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CLINICAL STUDY

Immunohistochemical study of tubular epithelial cells and vascular endothelial cells in glomerulonephritis

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Abstract

Background and aims: In order to assess the role played by tubular epithelial cells (TEC) and interstitial vascular endothelial cells (VEC) in interstitial fibrogenesis in human glomerulonephritis, we studied the expression of markers of activated fibroblasts (α-smooth muscle actin (αSMA) and vimentin (Vim)) and of the transforming growth factor β (TGFβ), at the level of these cells. *Methods*: We studied retrospectively 41 renal biopsies from patients with primary and secondary glomerulonephritis [24 males, 17 females, mean age 45.5 ± 12.9 years]. Immunohistochemistry using monoclonal antibodies (SMA, Vim, TGFβ) was assessed using a semiquantitative score, that was correlated with biological and histological data (quantified using a scoring system in order to assess active-inflammatory and chronic–sclerotic/fibrotic lesions). *Results*: The presence of SMA and Vim as markers of myofibroblasts was found in TECs and VECs. TEC Vim expression correlated with interstitial Vim expression ($r = 0.38$; $p = 0.008$), interstitial infiltrate ($r = 0.31$; $p = 0.027$), interstitial fibrosis ($R = 0.25$; $p = 0.042$), GFR ($r = -0.35$; $p = 0.016$), SMA ($r = -0.42$; $p = 0.015$), TGFβ ($r = 0.25$; $p = 0.046$), and hemoglobin ($r = -0.55$; $p < 0.001$). VEC Vim expression showed indirect correlations with interstitial infiltrate ($r = -0.32$; $p = 0.023$) and interstitial fibrosis ($r = -0.34$; $p = 0.017$). *Conclusion*: Our study reflects the complexity of the involvement of VEC and mainly of TEC in fibrosis. The expression of mesenchymal markers at the tubular cell level (especially Vim) correlates with histological interstitial changes, with the decrease of renal function and more strongly with anemia.

Keywords

Alpha smooth muscle actin, TGFβ, tubular epithelial cells, vascular endothelial cells, vimentin

History

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Introduction

Chronic kidney disease regardless of its etiology progresses towards end stage renal disease, and the final common pathway in this process seems to be fibrosis.[1] An important role is played by tubulo-interstitial fibrosis, which is the strongest morphological predictor of clinical outcome and is most tightly linked to progression of disease, even though the primary disease may be of glomerular origin.[2]

In a previous study, we have shown that the interstitial histological changes, especially the scores indicating sclerotic/fibrotic lesions, correlate with the presence of interstitial myofibroblasts, with an important role played by TGFβ.[3] Thus the main effector cell in this process is the interstitial myofibroblast, being most responsible for interstitial matrix accumulation. Other cells present at this level (tubular epithelial cells (TEC) and vascular endothelial cells (VEC)) are also involved in fibrogenesis.

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In the current study performed on human renal biopsies, we have set ourselves to investigate by means of immunohistochemistry, compared with histological and biological data, the possible role of TEC and VEC in interstitial fibrosis.

As shown in experimental studies, these cells are considered possible progenitor cells for myofibroblasts. During the course of kidney fibrosis in mice, about 30% of myofibroblasts are derived via epithelial–mesenchymal transition (EMT) from the TEC of the kidney. In addition, it has been shown that another 35% of myofibroblasts are derived via endothelial–mesenchymal transition (EndMT) from the endothelial cells normally residing within the kidney. The remaining portions are speculated to arise via activation of resident fibroblasts or other mesenchymal cells, such as perivascular smooth muscle cells/pericytes and fibrocytes in the circulation, or are fibroblasts derived from the bone marrow.[4,5] Despite these experimental data, the origin of interstitial myofibroblasts remains debatable, because data from *in vivo* studies is scarce.

In order to assess the role played by TEC and interstitial VEC in human glomerulonephritis, we studied at the level of these cells the expression of mesenchymal markers that are

markers of activated fibroblasts (α-smooth muscle actin (αSMA) and vimentin (Vim)) and of the transforming growth factor-β (TGFβ), a growth factor that is involved in this activation.

