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Comparison of the Morphology of Renal Cysts and Cystic Renal Tumors

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Renal tumors appear uncommonly with cystic changes. They may develop due to necrosis though well-formed renal cysts are also known. Such lesions may present problems in distinguishing them from benign renal cysts. Conditions leading to cyst formation are not known, however cell proliferation, altered extracellular matrix production and oncoprotein expression have been reported in cystic renal disorders. In the present study, we analysed the morphological features of 23 cystic renal tumors in comparison with 16 benign cysts using immunohistochemical and lectin binding methods. By our knowledge there has not been any publication on such studies. The cystic renal tumors were represented predominantly in males and the size of the cysts was slightly larger than that of benign cysts. Tumorous cysts shared similar morphological appearance to solitary and multilocular cysts. They

all showed strong epithelial membrane antigen reactivity on the luminal surface of the cells indicating distal tubular origin. Cell proliferation and p53 expression proved to be low excluding their role in the formation of the cysts. The amount of extracellular matrix and basement membrane was increased with an elevated type IV collagen and reduced fibronectin content. Polycystic kidney disease is different from tumorous cysts as cell proliferation, p53 oncoprotein expression and the composition of extracellular matrix proved to be the opposite. As renal cell tumors arise from proximal tubules, neoplastic or metaplastic differentiation toward distal tubular direction seems to be the key event in cyst formation. Altered cell-matrix or cell-cell contact can modulate this transformation providing a basis for further results. (Pathology Oncology Research Vol 3, No 4, 272-277, 1997)

Key words: renal cysts, renal tumors, tubular markers, cell proliferation, immunohistochemistry

Introduction

Cystic renal tumors may represent benign and malignant conditions including multilocular cystic nephroma, renal cell carcinoma and papillary adenocarcinoma. Multilocular cystic nephroma is usually a benign tumor but can also be considered as a multicystic variant of clear cell carcinoma. Renal cell carcinoma and papillary adenocarcinoma may also appear with cystic patterns although it is rather uncommon for the renal cell carcinoma.¹ The incidence of such tumors varies between 6 and 15%.^{2,3} Acquired renal cysts can also be complicated with malig-

nant changes. Prolonged survival by dialysis treatment is supposed to increase the risk of malignancy.⁴ There are contradictory data on the role of renal transplantation in the incidence of malignancy.^{5,6} Cystic renal tumors may also occur in Hippel-Lindau disease, tuberous sclerosis and Wilms' tumor.^{7,8,9} Diagnostic procedures for cystic renal tumors is the same as for solid ones and solitary renal cysts; including plain X-ray, scintigraphy, arteriography, computer tomography and ultrasound.¹⁰ Differentiation of unilocular and multilocular cysts from cystic tumors is often difficult. Cytology gives common negative results and imaging techniques usually do not allow differentiation between benign and malignant conditions although computed tomography provide better diagnostic yield.^{1,2,11,12,13} In most cases, only a systematic histological examination reveals the presence of a malignant cyst or cystic renal tumor.¹¹ Many factors are known to be included in the development of renal cysts, especially in poly-

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cystic kidney disease. There is evidence to suggest that cyst and tumor formations are in some way interrelated. The role of growth factors and oncogenes are known in both mechanisms. Overexpression of c-erb oncogene has been noted both in acquired or inherited cystic conditions and renal cell neoplasms.¹⁴ The role of these factors has been studied in polycystic kidney disease and experimentally induced renal cysts but there are no data on the morphologic characteristics of the cysts developed within tumors. The aim of our study was to delineate the morphological similarities and differences between renal cysts and cystic renal tumors.

Materials and Methods

Patients

169 (78.6%) out of 215 nephrectomies and 2 partial resections were performed for neoplasm at the University Medical School of Debrecen between January 1994 and June 1997. 16 non-tumorous renal cysts were derived from autopsies. The histological data of patients who underwent surgery and their renal cysts are seen in *Table 1*.

