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Shift of Keratin Expression Profile in End-stage Kidney Increases the Risk of Tumor Development

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Abstract. *Background/Aim: Pre-neoplastic lesions and renal cell tumors of distinct pheno- and genotypes occur frequently in end-stage kidneys. The aim of this study was to investigate the role of KRT7 and KRT19 in this process, the expression of which was previously detected by Affymetrix GeneChip analysis. Patients and Methods: Twelve end-stage kidneys were analyzed to find pre-neoplastic lesions and tumors and expression of KRT7 and KRT19 was examined by immunohistochemistry. Results: A total of 17 tumors, 149 pre-neoplastic lesions, 179 simple or proliferative cysts >2 mm were identified. Diffuse expression of KRT7 and KRT19 was seen in all end-stage kidneys as well as in the vast majority of cysts, pre-neoplastic lesions and tumors. Conclusion: Our data indicates that de novo expression of KRT7 and KRT19 resulting in altered plasticity and stem cell characteristics of epithelial cells might be a crucial factor in increasing the risk of tumor development in end-stage kidneys.*

Chronic renal disease (CRD) is characterized by a decrease in kidney function to a degree requiring renal replacement therapy (RRT). The long survival of patients with non-functioning kidneys results in remodeling of kidney structure, so-called end-stage renal disease (ESRD). In spite of atrophic structures, ESRD kidney shows a remarkable proliferative activity (1). In sclerotic end-stage kidneys diffuse cystic changes may develop, especially after long intermittent maintenance haemodialysis, and this condition is defined as acquired cystic renal disease (ACRD) (2). Remodelling of kidneys in ESRD/ACRD is frequently accompanied by pre-neoplastic lesions and renal cell tumors (RCT) of distinct pheno- and genotype (3-8). Clinically recognized renal cell carcinoma (RCC) has been found in 3.8% of native end-stage kidneys which contrasts with a detection rate of 0.04% to 0.3% lifetime risk of developing RCC in the general population (9, 10). Malignancy is currently reported as a cause of death in 3 to 4% of ESRD/ACRD patients, largely as the result of an increasing incidence of RCC (11).

The underlying molecular mechanism of structural remodeling of ESRD/ACRD kidneys, as well as of development of tumors is not yet known. RCTs arising in the general population are characterized by specific genomic alterations. It has been proposed that in ESDR/ACRD the microenvironment may trigger the development of tumors of unusual pheno- and genotype (12). Expression of growth factors and hypoxemia have been suggested to be responsible for cystic changes and tumors (13-15). Global gene expression analysis disclosed a Bunique gene expression signature including a group of cytokines indicating an inflammatory microenvironment in ESRD/ACRD kidneys (14). Another well-defined group of functionally related genes expressed in ESRD/ACRD kidneys encodes intermediate filament proteins including the keratins KRT7 and KRT19 (14). It was demonstrated that KRT7 and KRT19 are up-regulated during renal epithelial injury (16). KRT19 has also been described as a putative marker of epidermal stem cells and its overexpression was associated with metastatic capacity of tumours (17-19).

The aim of this study was to analyse the morphological changes and KRT7 and KRT19 expression in ESRD/ACRD kidneys, pre-neoplastic lesions and associated tumours by immunohistochemistry. A diffuse KRT7 and KRT19 expression was found in ESRD/ACRD kidneys and associated tumors indicating their role in structural remodelling and tumorigenesis.

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Key Words: End-stage renal disease, KRT7, KRT19, immunohistochemistry, tumor development.

