Cellular Senescence Is Associated with Faster Progression of Focal Segmental Glomerulosclerosis

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Keywords

Cell senescence · Focal segmental glomerulosclerosis · Chronic kidney disease

Abstract

Background: A current, albeit unproven, hypothesis is that an acceleration of cellular senescence is involved in impaired renal repair and progression of glomerular diseases. Focal segmental glomerulosclerosis (FSGS) is a glomerular disease with a substantial risk for progression to ESRD. However, if and to what extent cell senescence predicts a negative outcome in FSGS is still unknown. **Methods:** The hypothesis that cell senescence represents a proximate mechanism by which the kidney is damaged in FSGS (NOS phenotype) was investigated in 26 consecutive kidney biopsies from adult FSGS cases (eGFR 72 ± 4 mL/min, proteinuria 2.3 ± 0.6 g/day) who were incident for 2 years in a Northern Italian nephrology center and had a 6-year clinical follow-up. **Results:** Cell senescence (p16^{INK4A}, SA- β -galactosidase [SA- β -Gal]) was up-regulated by ~3- to 4-fold in both glomerular and tubular cells in kidney biopsies of FSGS as compared to age-matched controls ($p < 0.05$ – 0.01). Tubular SA- β -Gal correlated with

proteinuria and glomerulosclerosis, while only as a trend, tubular p16^{INK4A} was directly associated with interstitial fibrosis. At univariate analysis, basal eGFR, proteinuria, and tubular expression of SA- β -Gal and p16^{INK4A} were significantly directly related to the annual loss of eGFR. No correlation was observed between glomerular p16^{INK4A} and eGFR loss. However, at multivariate analysis, eGFR, proteinuria, and tubular p16^{INK4A}, but not SA- β -Gal, contributed significantly to the prediction of eGFR loss. **Conclusions:** The results indicate that an elevated cell senescence rate, expressed by an up-regulation of p16^{INK4A} in tubules at the time of initial biopsy, represents an independent predictor of progression to ESRD in adult patients with FSGS.

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Introduction

Aging is associated with accelerated cell senescence, a stress-response process during which cells undergo an irreversible cycle arrest and produce a proinflammatory secretory phenotype [1–3]. In several chronic diseases, including diabetes and obesity, senescent cells accumulate in tissues, where their inflammatory phenotype disrupts

structure and function, inducing fibrosis and, possibly, cancer [3, 4].

A current, albeit unproven, hypothesis is that an acceleration of cell senescence is involved in impaired renal repair and progression of glomerular diseases [3–5]. In a seminal study performed in 35 patients affected by different glomerular diseases, Sis et al. [6] demonstrated an increase in the cycle regulator and tumor suppressor p16^{INK4A} in the glomeruli, tubules, and interstitium, which correlated with the degree of interstitial fibrosis and tubular atrophy, suggesting a role for somatic cell senescence as a mediator of progression of glomerular diseases. In addition, we previously observed that p16^{INK4A} and senescence-associated β -galactosidase (SA- β -Gal) were markedly upregulated in both the glomeruli and tubuli in diabetic nephropathy and correlated with several clinical parameters of metabolic stress [7]. More recently, Liu et al. [8] showed an upregulation of p16^{INK4A} in renal tubular cells that correlated with the expression of collagen III in IgA nephropathy.

Focal segmental glomerulosclerosis (FSGS) is a common glomerular disease with a substantial risk of progression to ESRD [9]. In addition to the pathognomonic segmental glomerular sclerosing lesions, the renal histopathology in progressive FSGS is characterized by widespread tubular atrophy and interstitial fibrosis, and both proteinuria and the extent of tubulointerstitial disease correlate well with the degree of functional renal impairment [10, 11]. Despite recent progress in our understanding of genetic and environmental factors in the pathogenesis of FSGS [11–14] and in the molecular pathways that can differentiate etiologically distinct subtypes, translating basic research into clinical care appears to be insufficient [13–15]. Although cross-sectional studies have demonstrated an upregulation of somatic senescence in glomerular diseases, including FSGS [6], the functional role of the changes observed is unknown, and whether senescence is really associated with more disease progression is still not understood. A better knowledge of the role played by cell senescence in FSGS, and of the different cell types involved by this process, may be helpful also in view of the new “senolytic” therapies which, by eliminating accumulated senescent cells, could potentially recover tissue function [16].