Immunohistochemistry

Surgically removed and autopsy specimens were fixed in 10% neutral formalin embedded in paraffin and processed by the usual histological technique. Tissue slides were stained with HE. To demonstrate the tubular origin of the cystic tumors, proximal tubular marker lysozyme (DAKO, 1:100), distal tubular marker epithelial membrane antigen (EMA) (DAKO, 1:100) and collecting duct marker Dolichos Biflorus Agglutinin (DBA, 1:200) (DAKO) were used.¹⁵ Extracellular matrix proteins were detected by using type IV collagen antibody (DAKO, prediluted) and anti fibronectin (ATAB, 1:100). Cell proliferation was determined by anti Proliferating Cell

Table 2. Characteristics of cystic renal tumors and renal cysts

	Cystic Renal Tumors	Renal Cysts
Male*	24	7
Female	8	9
Age (years)**	51.45 ± 1.89	64.75 ± 22.03
Weight of kidney (g)**	374.64 ± 0.04	445.9 ± 656.8
Size of cysts (cm)**	4.22 ± 0.11	2.76 ± 2.35
Clear cell carcinoma	25	Polycystic kidney 3
Adenocarcinoma	5	Multilocular cysts 5
Oncocytoma	1	Soliter cyst 4
Wilm's tumor	1	Tubular dilatation 4

± SD, standard deviation; * – significant ($p < 0.05$); ** – not significant

Nuclear Antigen (PCNA) (DAKO, prediluted). Screening for the presence of oncogen expression, we used anti p53 antigen (DO7, DAKO, 1:100). Sections were pretreated either with 1% trypsin or microwaved in 10 mmol/l citrate buffer (pH 6.0) for 5 minutes 3 times each. The reactions were visualized by the avidin-biotin peroxidase complex method and by Vector Red as chromogen. For the fibronectin reaction, FITC labelled antigen was used. Endogenous biotin was blocked by Avidin-Biotin Blockage kit in all cases.

Results

Within the renal tumors 32 (18.9%) were found to be cystic by morphological and histological examination. 23 (13%) of 32 proved to be real cysts by a tumor cell lining and without any necrosis. Characteristics of the cystic renal tumors and renal cysts are shown in *Table 2*. There was a male predominance among patients with cystic renal tumor but no sex-difference was found in patients with renal cysts. The size of the cysts was larger in the tumorous cases.

Cystic renal tumors

The majority of neoplastic cysts proved to be unilocular with hemorrhage in some place while multilocular lesions were also found in 35 % of the cases (*Figure 1*). The cysts were lined by a single or stratified layer of tumor cells and papillary projections were seen in 17 %. Many of the cysts contained amorphous material in their lumen. Cysts in the neighbouring non-tumorous kidney tissue were found in only two cases. Distal tubular marker EMA revealed intensive positive reaction, especially on the luminal surface of the cysts, while solid areas showed focal, moderate reaction (*Figure 2A*). Proximal tubular marker lysozyme presented a diffuse, strong pos-

Table 1. Histological findings in nephrectomy and autopsy specimens.

Renal Tumors		Renal cysts	
Histology	No	Histology	No
Clear-cell carcinoma	118	ADPKD	3
Adenocarcinoma	21	Chronic nephritis	3
Uroepithelial carcinoma	8	Transplant rejection	2
Anaplastic tumor	2	Incidental finding	8
Oncocytoma	3		
Other*	65		

Others* – metastatic tumors, Wilms' tumor, soft tissue tumors, lymphomas, ADPKD – Autosomal dominant polycystic kidney disease