Materials and Methods

Tissue samples. From 11 ESRD/ACRD cases 12 entire kidneys were available and processed in several hundreds of paraffin blocks for histological analysis. ACRD was diagnosed when the secondary cystic changes replaced at least 40% of the kidney parenchyma. The

hematoxylin and eosin (H&E) stained slides were scored for cysts, small precursor lesions and tumors. The diagnosis of tumors was established according to the Heidelberg Classification (20) and Tickoo *et al*. (5). Tissue multi array (TMA) was constructed from paraffin embedded ESRD/ACRD associated tumors after marking the areas of interest on H&E-stained slides by one of the authors (GK). Core biopsies of 0.6 mm in diameter were placed within a recipient block by Manual Tissue Arrayer (MTA1, Beecher Instruments, Inc., Sun Prairie, WI, USA). The collection and use of tissue samples for this study was approved by the Ethics Committees of the University of Heidelberg, Germany and University of Pecs, Hungary.

Immunohistochemistry. Paraffin blocks of normal and ESRD/ARCD kidneys as well as a TMA containing ESRD/ARCD associated tumours were used for immunohistochemistry. Serial sections of ESRD/ACRD kidneys were used to compare the results obtained by different antibodies. After dewaxing and rehydration of the slides, antigen de-masking was performed in 10 mM sodium citrate buffer, pH 6,0 or TE buffer, pH 9,0 in 2100-Retriever (Pick-Cell Laboratories, Amsterdam, The Netherlands). Endogenous peroxidase activity and unspecific binding sites were blocked with 0.3% hydrogen peroxide containing 1% normal horse serum for 10 min at room temperature. Slides were incubated overnight with KRT7 (mouse monoclonal antibody, ab9021, Abcam, Cambridge, UK) in 1:300 dilution, KRT19 (rabbit polyclonal antibody, ab14363-1, Abcam,) in 1:100 dilution, TMEM27 (rabbit polyclonal antibody, ab200664, Abcam,) in 1:1,000 dilution and AQP2 (rabbit polyclonal antibody, PA5-38004, Thermo Fisher, Budapest, Hungary) in 1:200 dilution. HRP conjugated secondary antibody (MACH4 Universal HRP-Polymer, Biocare Medical, Concord, CA, USA) was applied for 30 min. The signal was visualized with AEC (Amino-ethyl-carbazol) and DAB (3,3'-Diaminobenzidin). Tissue sections were counterstained with Mayer's haematoxylin. In negative control the primary antibody was omitted.

Results

Cysts, precursor lesions and tumours in ESRD/ACRD. Analysis of 393 paraffin blocks obtained from 12 entire kidneys of 11 patients with ESRD/ACRD revealed 17 tumors with a size of 2-41 mm in diameter (Table I). The size of main tumours varied between 8 and 41 mm. Five tumours were diagnosed as papillary renal cell tumor (pRCT) and another 6 as conventional renal cell carcinoma (cRCC), all showing similar histology to those occurring in the general population. One oncocytoma, two ACRD-associated eosinophil-vacuolated tumors, two chromophobe-like tumors, and one clear cell papillary RCC were also found (Table I).

By detailed microscopic analysis of the 393 slides we detected 143 small cysts, 26 proliferative cysts with solid-papillary growth of cells within the cysts. Ten of the proliferative cysts displayed solid growth of large eosinophil cells with cytoplasmic vacuoles. Overall, 65 small papillary precursor lesions of small or medium sized cells revealed similar growth pattern, which was earlier described in kidneys with papillary RCC in the general population (21). The overwhelming majority of the 42 pre-neoplastic lesions with chromophobe RCC-like pattern occurred in the two kidneys of one patient (173R and 173L). Nearly all of the 24 small solid lesions with large eosinophil vacuolated cells were located in one kidney (209). The number of cysts and distinct types of pre-neoplastic lesions are shown in Table II. A good correlation was found between the phenotype of pre-neoplastic lesions and tumors, the majority of pre-neoplastic lesions displayed a similar morphology to the tumor observed in the same kidney (Figure 1).