In this study, we tested the hypothesis that acceleration of senescence represents a relevant mechanism by which the kidney is injured in FSGS. With this in mind, we studied the expression of 2 different markers of senescence, p16^{INK4A} and SA- β -Gal, in kidney biopsies from a cohort of incident adult FSGS patients who were as for the standard of care followed up for 6 years.

Table 1. Clinical characteristics of FSGS subjects.

Number of subjects	26
Age, years	52 (21)
Gender, M/F	12/14
BMI, kg/m ²	25 (9)
Proteinuria, g/day	2.3±0.6
eGFR, mL/min 1.73 m ²	72±7
SBP, mm Hg	138±21
DBP, mm Hg	82±9
Globally sclerosed glomeruli, %	10±3.8
Interstitial fibrosis, %	17±3.7
Tubular atrophy, %	8±2.8
Serum albumin, g/dL	3.5 (3)
Total cholesterol, mg/dL	207 (118.5)
Triglycerides, mg/dL	157 (152)
eGFR loss, mL/min/year	4.7±1.55
Follow-up, years	6±0.56

Data are mean ± SEM or median (IQR). FSGS, focal segmental glomerulosclerosis; eGFR, estimated glomerular filtration rate; SBP, systolic blood pressure; DBP, diastolic blood pressure.

Methods

Patients

Cell senescence was investigated in 26 consecutive kidney biopsies from FSGS patients who were incident from May 2007 to June 2009 at the Department of Internal Medicine, Nephrology Division, Genoa University, and IRCCS AOU San Martino, Genoa, Italy. All biopsies were not for research but for clinical diagnosis. The inclusion and exclusion criteria were defined to select cases with FSGS as the primary disease. During the screening, 12 FSGS cases were not included in the analysis because they were recognized to be affected by a secondary disease. The procedures were in accordance with the Declaration of Helsinki. The study was part of a larger study on aging and the kidney sponsored by the Ministero dell'Università e della Ricerca Scientifica (MIUR) (Progetti Finalizzati per la Ricerca di Base-FIRB 2002) and approved by the Ethical Committee of the Department of Internal Medicine [7, 17]. All kidney biopsy specimens were analyzed by the same renal pathologist (Dr. Gennaro Salvadio). The morphologic changes in kidney biopsies were considered for the following categories: glomerular sclerosis (global/segmental), glomerular cellularity, interstitial fibrosis, interstitial inflammation, tubular atrophy, and atherosclerosis. All the included cases belonged to the not otherwise specified FSGS subtype [13]. Normal portions of kidney tissue from nephrectomies for renal carcinoma ($n = 13$, 8 males and 5 females, 60 ± 4 years) were examined as a control group.

Baseline clinical and demographic characteristics of subjects are shown in Table 1. The average protein excretion was 2.3 ± 0.6 g/day; 5 subjects presented with nephrotic (> 3 g/day) proteinuria. The mean eGFR was 72 ± 7 mL/min 1.73 m². In FSGS, the percentages of globally sclerosed glomeruli, tubular atrophy, and interstitial fibrosis were 10 ± 3.8 , 8 ± 3 , and $17 \pm 3.7\%$, respectively. In control tissue, the percentages of globally sclerosed glomeruli, tubular atrophy, and interstitial fibrosis were 3 ± 1 , 5 ± 2 , and $4 \pm 1\%$,

Fig. 1. Expression of p16^{INK4A} in the glomeruli and tubulointerstitium of control subjects and patients with FSGS. The percentage of glomerular p16^{INK4A}-positive nuclei (**a**), as well as the percentage of podocyte p16^{INK4A}-positive nuclei (**b**), was higher in pa-

tients with FSGS than in controls. FSGS patients showed also a strikingly increased p16^{INK4A} expression in tubuli (**c**). * = $p < 0.05$ or less; ** = $p < 0.01$ or less versus controls. FSGS, focal segmental glomerulosclerosis; AU, arbitrary units.