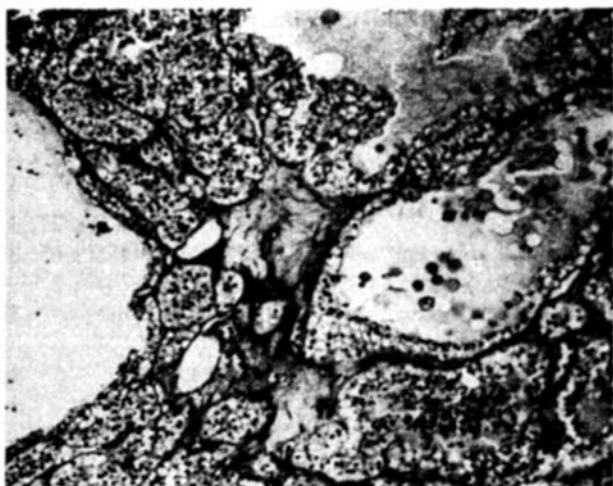


Figure 1. Multiple cystic cavities in renal cell carcinoma. HE, X200

itive reaction in both the cyst lining and other cells except in the oncocytoma where it was negative. DBA, characteristic to the collecting ducts remained negative with some focal, mild positive reaction within the solid areas of the tumor in 4 cases (*Figure 3B*). Basement membrane, type IV collagen was expressed in a variable fashion. In the solid parts of the tumor the reaction was weaker than around the cysts. The extent of the reaction increased with the appearance of the multilocular cystic pattern (*Figure 4A*). Fibronectin failed to present positive reaction in the majority of the cases. In a few cysts we could find mild, focal positivity (*Figure 4B*). Cell proliferation was intensive but the reaction was weaker in tumor cells lining the cystic cavities (*Figure 5*). Normal renal tubules were characterised by a lower proliferating activity or even by the absence of it. Only a few cells

demonstrated the presence of p53 oncoprotein in half of the cases, and these positive cells were found in the solid areas. Nonneoplastic neighbouring tubules remained negative. Proliferating activity of cyst lining tumor cells was slightly increased in those tumors displaying p53 expression but it still remained less intensive than in cells of solid tumors.

Renal cysts

Distal tubular marker EMA gave a uniform, strong reaction on the surface membrane of cells lining the cysts (*Figure 2B*). Proximal tubular marker lysozyme was detected in 25% of the cysts with a moderate appearance, but no reaction was found in the multilocular cysts. Collecting duct marker DBA revealed mild reaction in 75% of the cases (*Figure 3A*). The extracellular matrix production was strong around the multilocular and solitary cysts while the reaction was weaker in polycystic kidney disease and in kidneys with tubular dilation. Cell proliferation within the cystic epithelium was present in one third of the cysts while the neighbouring non-dilated tubules and the interstitial cells demonstrated increased activity. p53 oncoprotein was expressed within the non-dilated tubular cells in 2 out of 3 cases of polycystic kidney disease. The other types of cysts remained negative.

Discussion

Renal cysts are either congenital, inherited or acquired lesions. Their development is still uncertain but many factors like cell proliferation, altered basement membrane and extracellular matrix production, growth factors and oncogenes are thought to be responsible in cystogenesis. There is an increased risk for malignant transformation of renal