Expression of KRT7 and KRT 19 in ESRD/ACRD kidneys, pre-neoplastic lesions and tumours. In normal kidney KRT7 and KRT19 proteins were expressed in principal cells of the collecting duct. However, in ESRD/ACRD kidneys a diffuse *KRT7* and *KRT19* staining was seen in small atrophic as well as in dilated tubules lined with flat or cuboidal epithelial cells (Figure 2A and B). To clear the origin of cells stained positively with both KRT7 and KRT19, we have analysed ESRD/ACRD kidneys for expression of structural proteins of proximal tubules (TMEM27) and principal cells of the collecting duct (AQP2). No expression of TMEM27 and AQP2 or expression only in single tubules were observed (Figure 2C and D).

There was a strong immunostaining in dilated tubules showing papillary growth within the lumen (Figure 2E). All papillary RCTs developed in ESRD/ACRD kidneys showed diffuse positivity with *KRT7* antibody (Figure 2F). A membrane attenuated positive staining was seen in each small chromophobe-like pre-neoplastic lesion in kidneys 173R and 173L as well as in the chromophobe-like cancer (Figure 2G and H). ACRD associated eosinophil vacuolated tumors displayed only scattered positivity with KRT7. Conventional and clear cell papillary RCC were consequently negative for *KRT7*.

All cells of tubular-papillary growing tumours including those with broad papillary stalk displaying strong inflammation exhibited a strong *KRT19* positive immunostaining (Figure 2I and J). Proliferative cystic pre-neoplastic lesions for ACRD-associated eosinophilic-vacuolated tumor and also the tumor itself displayed focal cytoplasmic staining for KRT19 (Figure 2K). The chromophobe-like precursor lesions and tumours also showed a strong KRT19 immunoreaction. Of interest, 4 of the 6 conventional RCCs showed scattered positivity for KRT19 (Figure 2L), whereas the clear cell papillary RCC was negative.

Discussion

In chronic renal disease nephrons gradually lose their function and undergo an extreme structural remodeling. There is a long-debated question on the origin of atrophic

Table I. *Pertinent clinical and histological data of ESRD/ACRD tumours.*

Tumour samples	Patient age/gender	Renal disease	Size of kidney (cm)	Size of tumor (cm)	Histological diagnosis
94A	76/M	ESRD/NS	9.5×6×3	0.8	cRCC
94B				0.2	pRCT
94C				0.3	pRCT
105	71/F	ACRD/?	12×6.5×5	4.0	RO
123A	77/M	ACRD/GN	15×8×7	3.1	ACRD-T
123B				2.6	cRCC
123C				2.2	ccpRCC
137	61/M	ESRD/NS	6.5×3.0×3.0	3.0	cRCC
173 L	68/M	ESRD/GN	5.5×3.0×1.5	2.5	chRCC-like
173 R			5.5×3.0×2.0	1.5	chRCC-like
192	49/M	ACRD/NS	10×5.5×3	3.5	cRCC
195	43/M	ACRD/?	9×5x4	3.0	cRCC
203 A	54/M	ESRD/?	7×3.5×2.5	4.1	pRCT
203 E				0.3	pRCT
209	49/M	ACRD/GN	9.5×5.5×4	3.2	ACRD-T
217	71/M	ESRD/?	6.5×3.5×3	2.8	cRCC
1926	33/M	ACRD/?	10×5×4	3.5	pRCT

NS: Nephrosclerosis; ?: unknown; GN: glomerulonephritis; cRCC: conventional RCC; pRCT: papillary RCT; RO: renal oncocytoma; ACRD-T: ACRD associated large eosinophilic cells with cytoplasmic vacuoles; ccpRCC: clear cell papillary RCC; chRCC-like: similar morphology to chRCC.