Materials and methods

Patients

We studied retrospectively the renal biopsies of 41 patients admitted at the Department of Nephrology, Timisoara, with chronic glomerulonephritis (17 females, 24 males; mean age 45.5 ± 12.9 years, range 18–74). Only those cases were included that presented enough paraffin wax embedded in the biopsy material to permit the cutting of additional sections for immunohistochemistry. Cases with fewer than five glomeruli were excluded from the study.[6]

The patients had either primary (30 cases) or secondary glomerulonephritis (systemic vasculitis, four cases; infectious, three cases; collagenoses, two cases; neoplasia, two cases). The histopathological diagnoses were mesangial proliferative glomerulonephritis (12 cases), mesangiocapillary glomerulonephritis (1 case), membranous nephropathy (5 cases), minimal change disease (5 cases), focal and segmental glomerulosclerosis (15 cases), and crescentic glomerulonephritis (3 cases).

We used as controls renal tissue samples obtained from four patients who underwent nephrectomy for kidney tumors. The samples were obtained from the normal renal tissue. All control patients had normal serum creatinine, eGFR > 60 mL/min, and no proteinuria, at the moment of nephrectomy.

All biopsies were performed after obtaining an informed consent from patients regarding the procedure and the possible use of the obtained material for scientific purposes. The present study has the approval of the local ethical committee.

Histology

Routinely fixed and processed sections of kidney were processed for light microscopy and stained with hematoxylin and eosin (HE), periodic acid-Schiff (PAS), and Gomori's trichrome techniques using routine methods. All stained slides were assessed separately by two pathologists. In order to better quantify the histological lesions, a scoring system adapted by Neumann et al.[7] for ANCA-associated vasculitis, based on the standardized scoring system for activity and chronicity developed for lupus nephritis, was employed. At the tubulo-interstitial level, inflammatory lesions (edema, interstitial infiltrate) and sclerotic/fibrotic lesions (interstitial fibrosis, tubular atrophy) were assessed. Tubulo-interstitial lesions were assessed semi-quantitatively: <30% of tubules or interstitial area affected was considered as mild (1 point), 31–60% affected as moderate (2 points), and >60% affected as severe (3 points).[7]

Immunohistochemistry

The detection of αSMA, Vim, and TGFβ was performed on 4 μm-thick formalin-fixed, paraffin-embedded sections using a horseradish peroxidase-labeled streptavidin–biotin (LSAB2-HRP) method (a system intended for use with primary antibodies for the qualitative identification of antigens in paraffin-embedded tissues).

The primary antibodies used were ready-to-use monoclonal mouse anti-Vim (Vim3B4 antibody, DAKO, Carpinteria, CA); monoclonal mouse anti-smooth muscle actin (clone 1A4, DAKO, Carpinteria, CA); and concentrated monoclonal mouse anti-TGFβ (MCA 797, Serotec, Raleigh, NC).

Sections were first deparaffinized and rehydrated by routine protocol, then incubated with 3% hydrogen peroxide in distilled water for 5 min and afterwards rinsed with distilled water and placed in Tris-buffered saline (TBS) for 5 min. The next step was incubation with a primary antibody, diluted 1/75, for 10–30 min, followed by sequential 10-min incubations with a biotinylated link antibody and peroxidase-labeled streptavidin (both purchased ready-to-use, DAKO, Carpinteria, CA).

The labeling of TGFβ, αSMA, and Vim immunoreactivity, at the level of TEC and VEC, was graded for statistical evaluation using a semi-quantitative intensity scale from 0 to 3, similar to that used by Alexopoulos et al.[8] We considered 0 = no labeling (negative), 3 = the most intense labeling, whereas 1 and 2 are labeling of an intermediate degree. The same semi-quantitative intensity scale was used for assessing interstitial Vim staining.

Clinical parameters

In addition to the histological data, we obtained clinical and biological parameters at the time of the biopsy from the patients' files. In all patients, renal function (serum creatinine and glomerular filtration rate (eGFR)), blood pressure, proteinuria (24 h urine specimen), and hemoglobin count were taken. GFR was estimated using the MDRD4 formula.[9]

Statistical analysis

Data were recorded in a file created in Microsoft Excel, which was organized and managed as a database. Correlations between histological and immunohistochemical parameters were performed using the non-parametric Spearman's rank order test, while correlations among clinical, biological data, and immunohistochemistry were performed using parametric Pearson's test.

