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Podocyte dysfunction in aging - related glomerulosclerosis

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1. ABSTRACT

We review podocyte molecular structure and function, consider the underlying mechanisms related to podocyte dysfunction and propose that podocyte dysfunction be considered in the evaluation and management of age-associated glomerulosclerosis. With aging, progressive sympathetic activation, increased intrarenal renin-angiotensin system (RAS) activity, endothelin system and oxidative stress and reduced nitric oxide (NO)-availability can damage podocytes. Apoptosis and proliferation are the principal podocyte changes following injury with the latter leading to sclerosis and loss of nephrons. Podocyte loss can be evaluated by either determining their average number in biopsed glomeruli or by estimating podocyte number or their associated molecules in urine sediment. Podocyturia may be considered a marker of active glomerular disease. Preliminary data suggest that antiadrenergic drugs, angiotensin converting enzyme (ACE) inhibitors, RAS blocking drugs, endothelin system inhibitors and reduced oxidative stress can protect podocytes. Thus podocytes appear to play an important role in the pathogenesis, evaluation and therapy of age related glomerulosclerosis.

2. INTRODUCTION

Glomerulosclerosis is closely associated with aging, indeed most individual after 40 years of age have sclerotic glomeruli which further increase with aging (1-3). In rats, development of glomerulosclerosis occurs in relation to aging (4,5) and in this animal model glomerulosclerosis is considered to be a "podocyte disease"(6). Podocytes or visceral epithelial cells are highly differentiated epithelial cells of the visceral glomerular epithelium and form a crucial component of the glomerular filtration barrier and maintain a massive filtration surface (7). Wiggins hypothizes that human glomerular diseases is a consequence of podocyte dysfunction caused by genetic and/or environmental factors; aging-related glomerulopathy is included among the clinical glomerular diseases associated with podocyte dysfunction (8). Podocyte number is reduced by a decrease in proliferation due to lack of DNA synthesis, DNA damage or cellular glomerular hypertrophy,and/or an increase in podocyte loss owing to detachment and apoptosis (8). In the present review we examine podocyte molecular structure and pathology, consider the underlying mechanisms related to podocyte dysfunction and propose that podocyte

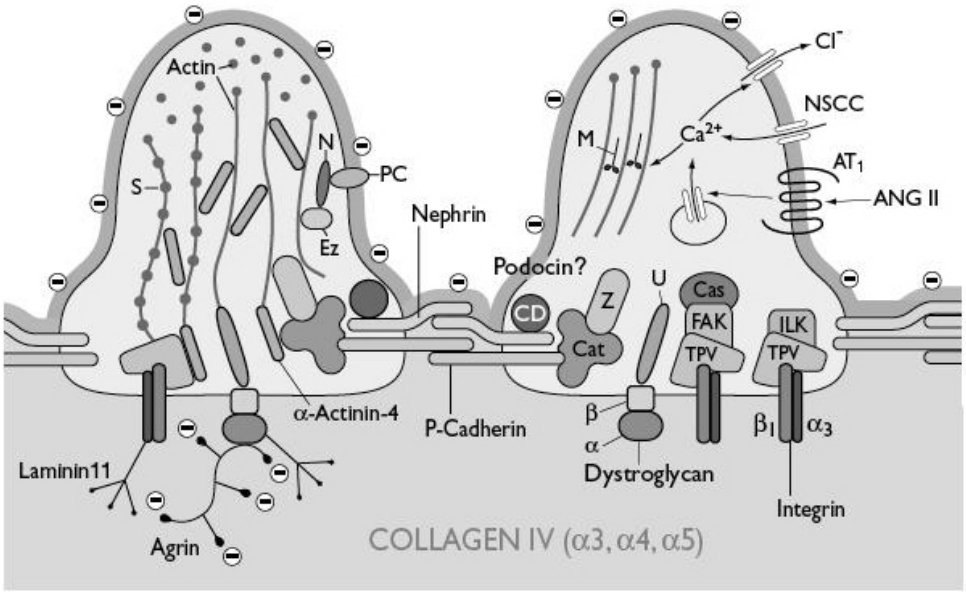

Figure 1. See text for details.

dysfunction be considered for the evaluation and management of age-associated glomerulosclerosis.