respectively. Routine clinical visits, including clinical labs, proteinuria, and eGFR (CKD-epi) estimate, were carried out basally, after 1–3 months, and then at least annually for 6 years. These results represent collection from standard practice. All but 1 subject received single or double RAS blockade. Fifteen subjects received steroids/immunosuppressive therapy. Seven of 26 patients (28% of cases) doubled their serum Cr or reached ESRD at the end of follow-up. The mean eGFR loss per year was 4.7 ± 1.55 mL/min.

Expression of Nuclear p16^{INK4A} and SA- β -Gal

SA- β -Gal-positive cells were detected in frozen tissue as described by Dimri et al. [18, 19] as cells showing a bright cytoplasmic blue precipitate. Samples were analyzed for p16^{INK4A} as previously described [17]. Paraffin sections (5 μ m) of 2% paraformaldehyde-fixed renal biopsies were deparaffinized, hydrated, and treated with 3% methanol hydrogen peroxide solution. Slides were exposed to primary antibody for 1 h (1:100 dilution in PBS), followed by incubation in biotinylated anti-rabbit antibody (1:100 dilution in PBS) for 30 min. At least 5 (range 5–10) glomeruli from each biopsy were systematically serially sectioned to estimate cell number by light microscopy. Positive glomeruli were defined by the presence of at least 1 positive nucleus/cell. The percentage of p16^{INK4A}-positive nuclei (nuclear p16^{INK4A}) was assessed for glomeruli and tubules and expressed as percent positive cells. On average, 900 glomerular and 1,300 tubular nuclei were counted. The nuclear cytoplasmic expression of p16^{INK4A} in tubules was examined by image analysis and expressed as percent positive areas [17]. SA- β -Gal tubular cytoplasmic staining was assessed by counting the percentage of tubular cross sections that showed positive cytoplasmic staining. For immunofluorescence studies, slides were incubated with anti-podocin and anti-p16^{INK4A} and subsequently with Alexa Fluor[®] 594 goat anti-rabbit IgG and Alexa Fluor[®] 488 goat anti-rabbit IgG.

Reagents

5-Bromo-4-chloro-3-indolyl- β -D-galactopyranoside was purchased from Merck Group (Milano, Italy), antihuman polyclonal p16^{INK4A} Ab was obtained from Santa Cruz Biotechnology (DBA Italia s.r.l., Seregno, Italy) and from St John's Laboratory (DBA

Italia s.r.l.), the biotin-streptavidin-amplified detection system was from Vector Laboratories Inc. (DBA, Italia s.r.l.), and DAB and Eukitt were from Merck Group. Anti-podocin, Alexa Fluor[®] 594 goat anti-rabbit IgG, and Alexa Fluor[®] 488 goat anti-rabbit IgG were purchased from Thermo Fisher Scientific (Milan, Italy).

Statistical Analysis

Data are presented as means $\pm$ SEM. Parameters not normally distributed were logarithmically transformed for statistical analysis and expressed as median (interquartile range). The Statview statistical package (Cary, NC, USA) was used for the analysis. Comparisons between groups were made by ANOVA. Comparisons of proportions were made using the χ^2 -test or Fisher's exact test whenever appropriate. Relationships between parameters were analyzed using simple regression analysis or the Spearman test, as required. Multivariate logistic regression analysis was performed to assess which parameters significantly related in the simple regression analysis were independent predictors of eGFR loss at follow-up. A 2-tailed p value < 0.05 was considered statistically significant.

Results

Cell Senescence Is Upregulated Both in the Glomeruli and in the Tubular Compartment of Patients with FSGS

In accordance with previous findings [6, 7], normal kidneys showed p16^{INK4A} staining in $1.1 \pm 0.57\%$ of glomerular cells, $1.4 \pm 0.17\%$ of tubular cells, and $0.2 \pm 0.5\%$ of vessels. Only scanty interstitial cells were p16^{INK4A} positive ($0.1 \pm 0.1\%$).