Correlation coefficients of linear regression analysis are presented in relation with p values. The significance of the correlation coefficient (r) is as follows: $r = 0$–0.25 indicates little or no correlation; $r = 0.25$–0.50 indicates a fair degree of relationship; $r = 0.5$–0.75 indicates moderate to good correlation; $r = 0.75$–1 indicates very good to excellent correlation.[10] In order to perform these tests, we used WinStat for Microsoft Excel and Epi 3.2.2.

Results

In 24 of the studied cases, we found positive Vim immunostaining at the level of TEC; in 5 of the cases, Vim staining was present either at a proximal (two cases) or at a distal (three cases) tubular level. The mean expression of Vim in TEC was 0.77 ± 0.87 in proximal tubules and 0.71 ± 0.76 in distal tubules. There was, however, no statistically significant difference between the two tubular segments overall.

Vim expression was low or moderate at the level of TEC in our patients; in only one patient (with mesangiocapillary GN), we had to use the degree 3 from the semi-quantitative scale to express a strong Vim staining. Vim expression was positive in patients with focal segmental glomerulosclerosis (nine patients at proximal level and seven patients at distal level), minimal change disease (two patients at proximal and three at distal level), membranous nephropathy (three at proximal and four at distal level), mesangioproliferative GN (five patients at distal and proximal levels), and crescentic GN and mesangiocapillary GN (in both types: one patient at distal and proximal levels) (Figure 1).

In 13 cases, αSMA immunostaining was present at the level of the TEC. The mean expression of αSMA was 0.55 ± 0.64 in proximal TEC and 0.57 ± 0.69 in the distal ones. This positive αSMA immunostaining occurred in six patients with mesangioproliferative GN, in three patients with focal segmental glomerulosclerosis, in two patients with minimal change disease, in one patient with membranous nephropathy, and in another one with crescentic GN (Figure 2).

TGFβ TEC immunostaining was present in 29 of the studied cases, with no statistically significant difference between the proximal and distal segments of the tubules. The mean expression of TGFβ was 0.97 ± 0.85 in proximal TEC and 1.04 ± 0.86 in distal TEC. Similar to Vim, TGFβ tubular immunostaining was moderate or low, with the exception of two patients in whom we found a strong immunostaining (degree 3). A positive TGFβ immunostaining at TEC level was found in 10 patients with mesangioproliferative GN, in 9 patients with focal segmental glomerulosclerosis, 5 patients with minimal change disease, in 3 patients with membranous nephropathy, in 1 patient with crescentic GN, and in 1 patient with mesangiocapillary GN (Figure 3).

At the level of the VEC, Vim staining was present in 27 cases, while TGFβ in 35 cases. The mean expression of Vim was 0.78 ± 0.58 and, for TGFβ, it was 0.92 ± 0.46. At the level of VEC, Vim was positive in 11 patients with mesangioproliferative GN, in 10 patients with focal segmental glomerulosclerosis, in 5 patients with minimal change disease, in 4 patients with membranous nephropathy, in 1 patient with mesangiocapillary GN, and none with crescentic GN. TGFβ was present in 11 patients with mesangioproliferative GN, in 13 patients with focal segmental glomerulosclerosis, in 4 patients with minimal change disease, in 3 patients with membranous nephropathy, 1 patient with mesangiocapillary GN, and in 3 patients with crescentic GN.

In the normal control patients, immunostaining was negative for Vim, αSMA, and TGFβ at the level of TEC and VEC. In these renal samples (from normal controls), we found αSMA in the media of the interstitial vessels.

As mentioned above in the patients with glomerulonephritis, the presence of αSMA and Vim as markers of the myofibroblasts, as well as of TGFβ involved in this process with the formation of active fibroblasts, was found at the level of TEC and VEC. The process shows a great variability in each patient, fact that makes a statistical correlation with biological and histological data difficult; however, some correlations have been found.

First we studied the correlations with tubulo-interstitial histological data assessed by light microscopy.

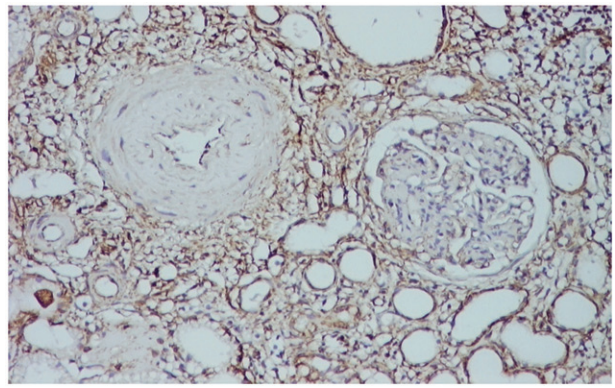

Figure 1. Intense positive vimentin immunostaining at the level of the interstitium, peritubular, and periglomerular. Vimentin stain LSAB2-DAB × 100.