3. PODOCYTE MOLECULAR STRUCTURE AND PATHOLOGY

During glomerular development (glomerulogenesis) podocytes can be distinguished from the S-shaped stage to the capillary loop stage of glomerulogenesis (9). Transition of the glomerulogenesis from the S-shaped stage to the capillary loop stage represents for the podocyte the transdifferentiation of an epithelial to a mesenchymal phenotype characterized by the disappearance of epythelial markers and the appearance of vimentin, a characteristic intermediate filament protein of mesenchymal cells, and regarded as a marker of podocyte maturity (10). This maturation process is associated with the expression of further podocyte markers including Wilms Tumor (WT)-1 (transcription factor), alpha3beta1 integrin,alpha-beta dystroglycan complex (podocyte adhesion molecules), podocalyxin, Glomerular Epithelial Protein (GLEPP)-1 (podocyte cell surface proteins) synaptodin, alpha-actinin4 (podocyte cytoskeleton protein) and nephrin, CD2-AP, podocin, Zonula Occludens (ZO)-1, P-cadherin, Fatty Acid Transporter (FAT) (podocyte slit-diaphragm proteins) (see Figure 1).

Table1 summarizes the podocyte molecular structure and function. During injury podocyte reverts to a dedifferentiated phenotype characterized not only by podocyte detachment/apoptosis/hypertrophy/proliferation but also by epithelial-mesenchymal transition with developing fibrosis (12-14). Furthermore, podocyte dysfunction and injury is linked to the loss of nephrons. Noninvasive methods to quantify podocyte damage are emerging and they include detection of podocyturia and podocyte-associated specific proteins in urinary sediment (15,16). To date, three different pattern of changes initiated by podocyte injury have been reported: degenerative changes that progress to classic focal segmental glomerulosclerosis (15); inflammatory changes that progress to crescent formation (16) and changes indicating differentiation leading to collapsing focal segmental glomerulosclerosis (17,18).

4. PATHOGENETIC FACTORS WHICH CAN IMPAIR PODOCYTE FUNCTION IN AGING RELATED GLOMERULOSCLEROSIS

The pathogenetic factors which impair podocyte function in aging-related glomerulosclerosis are shown in table 2 with the correspondent therapeutic treatment.

Sympathetic nervous system (SNS) and the podocyte: Human SNS is deranged by aging and involves the adrenergic receptors and the outflow of sympathetic neural traffic to individual organs (20). Microneurographic recording from sympathetic fibres and measurement of the spillover of the sympathetic neurotransmitter norepinephrine to plasma demonstrates unequivocal evidence that progressive sympathetic activation occurs with aging. The nature of the underlying perturbation in central nervous system sympathetic control remains unknown.

Sympathetic stimulation appears to involve the sympathetic outflow to the heart, gut, liver and kidneys (21,22). Increases in renal sympathetic nerve activity regulate the function of the nephron, the vasculature and the renin-containing juxtaglomerular cells (23). Because systemic RAS activity decreases with age (24) one can speculate that the slow progression of ischemia in nonrenal tissues forms the basis for the "physiologic" increase in sympathetic activity. Although adrenergic fibers do not

penetrate the glomerulus (25), podocytes have adrenergic as well as Angiotensin-II (Ang-II) receptors (26) and podocyte injury is a pivotal step in development of glomerulosclerosis.

There is experimental evidence to show that catecolamines are involved in the development of kidney damage independent of their effect on blood pressure (27). These detrimental effects include vascular and glomerular injury. In hypertensive stroke-prone rats chronic carvedilol administration reduces mortality and renal damage by decreasing vasoconstriction and proliferation of smooth muscle cells and adventitial fibroblats in the vascular wall (28). In normotensive humans moxonidine (a sympaticoplegic agent) reduces albuminuria without affecting blood pressure (29).

The mechanisms by which sympathetic activity damage the glomerulus has not been identified**.** Catecolamines induce proliferation of smooth muscle cells and adventitial fibroblasts in the vascular wall (30). We speculate that sympathetic activity leads to podocyte constriction by calcium influx and hence decreased selectin glomerular permiability resulting in proteinuria (26). Meta-analysis of the effect of antiadrenergic drugs on proteinuria in both diabetic and non-diabetic renal disease shows that beta blockade is approximately half as effective as ACE inhibition (31).

