Hypertensive nephropathy. Moving from classic to emerging pathogenetic mechanisms

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Hypertensive kidney disease classically entails nephroangiosclerosis and hyalinosis with glomerular damage. However, in recent years, several evidences showed that high blood pressure also injures tubular cells, inducing epithelial-to-mesenchymal transition and tubulointerstitial fibrosis. Recently investigated mechanisms are also podocyte effacement and loss, which lead to denudation of the glomerular basement membrane and focal adhesion of the tufts to the Bowman's capsule, with reduced filtration and scars. Starting from the classic concept of nephroangiosclerosis, this review examines the recently emerged knowledge of new biochemical and molecular mechanisms underlying the kidney damage in hypertension and discusses how viable podocytes or podocyte-deriving proteins are promising tools for early diagnosis of renal remodelling in hypertension.

Keywords: epithelial-to-mesenchymal transition, hypertension, kidney, nephroangiosclerosis, podocyte

Abbreviations: ACE, angiotensin-converting enzyme; Ang II, angiotensin II; BP, blood pressure; ECM, extracellular matrix; EMT, epithelial-to-mesenchymal transition; ESRD, end-stage renal disease; FSGS, focal segmental glomerulosclerosis; GBM, glomerular basement membrane; HT, hypertension; MYPT, myosin phosphatase target protein-1; NGAL, neutrophil gelatinase-associated lipocalin; RAS, renin–angiotensin system; ROS, reactive oxygen species; TIF, tubulointerstitial fibrosis

Hypertensive kidney disease is the second leading cause of end-stage renal disease (ESRD) after diabetes mellitus [1–3]. Most hypertensive patients develop mild-to-moderate hypertensive nephrosclerosis that, however, progresses only in a relatively small percentage into ESRD. Nevertheless, the percentage of patients that develops ESRD dramatically increases when blood pressure (BP) values are uncontrolled for long time or kidney disease preexists. Given that hypertension entails 30 to 45% of adult population, it determines kidney damage in a large number of patients [3–5]. In addition, hypertensive kidney disease and ESRD are expected to further increase in next decades, mainly due to ageing and improved survival from cardiovascular diseases [1].

High BP can affect each renal compartment: vessels, glomeruli and tubulointerstitium [6]. Starting from the seminal description of nephrosclerosis by Theodor Fahr and Franz Volhard in 1918, a bulk of studies has been published on hypertensive kidney disease (reviewed in [6,7]). For decades attention was mostly focused on the damage in the capillary tuft causing nephroangiosclerosis and hyalinosis, and the renin–angiotensin system (RAS) [8,9]. More recently, light was shed on other histologic aspects and molecular mechanisms so that the '*classic*' picture of hypertensive kidney disease known as nephroangiosclerosis has been wholly revisited. We therefore focus this review on the pathophysiology of hypertensive kidney disease integrating the '*classic*' picture of hypertensive nephrosclerosis with novel knowledge, which includes podocyte damage and loss, epithelial-to-mesenchymal transition (EMT) and tubulointerstitial fibrosis.

We used a search strategy in the Medline literature though PubMed by combining the terms 'hypertension' or 'high blood pressure', 'nephropathy' and arterioles', 'glomerular damage' and 'tubular damage' or 'tubulointerstitial fibrosis'. Only studies focused on histopathology and molecular mechanisms underlying nephropathy in primary (essential) hypertension were considered. Analysis was restricted to English-written studies.

DAMAGE IN SMALL VESSELS

A progressive intimal thickening of small arterioles, that is arteriolosclerosis, occurs along development and progression of hypertension. Smooth muscle cells, after changing into myofibroblasts, migrate from the media to the intimal layer and secrete collagen-inducing intimal thickening. Contrary to muscle cells in the media that have a circumferential arrangement, the media-migrated myofibroblasts are aligned along the long axis of the lumen. When contracting, longitudinally elongated myofibroblasts cause wall stiffness, with little or no effect on the lumen calibre.

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Hypertension also causes thinning of the media and, at the sites where smooth muscle cells are atrophic or missing, induces accumulation of hyaline material. Such process, known as hyalinosis, is characterized by loss of smooth muscle cells and, therefore, should be distinguished from arteriolosclerosis [9]. As a consequence of hyalinosis and cell loss, the wall of small vessels becomes more expansible favouring endothelial permeability and migration of plasma proteins to the media [9].