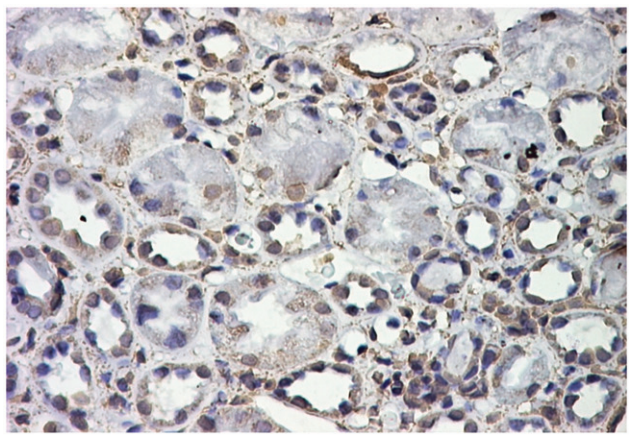

Figure 2. Positive αSMA immunostaining in the cytoplasm and nuclei of tubular epithelial structures. αSMA stain LSAB2-DAB × 200.

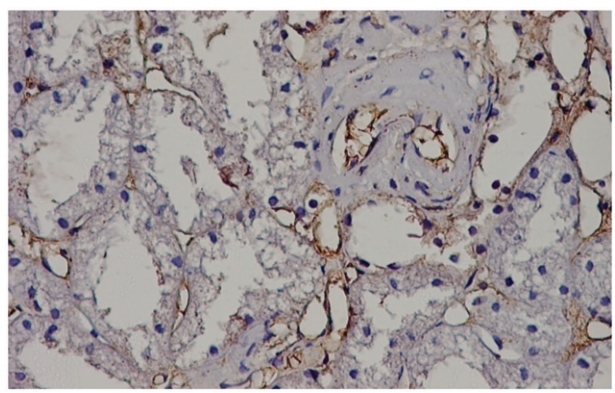

Figure 3. Interstitial capillary and arteriolar endothelium positive for TGFβ stain. TGFβ stain LSAB2-DAB × 200.

The tubulo-interstitial lesions were studied on standard stains in light microscopy (HE, PAS, and Gomori's trichrome) using the *scoring system* adapted from Neumann et al. As already mentioned in the Material and methods section, the following tubulo-interstitial lesions were assessed semi-quantitatively: inflammatory lesions (edema and interstitial infiltrate) and sclerotic/fibrotic lesions (interstitial fibrosis and tubular atrophy).

We found a statistically significant small degree of correlation between the scores for Vim staining at the level of the proximal TEC and interstitial infiltrate ($r = 0.31$,

$p<0.05$), on one hand, and interstitial fibrosis ($r=0.25$, $p<0.05$), on the other hand. As shown in Table 1, there are no other statistically significant correlations between immunohistochemical parameters at the level of the TEC and interstitial histological scores.

When comparing the different immunohistochemical parameters, we found the following correlations: Vim staining at the tubular level (both proximal and distal) correlated with interstitial Vim staining ($r=0.38$, $p<0.05$) and with TGFβ tubular staining (only for the distal tubules) ($r=0.24$, $p<0.05$). There was also an indirect correlation between αSMA and Vim at the level of the tubules: distal ($r=-0.42$, $p<0.05$) and proximal ($r=-0.30$, $p<0.05$).

We compared immunohistochemical data in all patients at the tubular level with clinical data (serum creatinine, eGFR, proteinuria, and hemoglobin) and we found that Vim staining at the level of distal TEC correlated statistically significantly with renal function: serum creatinine ($r=0.32$, $p<0.05$) and eGFR ($r=-0.36$, $p<0.05$). Proteinuria correlated indirectly with the proximal TEC Vim ($r=-0.27$, $p<0.005$) and TGFβ ($r=-0.30$, $p<0.05$) staining.