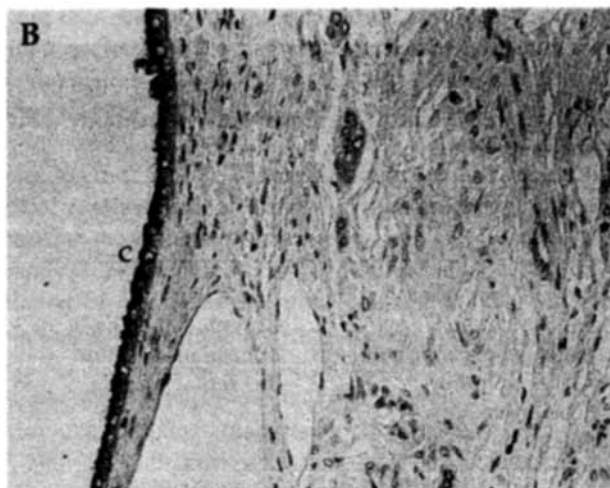
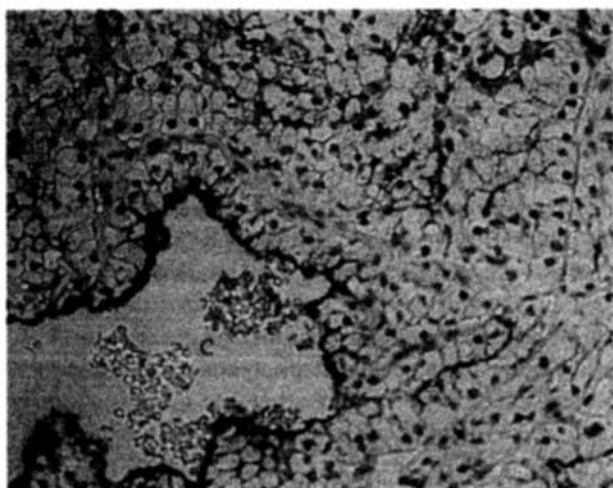


Figure 2. EMA is present on the luminal surface membrane of the cysts. (A) Tumorous cyst shows strong and diffuse while the solid part only weak reaction. (B) Epithelium of the renal cyst is positive and the surrounding non-dilated tubules are negative. X100; (c = cyst)

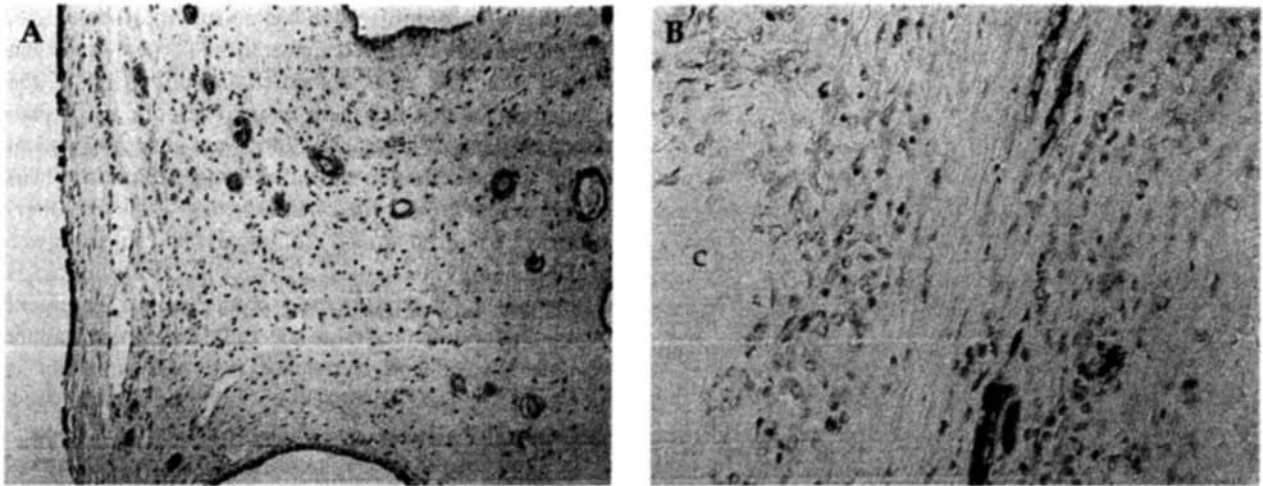


Figure 3. *Dolichus biflorus* agglutinin stains the epithelium of the renal cyst and of some distal tubules (A). Cystic cavities of the renal cell carcinoma fail to present reaction. There are a few positive cells within the tumor (B). X200; (c = cyst)

