 35 min. Slides were developed in 3,3'-diaminobenzidine (DAB) (D5637, Sigma) plus 0.3% hydrogen peroxide or Vector Red Alkaline Phosphatase Kit (SK-5100, Vector Laboratories, Burlingame, CA, USA) for 20 min, counterstained with light haematoxylin counterstaining, dehydrated, and mounted with DPX-type mountant. For each immunostaining protocol a known positive and a negative control was included to assure proper functioning of the staining system as well as for valid interpretation of results. Positive controls used were archival human tissues, which were known to contain the desired antigen and had provided positive staining previously, using these components. For negative controls, the primary antibody was omitted.

Double immunostaining for smooth muscle actin and for cell proliferation status

Sections were initially stained for SMA immunostaining using a streptavidin-alkaline phosphatase method, followed by MIB-1 immunostaining using the streptavidin-peroxidase method, then counterstained very lightly with haematoxylin. Finally, specimens were dehydrated and mounted, as above.

Cell counting

Stained sections were examined using a standard light microscope (Nikon Eclipse ME600, Tokyo, Japan). For cell counting after immunostaining, 20 consecutive non-overlapping fields per slide were examined under ×400 magnification. To assess cell proliferation (MIB-1 index), number of MIB-1-positive oncocytes was counted as a fraction of the total number of oncocytes in the same area. Similarly, to assess apoptosis (active caspase 3 index), number of active caspase 3-positive oncocytes was counted as a fraction of total number of oncocytes in the same area. Results are expressed as percentages (%).

Statistical analysis

All values described below are expressed as mean and standard deviation, per number of observations. Statistical analysis was conducted using the SPSS 11.0.4 for Mac (Chicago, IL, USA). Data were compared and analysed

Table 2. Protocols for immunostaining

	Proliferation	Apoptosis	Myofibroblasts	Wnt/ β -catenin signalling	Tumour stroma			
Antigen retrieval	Microwaving in 0.01 M citrate buffer at pH 6 for 20 min	Microwaving in 0.01 M citrate buffer at pH 6 for 20 min	Not necessary	Microwaving in 0.01 M citrate buffer at pH 6 for 20 min	Microwaving in 0.01 M citrate buffer at pH 6 for 20 min	Microwaving in 0.01 M citrate buffer at pH 6 for 20 min	Microwaving in 0.01 M citrate buffer at pH 6 for 20 min	Microwaving in 0.01 M citrate buffer at pH 6 for 20 min
Primary antibody	MIB-1	Active caspase 3	α -smooth muscle actin	β -catenin	E-cadherin	Collagen I	Collagen III	Fibronectin
Species	Mouse	Rabbit	Mouse	Mouse	Mouse	Goat	Mouse	Rabbit
Dilution	1:100	1:100	1:2000	1:100	1:30	1:300	1:600	1:300
Source	Dako	R&D System	Sigma	BD	Santa-Cruz	SouthernBiotech	ABcam	ABcam
Secondary antibody	Biotinylated rabbit anti-mouse	Biotinylated rabbit anti-mouse	Biotinylated rabbit anti-mouse	Biotinylated rabbit anti-mouse	Biotinylated rabbit anti-mouse	Biotinylated rabbit anti-goat	Biotinylated swine anti-rabbit	Biotinylated rabbit anti-mouse
Dilution	1:300	1:500	1:300	1:300	1:300	1:300	1:500	1:300
Source	Dako	Dako	Dako	Dako	Dako	Dako	Dako	Dako
Tertiary antibody	Streptavidin-peroxidase	Streptavidin-peroxidase	Streptavidin-alkaline phosphatase	Streptavidin-peroxidase	Streptavidin-peroxidase	Streptavidin-peroxidase	Streptavidin-peroxidase	Streptavidin-peroxidase
Dilution	1:500	1:500	1:50	1:500	1:500	1:500	1:500	1:500
Source	Dako	Dako	Vector Laboratories	Dako	Dako	Dako	Dako	Dako
Colour developer	DAB	DAB	Vector Red	DAB	DAB	DAB	DAB	DAB

using Student's *t*-test. *P*-values <0.05 were considered significant.