We found also a moderate indirect correlation between the hemoglobin and the Vim staining at the level of the proximal ($r=-0.51$, $p<0.001$) and distal tubules ($r=-0.55$, $p<0.001$).

Due to the relatively small number of patients with the different histological types of glomerulonephritis, we were not able to find some immunohistochemical expression patterns at the level of TEC. We studied, however, the two subgroups of patients that were better represented: patients with focal segmental glomerulosclerosis (FSGS, 15 patients) and patients with mesangioproliferative glomerulonephritis (MPGN, 12 patients).

In MPGN patients, we found a strong indirect correlation between tubular Vim and renal function: proximal TEC Vim with serum creatinine ($r=0.6$, $p<0.05$) and with eGFR ($r=-0.6$, $p<0.05$); distal TEC Vim with serum creatinine ($r=0.6$, $p<0.05$) and with eGFR ($r=-0.6$, $p<0.05$). For TGFβ at the level of distal TEC, we found the same correlation with eGFR ($r=-0.51$, $p<0.05$). Surprisingly in the same group of patients (MPGN), αSMA expression in TEC showed a direct correlation with eGFR both in distal ($r=0.59$, $p<0.05$) and in proximal tubules ($r=0.52$, $p<0.1$).

In FSGS patients, we found that proximal tubular Vim expression correlates directly with the score for interstitial fibrosis ($r=0.4$, $p<0.1$), while proximal tubular αSMA expression correlates indirectly with the scores for interstitial infiltrate ($r=-0.62$, $p<0.1$) and with interstitial fibrosis ($r=-0.58$, $p<0.1$). In this subgroup of patients, we found that TGFβ at the level of proximal TEC correlated indirectly with proteinuria ($r=-0.39$, $p<0.1$).

Regarding VEC, Vim staining at this level correlated indirectly with interstitial infiltrate ($r=-0.32$, $p<0.05$) and interstitial fibrosis ($r=-0.34$, $p<0.05$). Concerning clinical data, we found only an indirect correlation between proteinuria and TGFβ staining at the level of the VEC ($r=-0.25$, $p<0.05$) (Table 2). No statistically significant correlations have been found between Vim and TGFβ at VEC level with renal function or anemia.

Table 1. Correlation between immunohistochemical staining scores at the level of tubular epithelial cells (TEC) and interstitial histological scores and clinical data (prox, proximal; dist, distal).

Pearson correlation	Interstitial edema	Interstitial infiltrate	Interstitial fibrosis	Tubular atrophies	Proteinuria	Serum creatinine	eGFR
αSMA prox. TEC							
r	0.21009156	−0.08227098	0.03898	0.0850556	−0.0757	−0.12323	0.221282
p	0.14644391	0.34165263	0.42346	0.3365800	0.3566	0.27014	0.133666
αSMA dist. TEC							
r	0.1994508	−0.16858008	−0.0587	−0.1302433	0.063622	−0.15351	0.32015
p	0.1592839	0.20029769	0.38548	0.2586517	0.378748	0.222297	0.051761
Vimentin prox. TEC							
r	0.099812798	0.313162826	0.25943603	0.06529312	−0.2786578	0.21132465	−0.34791524
p	0.275512236	0.02778171	0.04279996	0.34846625	0.0474500	0.10139955	0.01616026
Vimentin dist. TEC							
r	0.256134317	0.223258053	0.05042303	−0.00074431	−0.14634197	0.32781289	−0.36793017
p	0.060307715	0.088932234	0.38184585	0.49823070	0.19371777	0.02225947	0.01152254
TGFβ prox. TEC							
r	−0.189164364	−0.172968478	−0.1383094	−0.1537955	−0.3027728	−0.02682417	0.01553297
p	0.118104471	0.139745243	0.19423911	0.16851610	0.02878059	0.43389092	0.46160562
TGFβ dist. TEC							
r	−0.083709401	−0.183802808	−0.1107815	−0.1665446	−0.23749043	0.05062329	−0.06327586
p	0.301413153	0.125000099	0.2452423	0.14900329	0.07002606	0.37663731	0.34714952

Table 2. Correlation between immunohistochemical scores for vascular endothelial cells (VEC) staining and interstitial histological scores and clinical data.