The percentage of p16^{INK4A}-positive glomeruli (19.8 ± 0.5 vs. $13.7 \pm 0.5\%$ in patients and controls, respectively; $p < 0.05$), as well as the percentage of glomerular p16^{INK4A}-

Fig. 2. p16^{INK4A} in human renal biopsies. Pictures are representative of p16^{INK4A} expression in controls (**a**, **b**) and FSGS (**c–f**). p16^{INK4A} was absent or very faintly expressed in controls (**a**, **b**), while it was detectable in the glomeruli of FSGS (**c**, **d**). In FSGS,

p16^{INK4A} was detected in glomerular cells, such as podocytes (**g**), and in tubular cells (**e–f**). **g** Podocin/p16^{INK4A} co-localization. Arrows indicate positive cells. Magnification, $\times 1,000$ = (**a**); $\times 400$ = (**b–g**). FSGS, focal segmental glomerulosclerosis.

positive nuclei (2.28 ± 0.52 vs. $1.1 \pm 0.57\%$ in patients and controls, respectively; $p < 0.05$), was higher in patients with FSGS with respect to controls (Fig. 1a, 2c, d). Particularly, podocyte nuclear p16^{INK4A} staining was increased in FSGS ($p < 0.02$) (Fig. 1b, 2c, d) as also depicted in Figure 2g representing podocin/p16^{INK4A} colocalization. In contrast, the p16^{INK4A} signal in the arterial vessels was similar to that of the controls (data not shown).

FSGS patients showed also a strikingly increased p16^{INK4A} expression in tubuli (~ 3 -fold increase; Fig. 1c, 2b, e, f). Also, SA- β -Gal staining was approximately 3-fold higher in the tubular compartment of FSGS than in the controls (32.6 ± 17 vs. $7.35 \pm 5.8\%$ positive tubuli in patients vs. controls; $p < 0.05$) (Fig. 3a, b). Upregulation of tubular senescence markers was also observed in FSGS subjects ($n = 16$) displaying mild ($< 10\%$) interstitial fi-

Fig. 3. a, b SA-β-Gal assay was approximately 3-fold higher ($p < 0.05$) in the tubular compartment of FSGS than in controls. Magnification $\times 200$. SA-β-Gal, senescence-associated β-galactosidase; AU, arbitrary units.

brosis ($p16^{\text{INK4A}}$ 0.30 ± 0.1 vs. 0.07 ± 0.1 AU in controls; SA-β-Gal 17 ± 3 vs. $7.35 \pm 5.8\%$ positive tubuli in controls; $p < 0.05$). In each individual biopsy, no association was observed between SA-β-Gal and $p16^{\text{INK4A}}$ ($r = 0.225$; $p = \text{ns}$) in tubuli.

Proteinuria Correlates with SA-β-Gal but Not with $p16^{\text{INK4A}}$ Expression in Kidney Tubules

Table 2 shows the associations between clinical findings and senescence markers in the kidney of subjects with FSGS. By univariate linear regression analysis, SA-β-Gal expression in the tubular compartment was directly and strongly associated with proteinuria ($r = 0.59$; $p < 0.01$). No association was found between senescence markers and eGFR at the time of biopsy, age, blood pressure, BMI, or other clinical/laboratory parameters.

Histological Associations of $p16^{\text{INK4A}}$ and SA-β-Gal Expression

When senescence markers were related to individual structural kidney changes (Table 3), we observed that tubular SA-β-Gal expression was directly associated with glomerulosclerosis and, only as a trend, glomerular $p16^{\text{INK4A}}$ was directly associated with interstitial fibrosis and atherosclerotic lesions.