Results

Haematoxylin and eosin and periodic acid-Schiff staining

Tumours consisted of uniform, non-anaplastic tumour cells (oncocytes) with abundant eosinophilic cytoplasm, growing in a nested fashion. No tumour haemorrhage nor necrosis was observed. Cells were mostly arranged in sheets, or in tubulocystic or combined patterns. Nuclei

appeared smooth and round, with minimal degree of mitotic activity or nuclear atypia. No capsular invasion nor renal vein thrombosis was observed, furthermore, tumour cells were negative for Hale's colloidal iron staining. Finally, clumps of oncocytes were separated by periodic acid Schiff-positive tumour stroma (Fig. 1a).

Collagen I, collagen III, fibronectin, desmin and human-caldesmon immunostaining

Tumour stromas were positive for collagen I (Fig. 1b), collagen III (Fig. 1d), and fibronectin (Fig. 1c), but

Figure 1. Analysis of tumour stroma of oncocytomas of the kidney. Renal oncocytomas were composed of two independent compartments, benign oncocytes and a pronounced fibrotic stroma (desmoplasia) and were associated with excessive deposition of extracellular matrix, which was periodic acid Schiff-positive (a, pink, asterisk), collagen I-positive (b, brown, asterisk), fibronectin-positive (c, brown, asterisk) and collagen III-positive (d, brown, asterisk), but negative for desmin (e) and human caldesmon (g) immunostaining. Archival smooth muscle tissue was used as positive control for desmin (f, brown, asterisk) and human caldesmon immunostaining (h, brown, asterisk) (magnification, $\times 400$).

negative for desmin (Fig. 1e) and human-caldesmon (Fig. 1h). Here, archival smooth muscle tissue was used as positive control for desmin (Fig. 1f) and human-caldesmon immunostaining (Fig. 1h).

MIB-1 and active caspase 3 immunostaining

Figure 2 shows analysis of cell proliferation and apoptosis in tumours and surrounding non-tumour parenchyma by MIB-1 (Fig. 2a,b) and active caspase 3 (Fig. 2e) immunostaining. As shown in Table 3, MIB-1 indices were low, but were higher in tumours than in non-tumour parenchyma (0.93 ± 0.09 versus 0.46 ± 0.07 , $P < 0.001$). Similarly, active caspase 3 indices were low, but were higher in tumour than in non-tumour parenchyma (0.76 ± 0.08 versus 0.41 ± 0.09 , $P < 0.001$). Similarly, archival colonic tissue was used as positive control for MIB-1 immunostaining (Fig. 2d). For negative controls, primary antibodies for MIB-1 (Fig. 2c) and active caspase 3 (Fig. 2f) were omitted.

Wnt/ β -catenin signalling

Figure 3 illustrates analysis of the Wnt/ β -catenin signalling pathway by E-catenin and β -catenin immunostaining. It seems that canonical Wnt/ β -catenin signalling pathway was not involved in this benign neoplasm, as there was no evidence of loss of membranous localization of E-cadherin (Fig. 3e) or intranuclear expression of β -catenin in the oncocytes (Fig. 3a,b). Likewise, archival colonic signet ring cell carcinoma tissue was used as positive control for intranuclear expression of β -catenin immunostaining (Fig. 3d). For negative controls, the primary antibodies for β -catenin (Fig. 3c) and E-cadherin (Fig. 3f) were omitted.

Double immunostaining, α -smooth muscle actin and MIB-1

Figure 4 illustrates that both SMA immunostaining (Fig. 4a) or SMA and MIB-1 double immunostaining

Figure 2. Analysis of cell proliferation and apoptosis in renal oncocytomas. Just few oncocytes were positive for MIB-1 (a, brown, asterisk) or active caspase 3 immunostaining (e, brown, asterisk). There were occasional MIB-1-positive cells (b, brown, asterisk) found in the stroma. Archival colonic tissue was used as a positive control for MIB-1 immunostaining (d, brown, asterisk). For negative controls, primary antibodies for MIB-1 (c) and active caspase 3 (f) were omitted (magnification, $\times 400$).