Renin angiotensin system and the podocyte: Ang-II receptors have been reported in the glomerular epithelial cells (42). There is a considerable body of evidence in the rat animal model that Ang-II directely modulates podocyte function by calcium and TRPC6 (Transient Receptor Potential Canonical) channels in podocytes (43,44). Within the kidney Ang-II can produce a multitude of non-hemodynamic effects including the induction of reactive oxygen substances, cytokine stimulation of collagen synthesis, apoptosis, proliferation and hypertrophy (40,41). Within the glomerulus Ang-II reduces the ultrafiltration coefficient and modulates glomerular capillary permselectivity leading to proteinuria, which, in turn, initiates tubulointerstitial injury. Acting as a growth hormone, Ang-II contributes to the pathogenesis of glomerulosclerosis.

An *in vitro* study shows the activation of the RAS within podocytes in diabetes (32). The systemic RAS is suppressed in normal aging (33,34), but the aging rat shows an intrarenal increase of RAS activity and responsiveness (35). In recent years pharmacological RAS blockade has shown compelling renoprotective effects in a variety of chronic nephropathies and recently in active immune complex-mediated glomerulonephritis (36). In conditionally immortalized podocytes mechanical strain leads to up-regulation of the AT1 receptor and increased Ang-II production: the activation of local tissue angiotensin system leads to an increase in podocyte apoptosis (37). The characterization of RAS enzyme activities in cultured mouse podocytes shows that podocyte express a functional intrinsic RAS characterized by neprilysin, aminopeptidase A, ACE-2 and renin activities which lead to Ang-[1-7] and Ang-[1-9] formation as well as Ang-II degradation (38). Ang-[1-7] and Ang-[1-9] peptides are produced from the metabolism of Ang-I by the action of ACE-2 and neprilysin (also called neutral endpeptidase) and have effects that are opposite of Ang-II (39). There is therefore experimental evidence to suggest a specific role of the podocyte in themaintenance of intraglomerular RAS balance, an imbalance of intraglomerular RAS may result in glomerulosclerosis.

4.1. NO and the podocyte

NO is a highly reactive, gas, formed during the conversion of L-arginine to L-citrulline by the action of nitric oxide synthae (NOS). Several cofactors are necessary for NOS activity. In addition, superoxide (O2-) which reacts with NO to form proxynitrate (ONOO-) also influences NO bioavailability. NO is an important neurohumoral modulator of glomerular ultrafiltration, renal hemodynamics and plays a role in the long-term regulation of blood pressure (46). Inhibition of intrarenal NO production increases blood pressure in the rat (47) but not the blockade of Ang-II. The regulation of glomerular hemodynamic renal vascular resistance is a complex process that involves the interplay of multiple neural-humural factors including vasodilators and vasoconstrictors (48). NO is an important within the kidney, largely to buffer the influences of a variety of potent vasoconstrictor systems such as Ang-II and renal adrenergic nerves (49,50). All three subtypes (isoforms) of NO synthase (NOS) exist within the kidney (51), they are inducible NO synthase (iNOS, NOS2), endothelial NOS synthase (eNOS, NOS3) and neuronal NO synthase (nNOS, NOS1). The mechanisms of synthesis and regulation of NO in the kidney remain to be clarified (52). The effect of asymmetric methylarginine (ADMA) in blocking NO formation by NOS and its metabolism by dimethylarginine dimethylaminohydrolase (DDAH), type 1 or 2, are unclear (53). NOS1 is expressed in podocytes of normal human kidney together with soluble guanylyl cyclase (sGC) (54) that is the physiologic NO target within the cell. NO activates sGC which catalyzes the conversion of GTP to cGMP(55): the NO-sensitive sGC is regarded as the most important receptor for the role of NO as a signalling molecule. In podocytes the cGMP signaling pathway has been reported but little is known about the implications of the NO/cGMP signalling cascade in these cells (56).We speculate that cGMP podocyte synthesis may have an important role for glomerular epthelial cell physiology because cGMP is a key signalling molecule that modulates the activities of cGMP dependent protein kinases cyclic nucleotides gated ion channels and phosphodiesterases (56,57). A recent study in mice shows that the regulation of actin cytoskeleton podocytes by the GTPase, dynamin, may have a role in the induction of proteinuria and associated foot process affacement in glomerular podocytes (58). Reduced NO bioavailability is considered a major factor in the multiple functional alterations associated with kidney aging, including reduced RPF, GFR and proteinuria as well as in the structural alterations manifest as glomerulosclerosis and a decreased number of nephrons. NO reduced bioavailability is linked to alterations of the L-arginine/NO pathway that

occur with ageing. Ageing is manifest by the reduction of circulating NO metabolites (59), changes in basal NO release as well as reduced renal NO metabolite excretion (60). While vascular NOS2 expression increases with ageing, NSO3 isoenzyme expression appears to be gender regulated in healthy humans (61,62).