Tubular Cell $p16^{\text{INK4A}}$ in FSGS Is an Early Marker for Progression

No association between the use of immunosuppressive therapy (steroids/mycophenolate or cyclophosphamide) and eGFR loss was observed ($r = 0.17$; $p = 0.45$). At univariate analysis, basal eGFR and proteinuria correlated with eGFR loss at follow-up, in accordance with previous findings [15]. In addition, tubular expressions of both SA-β-Gal and $p16^{\text{INK4A}}$ were significantly directly related to the annual loss of eGFR (Table 4). No correlation was observed between glomerular $p16^{\text{INK4A}}$ and eGFR loss. Using eGFR, proteinuria, age, tubular $p16^{\text{INK4A}}$, and SA-β-Gal for inclusion into multivariate logistic regression analysis models revealed that only eGFR, proteinuria, and tubular $p16^{\text{INK4A}}$ contribute significantly to the prediction of eGFR loss (Table 4).

Discussion

Cell senescence plays a major role in the physiology of tissue remodeling [3, 16], yet its role in the progression of glomerular diseases is still unexplored. In this study, we tested the hypothesis that accelerated senescence in adult subjects with primary FSGS is associated with faster dis-

Table 2. Associations between clinical findings and senescence marker in the kidney of subjects with FSGS ($n = 26$).

Clinical finding	Glomerular p16 ^{INK4A}		Tubular p16 ^{INK4A}		Tubular SA- β -Gal	
	<i>R</i>	<i>p</i> value	<i>r</i>	<i>p</i> value	<i>r</i>	<i>p</i> value
Age, years	-0.08	ns	-0.19	ns	-0.13	ns
Proteinuria, g/day	-0.18	ns	0.34	ns	0.59	0.01
eGFR, mL/min 1.73 m ²	0.10	ns	0.09	ns	0.10	ns
BMI, kg/m ²	-0.18	ns	-0.16	ns	-0.07	ns
Serum cholesterol, mg/dL	-0.14	ns	0.13	ns	0.34	ns
Serum triglycerides, mg/dL	-0.12	ns	0.07	ns	0.29	ns
Serum uric acid, mg/dL	0.28	ns	-0.40	ns	-0.21	ns

FSGS, focal segmental glomerulosclerosis; SA- β -Gal, senescence-associated β -galactosidase; eGFR, estimated glomerular filtration rate.

Table 3. Associations between structural changes and senescence marker expression in patients with FSGS ($n = 26$).

Histopathologic change	Glomerular p16 ^{INK4A}		Tubular p16 ^{INK4A}		Tubular SA- β -Gal	
	<i>r</i>	<i>p</i> value	<i>r</i>	<i>p</i> value	<i>r</i>	<i>p</i> value
Segmental lesions, %	0.19	ns	-0.06	ns	0.43	0.07
Global sclerosis, %	-0.14	ns	0.15	ns	0.59	0.01
Glomerular cellularity	-0.19	ns	0.26	ns	-0.05	ns
Interstitial fibrosis	0.42	0.06	0.31	ns	0.12	ns
Interstitial inflammation	0.33	ns	-0.02	ns	-0.07	ns
Tubular atrophy	0.29	ns	0.14	ns	0.34	ns
Atherosclerosis	0.46	0.04	0.07	ns	0.29	ns

FSGS, focal segmental glomerulosclerosis; SA- β -Gal, senescence-associated β -galactosidase.

ease progression. To this aim, we studied the expression of 2 different markers of senescence, SA- β -Gal and p16^{INK4A}, in 26 consecutive FSGS cases and observed changes in proteinuria and eGFR for 6 years. Three issues are addressed in this study, which bear discussion. The first is the overexpression of both senescence markers, SA- β -Gal and p16^{INK4A}, in kidney biopsies of patients with FSGS at the time of diagnosis, a finding that shows that cell senescence is an early mechanism which may play an independent role in kidney damage. The second is the association between the overexpression of the cycle regulator p16^{INK4A} in the tubular compartment and eGFR loss at the follow-up, a finding which gives strength to the hypothesis that cell senescence accelerates disease progression in FSGS. A third issue is the association between SA- β -Gal activity in tubular cells and proteinuria, but not with disease progression, which suggests that SA- β -Gal

activity is more expressive of proteinuria-induced tubular overload than disease progression.