Table 3. Analysis of cell proliferation and apoptotic indices in tumour and surrounding non-tumour parenchyma in renal oncocytomas ($N = 16$)

	Tumour parenchyma	Non-tumour parenchyma	<i>P</i>
MIB-1-positive cells, %	0.93 ± 0.09	0.46 ± 0.07	***
Active caspase 3-positive cells, %	0.76 ± 0.08	0.41 ± 0.09	***

*** $P < 0.001$.

(Fig. 4c–f) established the myofibroblastic nature of many of the stromal cells. As shown in Fig. 4d–f, some SMA-positive myofibroblasts were also positive for MIB-1 after double immunostaining with this antibody, indicating a proliferative role for myofibroblasts in the stromas of renal oncocytomas. Again, archival smooth muscle and urinary bladder carcinoma tissues were used as positive controls for SMA (Fig. 4g) and MIB-1 (Fig. 4h) immunostaining. For negative controls, primary antibodies for SMA (Fig. 4b) and MIB-1 were omitted.

Discussion

These data reveal that the kidney oncocytomas were composed of two independent compartments, benign oncocytes and a pronounced fibrotic stroma (desmoplasia) consisting of proliferating myofibroblasts (MIB-1 and SMA-positive) which were associated with excessive deposition of extracellular matrix (periodic acid Schiff-, collagen I-, collagen III- and fibronectin-positive, and desmin- and human caldesmon-negative). In this study, the SMA-positive cells were defined as myofibroblasts as reported elsewhere (24–33). Interestingly, although SMA is the usual marker for the myofibroblast, it is also recognized as an imprecise one and that mesenchymal spindle cells containing SMA can include myofibroblasts, smooth muscle cells, pericytes and endothelial cells. Nevertheless, mesenchymal cells positive for collagen I, collagen III and fibronectin and negative for H-caldesmon and desmin are very probably myofibroblastic.

The exact mechanisms of myofibroblast formation, as well as cells of their origin, have not yet been fully elucidated. The traditional view has been that myofibroblasts

Figure 3. Analysis of Wnt/ β -catenin signaling pathway in renal oncocytomas. There were no pieces of evidence of loss of membrane localization of E-cadherin (e, brown, asterisk) or intranuclear expression of β -catenin (a and b, brown, asterisk) immunostaining in the oncocytes. Archival colonic signet ring cell carcinoma tissue was used as positive control for intranuclear expression of β -catenin immunostaining (d, brown, asterisk). For negative controls, primary antibodies for β -catenin (c) and E-cadherin (f) were omitted (magnification, $\times 400$).

Figure 4. Analysis of proliferating myofibroblasts in stromas of oncocytomas of the kidney. Both SMA immunostaining (a) or SMA/MIB-1 double immunostaining (c–f) established the myofibroblastic nature (pink, asterisk) of many of the stromal cells. (d–f) show some SMA-positive myofibroblasts (pink, asterisk) were also positive for MIB-1 (brown, asterisk) after double immunostaining with this antibody, indicating a proliferative role of myofibroblasts in stromas of renal oncocytomas. Archival smooth muscle and urinary bladder carcinoma were used as positive controls for SMA (g, pink, asterisk) and MIB-1 (h, brown, asterisk) immunostaining. As negative control, primary antibody for SMA (b) was omitted (magnification, a–d, g, h $\times 400$; magnification, e, f $\times 600$).

arise from quiescent resident mesenchymal cells in surrounding tissues – fibroblasts being the mainly implicated progenitor, but also pericytes, smooth muscle cells and even endothelium (34). Increasingly however, attention is being focused on two other mechanisms, derivation from bone marrow-derived circulating mesenchymal fibrocytes and an origin from epithelial–mesenchymal transformation.