Table II. *Type and number of precuros lesions in ESRD/ACRD.*

Kidney samples	Nr. of blocks	Cyst	p-cyst	Conv	Pap	ch-like	l-eos	ccpap
94	27	11	3	0	**21**	2	1	0
105	21	13	2	0	5	0	0	0
123	95	18	4*	0	**13**	0	**4**	1
137	32	3	0	3	2	0	0	0
173R	28	14	2	0	2	**19**	0	0
173L	24	7	0	0	1	**21**	0	0
192	23	6	0	**4**	0	0	0	0
195	29	38	12	**5**	2	0	0	0
203	25	11	2	1	5	0	0	0
209	40	12	6*	0	1	0	**19**	0
217	27	2	3	**4**	1	0	0	0
1926	22	8	2	0	**12**	0	0	0

p-cyst: Proliferative cyst; conv: conventional-like lesion; pap: papillary lesion; ch-like: chromophobe-like lesion; l-eos: large eosinophilic cells with vacuoles; ccpap: clear cell papillary lesion; *proliferative cyst with large vacuolated cells. The dominant type of precursor lesion is marked in bold.

and proliferating cells in ESRD/ACRD kidneys (1, 14). KRT7 and KRT19 are expressed only in principal cells of the collecting duct and ascending loop of Henle. We found a diffuse positivity for KRT7 and KRT19 in atrophic tubules and single epithelial cells embedded in fibrous stroma as well as in dilated and cystic tubules. In this study, TMEM27 and AQP2 antibodies, which recognize structural proteins of proximal tubules and principal cells of the collecting duct, respectively, stained only single tubules in ESRD/ACRD kidneys. It was recently documented that SCEL, a marker of distal tubular cells, shows diffuse immune-reaction in nearly all cells of ESRD/ACRD kidneys (22). Therefore, we suggest that the vast majority of solid epithelial cell clusters, aberrant tubular and cystic structures in ESRD/ACRD kidneys correspond to proliferating distal tubules.

Strong arterial and arteriolar sclerosis leads to slow or blocked blood flow in ESRD kidneys (23). The reduced oxygen and nutrient delivery results in damage of epithelial cells especially of proximal tubules, which cannot easily convert from oxidative to glycolytic metabolism. Thus,