Elevated plasma levels of the endogenous NO synthase inhibitor ADMA are thought to contribute to the age-related alterations in the L-arginine/NO pathway (63). In aging rats high plasma concentrations of this compound are accompanied by reduced whole body NO generation (64) and by declining renal plasma flow and sustained proteinuria (59). In the rat chronic NO inhibition promotes severe and progressive arterial hypertension and renal structure injury consisting of glomerular ischemia, glomerulosclerosis, interstitial expansion and proteinuria (65). In this animal model proteinuria involves a glomerular size defect with depeletion of fixed negative charges at the glomerular wall. Chronic L-Name administration reverse proteinuria induced by impairing both glomerular size and charge selectivity (66). Data in elderly individuals (63) are in agreement with the observations in the rat suggesting that ADMA accumulation may play role in the decline of renal perfusion in the elderly. Moreover studies (67) show that ADMA accelerates cell senescence by increasing the tolemerase activity. The close relationship of NO, ADMA, DDAH and the kidney is well documented by histologic studies (53,68) and overexpression of TGF-beta by ADMA is thought to contribute to renal failure progression in rat (69). Finally, the NO system is a natural antagonist of catecholamines. Consequently, the decreased NO availability in chronic kidney disease and in the aging kidney may explain the progressive sympathetic activation that occurs with aging (70). NO is a neurotransmitter at synapses in autonomic ganglia of the peripheral nervous system (71) produced by neuronal NO synthase (nNOS) in proximity of the neuro-effector junction, it potentiates vagal transmission and decreases sympathetic transmission (72,73).

4.2. Endothelin and the podocyte

In kidney, the endogenous endothelin system controls water, sodium excretion and acid-base balance and mantains normal cell proliferation and tonic vasoconstriction under physiological conditions (74-77). The endothelin system is activated in ageing i.e. endothelin-1 (ET-1) expression increases in the absence of other risk factors (78,61). Human podocytes and mesangial cells are targets for ET-1 (79) and in podocytes of the rat kidney andothelin-B-receptors have been reported (80). Their stimulation results in a decrease of both glomerular blood flow and glomerular filtration rate. The endothelin system (ET-1,ET-2,ET-3) is present in kidney, but ET-1 is predominant and, biologically, the most relevant isoform which functions in both a paracrine and autocrine manner (80,75,76) via activation of ETA and ETB receptors. *In vitro*, the endothelin shows disruption of the podocyte actin cytoskeleton (81) and pretreatment of podocytes with an ETA receptor antagonist prevents disruption of the podocyte actin cytoskeleton after injury by puromicyn aminonucleoside (82). In the glomerulus the synthesis of ET-1 is stimulated by aging and ET-1, via activation of the ETA receptor, promotes podocyte injury. Podocyte ETA receptor activation promotes glomerular injury and sclerosis through the following mechanisms: the matrix metalloproteinase 9 (MMP-9) also known as gelatinase B collagen degradating, mitogen-activated protein kinase (MAPK p44/p42), cyclin-dependent kinase inhibitor p38; the growth promoter and cyclin-dependent kinase inhibitor p21 waf/cip1; the nuclear factor kB(NFkB). ET-1 causes disruption of the F-actin podocyte cytoskeleton and dysfunction of the slit diaphragm via activation of Rho kinase and PI3 kinase (83).