Bone marrow-derived circulating mesenchymal cells or fibrocytes have been demonstrated to have the ability to localize at and populate tissue sites. These include normal tissues (33,35), granulation tissue (25), fibrosing conditions (30,32) and tumour stroma (26,28). Direkze *et al.* (26) employed green fluorescent protein (GFP) staining of transplanted male cells into female GFP-negative recipients, to show how bone marrow-derived cells can populate tumour stroma. Furthermore, not all lesions involve repair by circulating cells; in atherosclerosis, while there is evidence that circulating progenitor cells regenerate endothelium (36), other data suggest that smooth muscle cells that heal atherosclerotic plaques are of local tissue origin (37). Finally, Kojc *et al.* (38) demonstrated that the stroma of normal mucosa and squamous intraepithelial

lesions contain scattered CD34-positive cells or fibrocytes, but there no SMA-positive myofibroblasts. In contrast, stroma of squamous cell carcinoma was shown to contain SMA-positive myofibroblasts, but no CD34-positive stromal cells. These results suggest that disappearance of CD34-positive stromal cells and appearance of stromal myofibroblasts are associated with transformation of laryngeal squamous intraepithelial lesions to squamous cell carcinoma, in those tissues. Similar results have been reported in invasive ductal carcinoma of the breast (39), invasive squamous cell carcinoma of the cervix uteri (40) and pancreatic neoplasms (41); normal stroma harbours CD34-positive stromal cells, whereas tumour-associated desmoplastic stroma was characterized by presence of myofibroblasts. Complete disappearance of CD34-positive stromal cells in laryngeal squamous cell carcinoma suggests that they might have been transformed into SMA-positive myofibroblasts (38).

Epithelial–mesenchymal transition or transformation is the second major mechanism suggested to explain the origin of myofibroblasts in fibrosing conditions (29) and tumour stroma (42). The proposed mechanism is based on findings of the kind typified in tubulointerstitial fibrosis.

In the study by Liu (31), stromal cells in the interstitium are SMA positive and are, therefore, interpreted as being myofibroblasts; the tubular epithelium concomitantly loses some of its cells of epithelial phenotype, which assume some mesenchymal features (for example, expression of SMA) and is hypothesized as perhaps developing into myofibroblasts.

The benign nature of oncocytomas is compatible with low MIB-1 index that we found. By pathological examination, we saw that MIB-1 indices were low, but were higher in tumours than in surrounding non-tumour parenchyma (0.93 ± 0.09 versus 0.46 ± 0.07 , $P < 0.001$), and clinically, all 16 of our patients remained free of tumour recurrence at follow-up. Rather than proliferating cell nuclear antigen (PCNA), this study used immunostaining for MIB-1 as a marker to identify tubular cell proliferation. MIB-1 is a nuclear antigen expressed in all phases of the cell cycle, with the exception of the G_0 and early G_1 phases (43). Expression of MIB-1 increases over cell cycle progression, and peaks during late S and G_2 phases. PCNA is a polymerase delta accessory molecule involved in nucleotide excision repair, and its maximum concentration occurs during the S and late G_1 phases. PCNA is a marker of G_1 , S, G_2 and M phases of the cell cycle, however, because of its relatively long half-life, PCNA is also detectable in cells that are in G_0 (44). Results obtained using PCNA must be treated sceptically, as number of positive cells is highly dependent on fixation time and tissue pre-treatment. In addition, even if these are standardized, PCNA is unreliable since large quantities of PCNA are visible in tissue around tumours or in tissue of animals fed growth factors, even in the absence of tritiated thymidine uptake (45). Thus, MIB-1 is a more accurate marker of cell proliferation.

Clinical and experimental data (18) support the hypothesis that myofibroblastic tumour stroma regulates metastatic invasion and spread. Therefore, an emerging question from this study concerns, how the benign status of renal oncocytoma is compatible with myofibroblastic stroma that is being recognized as a driver of invasive cancer growth? One possibility is that the morphologically identifiable myofibroblastic population might lack one or more critical molecules for promoting invasion – an area for future work on this group of tumours.

In summary, our data demonstrate for the first time that there was a proliferation of myofibroblasts in the stroma of oncocytomas of the kidney, which might serve as a novel target for treatment in future. In this regard, drugs that inhibit differentiation of fibroblasts to myofibroblasts, such as PPAR- γ agonists, might have potential not only as anti-fibrotic, but also as anti-oncocytoma therapies in the future. Finally, this study is limited by small

tissue samples and the lack of ultrastructural analysis, which can be addressed in the future.

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