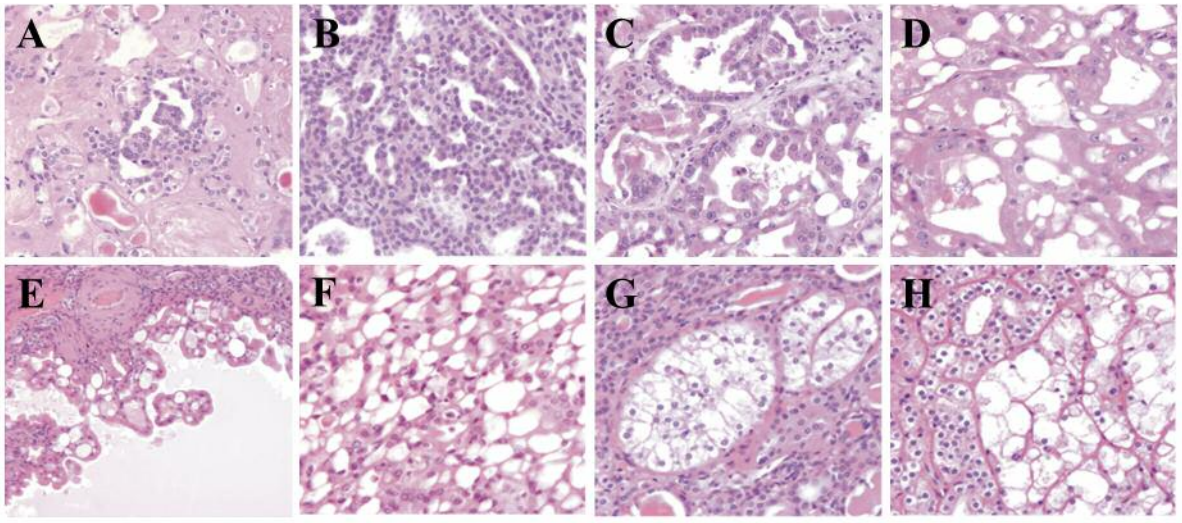

Figure 1. *Morphological heterogeneity of precursor lesions and ESRD/ACRD tumors. A, Tubulopapillary growth of small-cell pre-neoplastic lesion (A) and papillary RCT (B). A pre-neoplastic lesion showing a transition from small-cell papillary morphology towards large eosinophilic cells (C) and the ACRD-associated vacuolated eosinophil tumor (D) of the same kidney. A proliferative cyst lined with large vacuolated eosinophil cells (E) and corresponding tumor (F). Small chromophobe-like pre-neoplastic lesion (G) and tumor (H).*

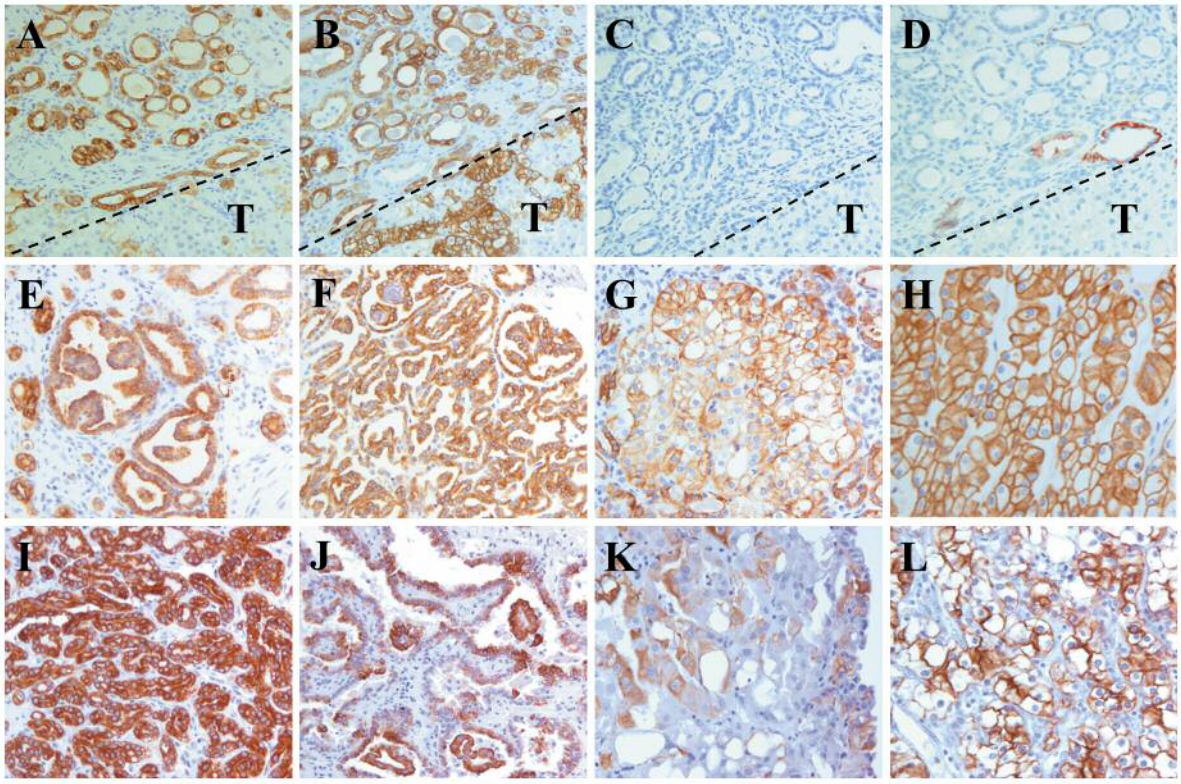

Figure 2. *Expression of KRT7 and KRT19 in ESRD kidney, pre-neoplastic lesions and tumors. Diffuse KRT7 (A) and KRT19 (B) positivity but no or scattered staining with AQP2 (C) and TMEM27 (D); the corresponding tumor (T) shows positive reaction with KRT19. KRT7 positive immuno-reaction in a papillary preneoplastic lesion (E), papillary RCT (F) as well as in a small chromophobe-like lesion (G) and corresponding tumor (H). Note the strong KRT19 staining in a tubulopapillary (I) and papillary (J) RCT, scattered positivity in a ACRD-associated tumor (K) and also in a conventional RCC (L).*