4.3. Oxidative stress and podocyte senescence:

Reactive Oxygen Species (ROS), such as the superoxide radical hydrogen peroxide and the hydroxyl radical, which are highly reactive due to one or more impaired electrons in their outer orbits, are generated endogenously. They cause oxidative damage to cellular macromolecules such as proteins, carbohydrates, lipids and nucleic acids and, as such, are cytotoxic (84,85). Oxidative stress reflects an imbalance between the formation of ROS and antioxidant defence systems, both enzymatic and nonezymatic. ROS arise from the mithocondrial electron transport chain as part of oxidative phosphorylation, from metabolism of arachidonate by either cyclooxegenase or lypoxygenase enzymes to prostaglandins or leukotrienes, from cytochrome P450 enzymes, oxidase enzymes such as NADPH oxidase (Nox) or from nitric oxid synthetases (NOS) (86).The free radical theory of aging postulates that the production of intracellular ROS is the major determinant of life span altering cellular protein, lipid and carbohydrate structure, DNA nucleus, the cytosolic and mytocondrial pathways (87). Nox have been reported in kidney with the distinct cellular localization in glomeruli: mesangial and podocyte. Distinct components and regulatory subunits of Nox have been analyzed in the podocyte and Nox2,p22phox,p47phox, p67phox have been reported (88). Cultured human podocytes produce ROS by the action of Nox after stimulation with vasoactive hormones. Nox activity in the kidney cortex is upregulated by of Ang-II and by a high salt diet (89-91). ROS generated by Nox activation act as second messangers for several transcriptions factors implicated in renal disease, hypertension and cellular senescence (92). The molecular basis of cellular senescence mechanisms remains largely unknown but environmental stress and genome changes such as loss of telomeres are believed to contribute (93). Oxidative stress shortens telomeres (94) and telomere have been shown to shorten in kidney with age (95). Tolemeres are specialized repetitive DNA sequences at the end of the linear chromosomes that serve to mantain the integrity of the chromosomes (96). Telomerase is a ribonucleoprotein DNA polymerase complex that mantains telomere length. This complex includes the protein telomerase reverse transcriptase (TERT) and a catalytic RNA (TERC) (97). In the absence of telomerase activity, telomeres progressively shorten followed by chromosome fusion and genomic instability (98). Telomere shortening is not the only mechanism that can lead to podocyte senescence. In many cell types overexpression of cyclin-dependent kinase

(CDK) inhibitor such as p16INK4a or p21CIP1/WAF1 (99) may lead to senescence. The CDK inhibitor, p16INK4a, is upregulated in epithelial and interstitial cells of both the aging rodent and human kidney with subsequent cellular senescence (100). CDK p21CIP1/WAF1 inhibitor expression leads to proliferative arrest, apoptosis and cellular hyperthrophy(101,102). Telomere shortening triggers the expression of this cell-cycle inhibitor by activation of the ATM (Ataxia Teleangectasia Mutated)/p53 pathway (93). Evidence for the *in vivo* human podocyte senescence involving telomere lenght are lacking.

5. PODOCYTE DAMAGE : POSSIBLE CLINICAL RELEVANCE

Podocytes are highly differentiated cells with a complex cellular morphology (foot processes and the slit diaphragm). Our understanding of podocyte structure and function continues to grow. Table 1 shows the functional molecular structure of the renal podocyte. Podocytes play a major role in the initiation and progression of glomerular diseases and are targets of both immune-mediated and non-immune-mediated injury (103). Independent of underlyng disease, podocyte injury, if not repaired, can lead to severe progressive glomerular disease. The specific responses to podocyte injury comprise the foot process effacement, engagement of the apoptic pathway, abnormal differentiation and proliferation, the detachment and loss in the urine together of specific podocyte molecules. Several methods are emerging to assess the podocyte damage and include the estimation of the average number of podocytes in glomeruli obtained from biopsy material (104) by immunostaining kidney sections with anti-C3bR antibody or by intra-renal mRNA expression of nephrin,podocin,synaptodin measured by real time quantitaive polymerase chain reaction. Recently, estimation of podocytes or podocyte-associated molecules in urinary sediments has been used to assess podocyte damage. Immunofluorescent staining of a podocyte specific marker with anti-podocalyxin,anti-C3bR,anti-alpha3 integrin antibodies has also been reported (105). Urinary messenger RNA(mRNA) expression of podocyte-associated molecules detected and quantified by real time quantitative polymerase chain reaction such as nephrin and podocin has also been used. Finally, evaluation by immunofluorescence staining of cultured podocytes derived from urine sediment has to be considered. Immunofluorescent staining of podocyte specific protein markers using monclonal antibodies requires experienced cytologists to count the urinary podocytes: resulting in a relatively time-consuming method of quantitation of renal damage. Immunofluorescent staining of cultured podocytes includes the potential of bacterial and fungal contamination, as well as proliferation of podocytes in cell culture. Urinary messenger RNA(mRNA) expression of podocyte-associated molecules requires fresh urine samples and, as such, is vulnerable to the presence of bacteria proteases and ribonucleases (RNAses) (106). In view of the above problems alternative non-invasive approaches to detect podocyte damage *in vivo* have been studied and these include quantification of urinary slit diaphragm molecules (nephrin) or atypical podocyte molecules (podocalyxin) by immonoblotting (ELISA) (107).