Even as low as 10% of senescent cells in tissues appear to be sufficient to cause damage and dysfunction [20]. In our study, both the glomeruli and tubuli showed a several-fold increase in senescent markers in FSGS. In the glomeruli, the expression of p16^{INK4A} showed a podocyte pattern, suggesting that an upregulation of p16^{INK4A} in glomeruli mainly affects this cell type. Glomerular podocyte depletion has a central role for glomerulosclerosis development [21–23], and increased appearance of podocytes and podocyte constituents in urine is associated with more rapid deterioration of renal function in patients with FSGS [21]. Failure of podocytes to cover the GBM filtration surface area results in denuded areas of GBM, which in turn triggers matrix accumulation and glomerulosclerosis [22, 23]. Even if podocytes exhibit un-

Table 4. Regression analysis for eGFR loss in FSGS patients ($n = 26$).

Characteristics	Univariate model		Multivariate model 1		Multivariate model 2	
	r	p value	t	p value	t	p value
Age, years	-0.22	0.27			-0.09	0.51
BMI, kg/m ²	0.34	0.21				
eGFR, mL/min/1.73 m ²	0.45	0.02	0.49	0.003	0.36	0.021
SBP, mm Hg	0.28	0.26				
DBP, mm Hg	0.21	0.41				
Proteinuria, g/day	0.59	0.002	0.40	0.016	0.34	0.02
LDL-cholesterol, mg/dL	0.11	0.33				
Triglycerides, mg/dL	0.42	0.17				
Global glomerulosclerosis, %	0.12	0.60				
Segmental glomerulosclerosis, %	-0.17	0.47				
Interstitial fibrosis, %	0.24	0.30				
Interstitial inflammation, %	-0.19	0.42				
Tubular atrophy, %	0.23	0.23				
Atherosclerosis, %	-0.13	0.58				
p16 ^{INK4A} (glomeruli), %	0.08	0.74				
p16 ^{INK4A} (tubuli), %	0.62	0.006	0.44	0.010	0.28	0.034
SA- β -Gal (tubuli), %	0.46	0.029			-0.20	0.19

FSGS, focal segmental glomerulosclerosis; eGFR, estimated glomerular filtration rate (CKD, epi CKD-epi); SBP, systolic blood pressure; DBP, diastolic blood pressure; SA- β -Gal, senescence-associated β -galactosidase.

der normal conditions a very low replication, the upregulation of CDK inhibitors could, in theory, lead with time to podocyte loss. However, the functional effect of CDK inhibitors on podocyte replication and loss is not completely recognized. While p16^{INK4A} has been little studied, podocyte quiescence may require the presence of the CDK inhibitors p27 and p57. In human glomerular diseases, a decrease in p27 and p57 appears to be permissive for an altered proliferative podocyte phenotype [24]. However, in our study, against a functional role of the overexpression of p16^{INK4A} in glomerular cells, glomerular p16^{INK4A} did not associate neither with worse renal prognosis nor with proteinuria.

Nuclear expression of p16^{INK4A} and cytoplasmic SA- β -Gal activity increase with age principally in tubular epithelial cells [25]. As a new finding, data reported here show that in adult FSGS patients with normal or slightly decreased GFR, better than glomerular cell senescence, it is tubular senescence that predicts the loss of renal function. Thus, the degree of tubular p16^{INK4A} in patients with FSGS may represent a negative prognostic factor and a novel marker for progression. Proteinuria and interstitial fibrosis have been shown to predict kidney survival in FSGS [15, 26]. However, in the earlier stages of the disease, there are a few light microscopy changes that are

predictive of the subsequent loss of renal function. In this study, when tubular SA- β -Gal and p16^{INK4A} were included in a multiple regression analysis, only tubular p16^{INK4A} (but not SA- β -Gal) was found to predict independently the loss of GFR. Thus, an elevated p16^{INK4A} at the time of initial biopsy comes forth as a novel and independent predictor of the loss of renal function in our cohort of patients with FSGS.