proximal tubular cells are not only the victims but actively participate in the inflammatory response to ischemia by releasing cytokines and chemokines which play an important role in progressive fibrosis which in turn increases the hypoxemic stress (24, 25). Cells of the distal tubules are more resistant to hypoxia and have greater capacity to shift from oxidative to glycolytic metabolism, which may explain the survival and preferential proliferation of cells of distal tubules.

Corresponding to the hypoxemic stage, high expression of hypoxia inducible gene HIG2, HIF1A and also NFkB triggering the expression of pro-inflammatory and inflammatory proteins, has been documented in ESRD/ACRD kidneys, cysts and epithelial hyperplasia (25). HIF1A is also expressed in conventional RCC whereas HIG2 in papillary RCTs. Using global gene expression analysis two groups of functionally related genes have been detected in ESRD/ACRD kidneys (14). The high expression of cytokines including IL-6, IL-8 and their receptors indicated a strong inflammatory microenvironment (14). The other group of functionally related genes included KRT7 and KRT19, which are known to be associated with increased plasticity of epithelial cells. Recently, it was also shown that expression of IL-6, TNFa and NFkB leads to up-regulation of cytokeratins in experimental renal disease models (26).

Highly specialized epithelial cells of kidney express, at normal state, only KRT8 and KRT18, which are crucial in keeping the integrity and mechanical stability of tubular epithelial cells. Recent studies showed that this structural support may be modulated to accommodate the changing needs of cells and pointed out a novel role of intermediate filaments influencing cell growth and death through dynamic interactions with non-structural proteins. Keratins are not only structural cell markers but are also stress-responsive proteins in tubular cells (26). For example, KRT7 and KRT19 are upregulated during renal epithelial injury and repair (16). Thus, upon various types of stress such as hypoxemia, inflammation, renal tubular injury or atrophy, each of them occurring in ESRD/ACRD, epithelial cells may switch on expression of KRT7 and KRT19, which appears to correlate with reduction in the degree of terminal differentiation.

A diffuse expression of KRT7 and KRT19 was found not only in ESRD/ACRD kidneys, cysts, pre-neoplastic lesions but also in tumors of distinct histological types. *KRT19* has been described as a putative marker of epidermal stem cells (17). The increased expression of KRT19 shows a good correlation with the invasive growth and metastatic potential of liver and cancer (18, 19). Serum levels of *CYFRA* 21-1, a *KRT19* fragment, can be used as a tumour marker in variety of cancers such as non-small cell lung cancer, breast, ovarian, liver and bladder cancer to detect tumour cell dissemination and presence of metastasis (27, 28).

In summary, our results indicate that tubular cells of end-stage kidneys suspend their program of terminal differentiation and shift their keratin expression profile from KRT8 and KRT18 to KRT7 and KRT19 which allows them to alter their shape and to increase their plasticity necessary for the remodeling and tumorigenic processes. ESRD/ACRD is associated with large number of proliferative cysts and pre-neoplastic lesions with distinct cellular characteristics similar to those observed in tumors from the corresponding kidney. Previous and present data indicates that ESRD/ACRD is a novel disease. The hypoxemic and inflammatory microenvironment alters the plasticity and leads to stem cell characteristics of epithelial cells which might be crucial factor in tumor development.

Conflicts of Interest

Authors have no conflicts of interest to declare.

Acknowledgements

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