Podocyte damage results in cell death and focal denudation of the glomerular basement membrane. Such denudation is the origin of focal adhesions of the glomerular tuft to the outher leaflet of Bowman's capsule. Estimation of average podocyte number per glomerulus quantiftes glomerular podocythopenia. Podocyturia which is the result of podocyte damage, can function as a marker of active glomerular disease different from proteinuria (108). In fact studies on podocyturia and protenuria in rats performed in puromycin aminonucleoside nephrosis (PAN), in anti-Thy 1.1 nephritis mode induced in male Wister rats,in 5/6 nepherctomy model, showed that the onset of proteinuria was concomitant to the onset of podocyturia. However, in the late disease state proteinuria persisted while podocyturia disappeared (109). This observation suggests that the detection of proteinuria does not distinguish between ongoing injury and a persistent defect of the glomerular barrier whereas podocyturia may be more specific for "active" injury of the glomerulus. SU Vogelmann and others (110) studied urinary excretion of viable podocytes in health individuals and patients with renal disease. Patients with active glomerular disease excreted up to 388 podocytes/mg of creatinine whereas healthy controls and patients with quiescent disease generally excreted less than 0.5 podocytes/mg of creatinine. Recently various methods of podocytopathy study have been applied in human studies of subjects with lupus nephritis, in children with glomerulonephritis, in type 2 diabetes patients with microalbuminuria, in differential diagnosis of idiopathic focal glomerulosclerosis and minimal-change nephrotic syndrome, in patients with focal segmental glomerulosclerosis and chronic renal failure (111,112,113). These studies show the following. Urinary podocytes are not detected in normal children and in subjects with nonglomerular diseases while they are detected in children with glomerular inflammatory diseases. Urinary podocytes are not detected in healthy subjects, in diabetic patients with normoalbuminuria and chronic renal failure while they are detected in diabetic patients with micro and macroalbuminuria. Finally, urinary podocyte loss is higher in primary focal segmental glomerulosclerosis than in minimal-change disease or membranous nephropathy. Therefore the study of podocyte injury may become important for understanding, early diagnosis and management of aged glomerulopathy.

6. DISCUSSION

Progressive sympathetic activity is an hallmark of aging, however the biological mechanism of this activation remains unknown. In experimental studies the renal sympathetic nerve activity shows important effects (23,25,115). In rats NO exerts a tonic inhibition of central SNS activity while ROS stimulate central and peripheral sympathetic nervous system activity (116,117). The blockade of the RAS provides renoprotection in both glomerulopathies and in aging kidney (118). Cultured mouse podocytes express a functional intrinsic RAS system where "nonclassic" RAS enzymes such as ACE2 and