	Interstitial edema	Interstitial infiltrate	Interstitial fibrosis	Tubular atrophies	Proteinuria	Serum creatinine	eGFR
Vimentin VEC							
r	−0.199299803	−0.329175908	−0.3469841	−0.4079876	−0.05671652	−0.17901234	0.20386943
p	0.118485114	0.023328144	0.01768722	0.0060939	0.3712464	0.14454750	0.11308439
TGFβ VEC							
r	0.203251664	0.17368135	0.19315847	0.16755051	−0.25851234	0.10722386	−0.23323048
p	0.101234127	0.138741544	0.1131387	0.14752826	0.0436287	0.2523020	0.07111387

Discussions

The results of our study showed at the level of tubules and interstitial vessels a great variability of the expression of the studied immunohistochemical markers (Vim and αSMA), and also of the growth factor TGFβ. Despite this great variability, some facts regarding a certain pattern of staining in these cell types can be discussed.

At the level of TEC, the expression of the mesenchymal markers αSMA and Vim could indicate a process of EMT, a biological process in embryological development.[11] In different studies (especially *in vitro* or in experimental animal models), it has been tried to establish whether EMT also occurs in renal epithelial cells, following kidney injury, and to show that the mesenchymal cells formed could give rise to myofibroblasts which populate the renal interstitium, causing fibrosis within it.[12,13]

In the cases studied by us, the expression of Vim as the mesenchymal marker at the level of proximal TEC correlated with histological interstitial markers of activity (interstitial infiltrate) and of chronicity (interstitial fibrosis—especially in our FSGS patients). We found also correlations between tubular and interstitial Vim expression. It has already been shown by other authors that in human biopsies of kidneys with fibrotic lesions, Vim is positive at the tubular level.[14] Rastaldi et al. have also shown in human biopsies that tubular Vim correlated with the interstitial infiltrate and with interstitial fibrosis, on one hand. In the same study, it was shown that, on the other hand, tubular αSMA was rare, despite the fact that its interstitial expression is a good marker of renal disease progression.[15] Our results were consistent with these data, αSMA was present in only 13 cases at the tubular level; moreover, when was assessed quantitatively, it correlated indirectly with Vim expression. We could also observe that especially in the subgroup of FSGS patients, tubular αSMA showed an indirect correlation with interstitial fibrosis, showing thus an opposite pattern to the tubular Vim expression.

TGFβ, the other marker used by us, has the ability to induce the expression of extracellular matrix proteins in mesenchymal cells and to stimulate the production of protease inhibitors that prevent enzymatic breakdown of the ECM. Elevated TGFβ expression in affected organs, and subsequent deregulation of TGFβ functions, correlates with the abnormal connective tissue deposition observed during the onset of fibrotic diseases.[16] Therefore, we used TGFβ antibodies in our study to find out if there is an involvement of this growth factor in the activation of TEC. According to Fragiadaki et al., there is no doubt that proximal TEC can undergo EMT *in vitro* in response to TGFβ-1 and also other inflammatory stimuli.[12] The consequence of TGFβ stimulation, due to injury, is an increased expression of Vim in the tubular epithelium.[17]

In our patients, TGFβ staining correlated with Vim staining at the level of the distal tubules. If we maintain the hypothesis, proven in experimental studies, that TGFβ can promote EMT at the tubular level, then the positive correlation between these two stainings (Vim and TGFβ) can be explained.

The expression of the mesenchymal markers at the tubular level could be correlated with the renal function at the moment of the renal biopsy. In the study performed by Rastaldi et al., tubular Vim correlated with serum creatinine.[15] In another study performed by de Matos et al. on 49 kidney transplant recipients, it was shown that a high expression of tubular Vim was associated with a reduction of graft function.[18] All these data are consistent with our results concerning the correlation between tubular Vim and renal function (serum creatinine and eGFR) at the moment of renal biopsy. This fact was especially true in the subgroup of patients with MPGN. In this same subgroup of patients, however, αSMA showed, surprisingly, a direct correlation with eGFR.

Urinary proteins from patients with minimal change disease and, especially FSGS, induce in cell cultures the expression of αSMA and Vim.[19] In the aforementioned study performed on human renal biopsies by Rastaldi et al., there was a strong correlation between proteinuria and tubular Vim.[15] Surprisingly proteinuria showed in our patients an indirect correlation with tubular Vim. Similar to our results, Yonemoto et al. found in patients with diabetic nephropathy that newly acquired Vim (at the mesangial level in their study) decreased in patients with heavy proteinuria.[20] Also, in a study performed on patients with congenital nephrotic syndrome of the Finnish type (NPHS1), it was shown that heavy proteinuria did not lead to the transition of the TEC into myofibroblasts, as shown by the expression of tubular Vim and αSMA.[21] Moreover, in our patients (especially the FSGS patients), there was also an indirect correlation between tubular TGFβ and proteinuria.