Surprisingly, our study shows that the tubular SA- β -Gal activity assay, the most commonly used analysis for senescence [27], was not able to predict kidney outcome. Senescent cells express a higher level of lysosomal β -galactosidase gene (*GLB1*) and show an increased β -galactosidase activity at a suboptimal pH of 6.0 (optimal pH level is 4–4.5), compared to normal cells [18]. It is interesting that in FSGS patients studied here SA- β -Gal was strongly accounted by a high degree of proteinuria at the time of the kidney biopsy, a finding in keeping with the role of proteinuria in inducing lysosome dysfunction in renal tubules [28]. According to our findings, SA- β -Gal activity appears to better correlate with the tubule overload produced by proteinuria than with the progression of disease.

In our study, we could not find an association between age and cell senescence in FSGS. As a matter of fact, we

studied an adult population, since younger subjects were not included in the study. In addition, eGFR, as also previously observed in patients with diabetic nephropathy [7], was not related to senescence, suggesting that renal failure per se is not responsible for senescence activation. However, upregulation of cell senescence was already observed in proteinuric patients with normal or subnormal GFR and only modest chronic changes at the time of their biopsy, suggesting that the acceleration of senescence is an early phenomenon.

The mechanism(s) driving cell senescence in the kidney of patients with FSGS cannot be explained by our study. Several cellular stressors, including DNA damage, oxidative stress, RAS induction, and epigenetic mechanisms, induce premature senescence in kidney cells [29–32]. The mechanism(s) underlying the effects of the overexpression of p16^{INK4A} in kidney tubules in FSGS could involve reduced tissue remodeling through different sequential processes, such as a stable proliferative arrest or a secretory phenotype that recruits immune cells and modifies the extracellular matrix and the mobilization of nearby progenitors that repopulate the tissue [3].

Cell senescence is potentially treatable. It has been shown that loss of p16^{INK4A} attenuates permanent damage, such as interstitial fibrosis and tubular atrophy, and allows for better functional recovery [33]. Therapeutic interventions targeting senescent cells could attenuate FSGS-related renal dysfunction and improve disease outcome. Recently, Baker and colleagues have shown that clearance of p16^{INK4A} expressing cells extends lifespan and attenuates age-dependent glomerulosclerosis [34].

This study has some limitations. Our cohort of incident patients with FSGS was mainly represented by patients with a low degree of proteinuria, a characteristic which is associated with less progressive disease [26]. Therefore, the results of the present study cannot be extended to patients with more aggressive disease, such as those patients with the collapsing FSGS variant [2, 35].

In summary, we demonstrated that senescence is up-regulated in both glomerular and tubular compartments in a cohort of adult patients with FSGS, and that p16^{INK4A} overexpression in the tubular compartment is associated with accelerated loss of renal function. Therefore, our results suggest that senescence, as expressed by p16^{INK4A} overexpression in the tubular compartment, contributes to accelerated loss of eGFR in FSGS. In addition, this study provides a clinical basis for in vitro studies of senescence regulatory molecules as a potential target for therapeutic intervention in a common clinical condition that is still associated with an unfavorable prognosis. With

this regard, the estimate of p16^{INK4A} in kidney biopsies could also be considered a sensitive index of disease progression.

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Statement of Ethics

This study was approved by the Institutional Research Ethics Committee of Department of Internal Medicine, University of Genoa, Italy. This study was performed in compliance with the Helsinki Declaration. All individuals provided informed consent prior to their inclusion in the study.

Conflict of Interest Statement

There is no conflict of interest to disclose.

Author Contributions

D.V. studied senescence marker expression in kidney biopsies; F.V. and G.G. designed the study; G.S. described the histology of kidney biopsies; E.R. and L.C. collected clinical data; M.S., L.P., and A.C. analyzed the data; G.G., P.E., and D.V. wrote the manuscript; and F.C., D.P., and Ga.Ga. contributed to the discussion and edited the manuscript. All authors approved the manuscript. G.G. is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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