Table 1. Podocyte molecular structure and function

Structure component	Site	Function
Podoendin	cell surface protein	unknown
Podocalyxin (Pdcx)	Highly charged cell surface protein	electrostatic charge repulsion Pdcx associates with the actin cytoskeleton by its interaction with ezrin and the Na/h exchanger regulatory factor-2 (NHERF-2)
Glomerular Epithelial Protein-1 (GLEPP-1)	Podocyte apical cell surface	unknown
CD2-associated protein	Basolateral-transmembrane protein	Adaptor protein mediating the connection of slit diaphragm to the actin cytoskeleton
Podocin	Basolateral-transmembrane protein	adaptor protein mediating the connection of slit diaphra gm to the actin cytoskeleton
Nephrin	slit diaphragm protein	integrity of slit diaphragm and amintenance of normal glomerular permeability
P-cadherin (FAT: Fatty Acid Tansporter)	slit diaphragm cadherin	unknown
Alpha,beta,gamma catenins	basolateral surface	cell junction-associated proteins
Zonula Occludens-1 (ZO-1)	basolateral protein	linker protein for the attachment of slit diaphragm to actin cytoskeleton. Membrane associated guanylate kinase protein
Alpha-3-beta1 integrin	basal cell podocyte	interaction between the podocyte and the glomerular basement membrane and maintenance of the integrity of the filtration slit
Megalin	cell surface protein	transmembrane receptor of LDL family of receptor
Alpha, beta dystroglycan complex	Baso-basolateral cell cell podocyte	Interaction between the podocyte and the glomerular basement membrane and maintance of the integrity of the filtration slit
Synaptodin	cytoskeleton	actin-binding protein with unknown function (coordination actin bundling ?)
Alpha-actinin 4	cytoskeleton	isoform of alpha-actinin and an actin-binding protein (actin cross-linking protein)
Vascular Endothelial Growth Factor (VEGF)	unknown	signaling molecule expressed by podocyte involved in the formation and maintenance of glomerular capillaries throghout life
Wilms Tumor-1 (WT-1)	Unknown inside the nucleus of the body of the podocyte	Zing finger transcription factor and RNA binding protein. Protein of podocyte development
13 A antigen	membrane protein	unknown
Actin	Protein filaments in foot processes only	Foot process mobilityand architecture and connected with slit diaphragm, glomerular basement membrane, cell surface foot process (Active regulator of podocyte shape and function)
Vimentin	protein filaments and microtubules confined to the podocyte cell body	Cytoskeletal protein
Ezrin	Protein linked to actin filaments	unknown.Probably attaches the actin cytoskeleton to podocalyxin via the linker protein NHERF2 (Na/H-exchanger regulatory factor 2)
Lmx1b	Unknown inside the nucleus of cell body	protein expressed in podocyte contributes to transcriptional regulation membrane collagen expression by podocytes
p21	unknown	cyclin-dependent kinase (CDK) inhibitor expressed in normal qiuescent podocyte. Inhibits cell podocyte proliferation.
p27	unknown	cyclin-dependent kinase (CDK) inhibitor expressed in normal qiuescent podocyte. Inhibits cell podocyte proliferation.
p57	unknown	cyclin-dependent kinase (CDK) inhibitor expressed in normal qiuescent podocyte. Inhibits cell podocyte proliferation.

neprylysin, also called neutral andopeptidase, may result in active metabolites(Ang1-9,Ang1-7) with biological effect opposite to those of Ang-II. This means that the beneficial effects of ACE inhibitor and angiotensin receptor blockers result from interference with podocyte RAS (38,119). The effect of RAS blockade on podocytes has been shown in a variety of experimental settings but further experiments are required to identify the underlying molecular mechanisms of podocyte protection from RAS blockade (120,121) as well as aldosterone blockade (122). In kidney aging reduced NO bioavailability is considered a major factor of multiple functional alterations such as reduced RPF and GFR with proteinuria. Because podocyte function is intimately linked to its complex cytoskeletal architecture, the podocyte foot process affacement-associated proteinuria is dependent upon disruption of the actin cytoskeletal network as an initiating event (123). Sever showed that actin podocyte cytoskeleton is regulated by the GTP system (58,124) and guanylyl cyclase is the physiological NO target inside the cell (54,56). Future investigation is necessary to understand the NO/GTP signalling cascade in glomerular visceral cells. In elderly subjects ADMA accumulation may have a prominent role in reducing NO bioavailability (63). Thus lowering plasma ADMA level concentrations in subjects at risk of glomerulosclerosis could be a major therapeutic goal. ADMA metabolism is under the control of DDAH enzymatic activity and oxidative stress, such as ROS increase, inhibits DDAH activity (53). Thus a pharmacological modulation of DDAH activity with ROS may offer a new therapeutic approach.

The effect of endothelin inhibition on established focal segmental glomerulosclerosis due to aging in normotenisve animals has been studied (82). Four weeks of treatment with a selective ETA antagonist, darusentan, not only reduced proteinuria but also reversed glomerulosclerosis (125). The expression of matrix metallo-proteinase 9, a marker of glomerulosclerosis, and associated podocyte injury was reduced in laser-microdissected aged glomeruli. In experimental diabetic nephropathy the administration of avosentan (ETA receptor antagonist) is followed by reversal of proteinuria and podosyte loss (126). Concerning the effects of endothelin system inhibition on proteinuria due to aging, clinical studies are lacking. Although ROS production is difficult to

Table 2. Pathogenetic factors which can impair podocyte function in aging-related glomerulosclerosis and probably protective drugs-intervention