cysts and renal tumors may also show cystic changes, which findings represent differential diagnostic problems. Many of the cystic cavities develop following necrosis of tumor cells, although epithelial lined well-formed cysts are also present. There is almost no explanation for their development. Some reports mention the role of obliteration by papillary proliferation¹⁶ or by involvement of the upper calyx.¹⁷ Increased cell proliferation and abnormal cell-cell contact are also theorised.^{3,18} In our study, renal tumors with cystic changes gave intensive reaction with lysozyme characteristic of proximal tubules therefore suggesting proximal tubular origin. Renal cysts did not show this reaction but instead, strong, uniform EMA expression and mild DBA positivity. These cysts arise from distal tubules or collecting ducts which is in accordance with other reports.^{19,20,21} The epithelial membrane antigen reaction

demonstrated interesting results. It was present on the luminal surface of cysts lining tumor cells while the solid areas remained negative or weakly positive. The heterogeneity of lectin binding within renal cell tumors has been reported.²² In our study, we found strong EMA reaction with lysozyme positivity only on the cells lining the cysts. This suggests, that cysts developed only in those areas where tumor cells reacted with both proximal and distal tubular markers. From these results, we assume that modified differentiation in the distal tubular direction seems to be important in cyst formation of tumors. The presence of some DBA positive areas within the tumor may indicate entrapped collecting duct elements or can simply be evaluated as another sign of heterogeneity.

The presence of cell proliferation in cystic renal disorders is controversial. Both increased and unchanged cell

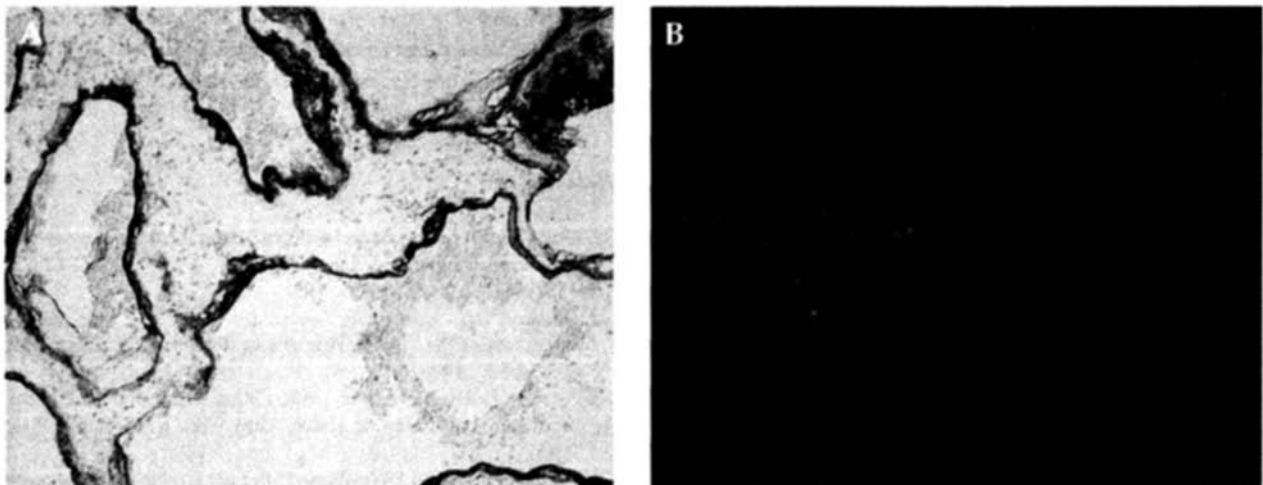


Figure 4. Basement membrane, type IV collagen is strong around the cystic cavities in tumorous kidney (A). Fibronectin can be detected around some of the tumorous cysts, while solid parts remain negative (B) X200