We found an interesting indirect correlation between Vim at the TEC level and hemoglobin. This could be due to the fact that EPO-producing fibroblasts transform into myofibroblasts at the cost of EPO production. This finding could clarify the link between renal fibrosis and anemia.

Studies using *in situ* hybridization and the transgenic mice approach indicate that EPO is mainly produced by the interstitial fibroblasts in the deep cortex and the outer medulla in the kidney. Asada et al. demonstrate the occurrence in the kidney interstitium of fibroblasts that produce EPO, and can transdifferentiate into scar-producing myofibroblasts, thus losing their EPO-producing activity after kidney injury.[22]

The fact that in the patients studied by us there was an indirect correlation between tubular Vim and hemoglobin could indicate the fact that EPO-producing fibroblasts have been replaced by myofibroblasts (with Vim expression). It is, however, interesting that for SMA (the other myofibroblast marker), these correlations have not been found.

The few studies performed using human renal biopsies, including the present one, show a correlation of tubular Vim staining with interstitial infiltrate and fibrosis, with renal function and with anemia. Our results regarding TEC could support the hypothesis shown mainly in experimental (*in vitro*) studies that these cells may acquire markers of mesenchymal cells, a process known as EMT. This possible origin of interstitial myofibroblasts remains debatable.

Humphreys et al. have shown *in vivo*, but in a mouse model of ureteral obstruction that after injury TEC do not migrate and do not transform into myofibroblasts.[23] In another study performed using proximal TEC cultures, it has

been shown that shear stress, as it occurs in hyperfiltration, leads to matrix generation and thus fibrogenesis, but inhibits the motility of tubular cells, thus excluding EMT. In contrast, incubation with TGFβ induces cell motility and Vim expression in the cultured tubular cells. The authors conclude that renal fibrosis and EMT could exclude each other.[24] This could be a possible explanation of the fact that in our patients with FSGS tubular αSMA correlated indirectly with interstitial fibrosis, or that it correlated directly with eGFR (in patients with MPGN). It remains, however, difficult to explain why in our patients Vim and αSMA showed different patterns of expression and correlations in TEC. It is possible that the two markers are not present at the same time in the different TEC that did undergo a transformation. The fact that we were not able to perform double staining (Vim and αSMA) could be considered a limitation of this study. It is also possible that there are different patterns of expression of these markers in TEC in different histological types of glomerulonephritis, as we have shown for FSGS or MPGN, but the reduced number of cases did not permit the drawing of a pertinent conclusion.

EndoMT, a newly recognized type of cellular transdifferentiation, has emerged as another possible source of tissue myofibroblasts. EndoMT is a complex biological process in which endothelial cells lose their specific markers and acquire a myofibroblastic phenotype and express mesenchymal markers. Similar to EMT, EndoMT can be induced by TGFβ.[25] It has been shown in experimental diabetic nephropathy in mice that endothelial–myofibroblast transition occurs and contributes to the early development and progression of renal interstitial fibrosis.[26] In the cases studied by us, however, we found that, unlike in TEC, Vim expression in VEC showed an indirect correlation with interstitial histological scores. This surprising fact together with the absence of other correlations between the expression of Vim or TGFβ at the level of VEC with histological and clinico-biological parameters could lead to the conclusion that these cells do not undergo a transition into mesenchymal cells, but the presence of these markers at this level opens the perspective to new studies in this field.

Conclusions

Our study performed in human biopsies reflects the complexity of the involvement of VEC and mainly of TEC in fibrosis. It cannot be stated that TEC are, without doubt, transformed into myofibroblasts during renal injury, but it has been shown that the expression of mesenchymal markers at the tubular cell level (especially Vim) correlates with histological interstitial changes, with the decrease of renal function and more strongly with anemia.

The possible link between renal fibrosis and anemia could be important in developing new treatment strategies that are aimed to target both the renal anemia and the reversibility of fibrosis.

Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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