Mechanism/site		**Podocyte lesion**	**Expected renal dysfunction**	**Probably protecting drug or intervention**
Increased sympathetic adrenergic activity	Adrenergic receptor activation	Podocyte constriction	Decreased glomerular permeselectivity	Sympathicolytic agent
Increased intrarenal Ang II activity	Increase in free cytosolic calcium	Podocyte depolarization	Proteinuria	Inhibitor TRPC6
Desreased NO bioavailability	Deranged actin cytoskeleton by GTPase dynamin	effacement	Proteinuria	Nitric oxide donors ADMA inhibitors
Increased ET-1 availability	Distruption actin cytoskeleton. Dysfunction slit diaphragm	Sclerosis effacement detachment	Renal failure proteinuria	Endothelin antagonists
Increased oxidative stress (ROS)	ROS as second messanger for several transcription factors such as nuclear factor kB. Telomerase shortnes	Apoptosis detachment hyperthrophy	Proteinuria sclerosis	Antioxidant
Telomeres shortening	Decreased telomerase activity	Impaired podocyte repair (senescence)	Proteinuria	Telomerase activity reactivation

measure in biological tissues there are various indirect signs of oxidative stress in old age including lipid peroxidation, DNA oxidation and protein oxidation (127). It is difficult to distinguish whether the ROS increase results from an age- related accumulation of oxidative damage or from an age-related increase in production. However antioxidant therapy may be an useful antidote. Because the rate of mitochondrial ROS production is significantly influenced by the availability of mitochondrial energy substrates it is not surprising that dietary restriction is today the best investigated and most promising experimental strategy to increase life span and to improve the quality of life in old age. Whereas rigorous caloric restriction may be an unattractive regimen for human subjects, endurance exercise may yeld similar effects with lower risk of malnutrition. Telomerase reactivation with the enzyme telomerase or p16INK4a or stem cells therapy for age-associated podocytopathy senescence may be a therapeutic target for aging-related glomerulosclerosis. Table 2 lists drugs potentially useful for the management of aging related glomerulosclerosis selected according to the principal disease mechanism which impairs podocyte function. The type of podocytopathy can be responsible for a specific glomerulopathy. In fact process affacement characterizes minimal change disease while apoptosis and abnormal differentiation or proliferation can be related to acute glomerulonephritis and focal segmental glomerulosclerosis. The podocyte damage can be simply evaluated by quantitative podocyturia.

7. CONCLUSION

Podocytes play a central role in aging-related glomerulosclerosis. They can be the target of different pathogenetic mechanisms, which are age related as well as drug or related by therapeutic interventions which interfere with these mechanisms. Podocyte damage can initiate the glomerulosclerotic process and can be detected and monitored by appropriate laboratory methods discussed above. Further investigation on podocytes may be useful to prevent or slow age related glomerulosclerosis.

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Abbreviations: RAS: Renin-Angiotensin System; NO: Nitric Oxide; ACE: Angiotensin Converting Enzyme; WT-1 : Wilms Tumor-1; GLEPP-1 : Glomerular Epithelial Protein-1; CD2-AP: CD2-associated protein; ZO-1: :Zonula Occludens-1; FAT :Fatty Acid Transporter; SNS: Sympathetic Nervous System; AngII: Angiotensin II; TRPC6 :Transient Receptor Potential Canonical; AT1 : Angiotensin receptor 1; NOS: Nitric Oxide Synthase; $(O2^-)$: anions suporoxide; $(ONOO^-)$: peroxynitrate; ADMA :asymmetric dimethylarginine; DDAH : dimethylarginine dimethylaminohydrolase; sGC :soluble Guanylyl Cyclase; RPF : Renal Perfusion Flow; GFR :Glomerular Filtration Rate; TGF : Transforming Growth Factor; ET- 1 : Endothelin-1; ETA :Endothelin receptor A; ETB: Endothelin receptor B; MMP-9: Matrix Metalloproteinase -9; MAPK: Mitogen-Activated Protein Kinase; NFkB : Nuclear Factor-kappa B; ROS: Reactive Oxygen Species; NADPH: Nicotinamide Adenine Dinucleotide Phopshate-reduced form; TERT: Telomerase Reverse Transcriptase; TERC: Telomerase RNA component; CDK:Cyclin-Dependent Kinase; ATM :Ataxia Teleangectasia Mutated/p53 pathway; PAN: Puromycin Aminonucleoside Nephrosis. Cas: p130Cas, Cat: Catnein, CD: CD2 associated protein, EZ: Ezrin, FAK: focal adhesion kinase, M: Myosin, N : NHERF2, NSCC: non selectrive cation channel, PC: Podocalyxin, S: Synaptopodin, TPV : Talin, paxillin, vinculin, U: utrophin, Z: ZO-1

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