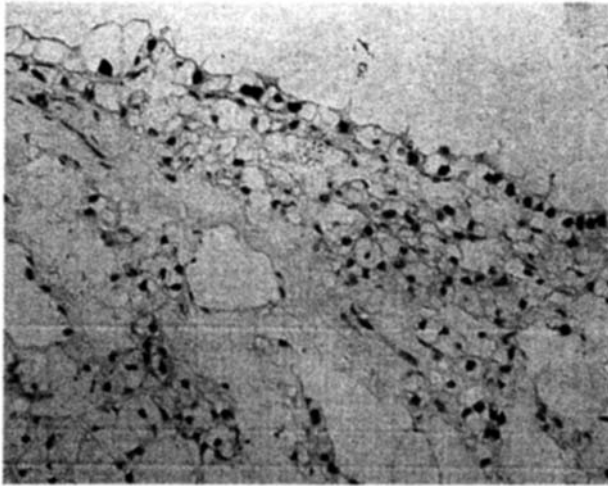


Figure 5. Proliferation activity in cells lining tumorous cysts. PCNA labeling. X200

growth have been reported.^{22,23,24,25} In our study we found increased cell proliferation within the normal tubular epithelium and the mesenchymal cells but not in the epithelium of cystic kidneys of various origin while cystic epithelium presented increased proliferation only in polycystic kidney disease. Renal tumors revealed increased cell proliferation by PCNA, especially in the solid areas, but cysts lining cells demonstrated a weaker reaction. From this finding, we think that cell proliferation is not important in cyst formation of renal tumors and of renal cysts and can be considered being an inductive factor only in polycystic kidney disease. However we cannot exclude the role of increased cell growth in the initiation of cyst formation as nondilated tubular cells in renal cysts and tumor cells of the neighbouring solid areas showed increased growth potential. Proliferation of cyst lining cells might decrease in time as the cysts are fully developed.

There are reports on the presence of oncogenes like *c-myc*, *c-fos* and *c-erb* in the inherited forms of cystic kidney diseases.^{14,26} Studying normal kidneys and tumors, expression of mutant p53 showed diverse reactions.^{27,28} We could detect weak positivity in normal and cystic tubular epithelium in polycystic kidney disease. In the neoplastic renal cysts, only half of the cases demonstrated weak positivity in some tumor cells lining the cavities and forming solid areas. Cystic renal tumors expressing p53 also showed a relatively increased cell proliferation compared to p53 negative cases. Mutant p53 can lead to an uncontrolled proliferation of cells. While cystic renal tumors with p53 expression presented a relatively increased cell proliferation compared to negative counterparts, it still remained lower than within the noncystic tumor cells. In renal cysts, p53 was found to be positive in nondilated tubular cells but cystic epithelium showed almost no proliferating activity. From these findings we conclude that

p53, like as cell proliferation has no role in cystogenesis. The moderate or increased appearance of type IV collagen around tumorous cysts compared to solid parts of the tumor implies that basement membrane collagen is produced by the cyst lining cells and is not generally typical to the tumor cells. On the other hand, fibronectin was rarely present around the cystic cavities. The same alterations were noticed around multilocular and solitary cysts of the nontumorous kidneys. Polycystic kidney disease is characterised by an increased production of basement membrane and extracellular matrix in which the amount of type IV collagen is reduced and of fibronectin is increased.^{29,30} The same reaction was found with polycystic kidney diseases and in kidneys with tubular dilation.

In summary, tumorous cysts share common features with nontumorous, especially solitary and multilocular cysts of various origin when they present distal tubular marker EMA on the surface membrane of the lining cells. Proliferating activity of the cyst lining cells is usually less than that of the solid areas and p53 expression was found to be restricted to a few cells just as in the benign renal cysts. The basement membrane antigen type IV collagen was increased, along with a reduced fibronectin component. Polycystic kidney disease is a distinct entity characterised by a higher cell proliferating activity and lower amount of type IV collagen. The formation of cysts in renal tumors may be related to changes in tubular phenotype. What is responsible for this modification is still not known. We suppose that the altered cell-matrix or cell-cell contact may be the key issue. If this was the case it would be interesting to determine whether it could lead to increased metastatic incidence or simply explains why cystic cavities develop in renal tumors.

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