In hypertension hyalinosis specifically entails the afferent arteriole leading to modest changes in the intraglomerular haemodynamics, whereas in diabetes hyalinosis affects the afferent and, to more extent, the efferent arteriole, thereby inducing an increase in the intraglomerular pressure [10]. Hyalinosis can be also found in ageing kidneys, indicating that hyalinosis is not a hallmark of hypertensive kidney disease [9,11]. However, in hypertensive patients, hyalinosis develops earlier and more markedly than in normotensive ageing, thus supporting the concept that hypertension causes an accelerated senescence [12,13].

Structural remodelling of arterioles associates with haemodynamic and structural changes in the aorta and elastic arteries. The progressive breakdown of elastic fibres in large arteries caused by increased pulse wave velocity was found to be associated with laminar-to-pulsatile flow shift in the arcuate, interlobular and afferent arterioles, and also with glomerular damage and microalbuminuria, supporting the role of mechanical stress in the glomerular damage [14].

GLOMERULAR DAMAGE

Any change that primarily involves the small vessels translates into glomerular damage [9,11]. Narrowing of afferent arteriole in hypertension causes partial ischaemia of the glomerular tuft that becomes smaller and gradually reduces the filtration [9], (Table 1 and Fig. 1). Hyperfiltration and hypertrophy of the remaining nephrons allows the kidney to function longer, thereby explaining why ESRD seldom occurs in hypertensive patients if other risk factors, including obesity, smoking or diabetes mellitus, lack.

When hyalinosis is the major change affecting afferent arteriole in hypertension, glomeruli become hypertrophic [6,7,9] with enlargement of the glomerular tuft and occlusion of capillaries by hyaline material, leading to accumulation of periglomerular extracellular matrix (ECM) and focal segmental glomerulosclerosis (FSGS) [6,15].

A large spectrum of glomerular lesions has been reported in hypertension. The opposite ends of this spectrum are represented by the small and sclerotic glomeruli mainly induced by arteriolosclerosis and ischaemia, and the large glomeruli embedded with ECM associated with FSGS [7]. Other patterns of hypertensive glomerulopathy include glomeruli with partially or wholly collapsed tuft and accumulation of extracellular material in Bowman space; glomeruli with expanded tuft entirely replaced by collagen (global glomerulosclerosis); glomeruli with capsular adhesion, segmental scars and hyalinosis [9].

PODOCYTE LOSS

Podocytes are epithelial cells that constitute a glomerular barrier against protein loss. Foot processes of neighbouring podocytes interdigitate and, via interconnection through specific cell-to-cell junctions called slit diaphragms, provide a size selective filtration barrier. Several podocyte proteins, including podocin, nephrin, synaptopodin and podocalyxin, maintain the structural and functional integrity of glomerular slit diaphragm through complex interactions [16,17]. As mature podocytes are terminally differentiated cells, their loss results into irreversible damage [18].

In hypertension the major mechanism of podocyte loss is the cell detachment from the glomerular basement membrane (GBM), a process that starts with podocyte injury and ends with cell death and denudation of GBM [19]. This causes focal adhesions of the glomerular tuft to the outer leaflet of Bowman's capsule and misdirected filtration to the renal interstitium.

The major factor driving podocyte detachment is capillary hypertension, which causes circumferential and axial capillary stress that, in turn, determines hyperfiltration and glomerular hypertrophy [20]. Hyperfiltration increases fluid shear stress, and glomerular hypertrophy compels podocytes to cover larger and more remote areas of GBM. Enlargement of podocyte cell surface determines flattening of cell ramifications, a process known as foot process effacement that, by enhancing the expansible forces, causes podocyte detachment and loss [20]. Intraglomerular pressure bulges outwards the denuded GBM, thereby favouring focal adhesions of the glomerular tuft to the outer leaflet of Bowman's capsule and glomerulosclerosis [21]. Podocyte

TABLE 1. Changes induced by high blood pressure in the vascular, glomerular and tubulo-interstitial compartments

Compartment	Changes	Effects
Vessels	Transition of smooth muscle cells into myofibroblasts and intimal thickening of small arterioles	Wall stiffness, with little or no effect on the lumen calibre
	Thinning of the media and hyalinosis of the afferent arteriole	Reduced filtration
	Occlusion of intraglomerular capillaries by hyaline material	Hypoxia
	Breakdown of elastic fibres in large arteries	Laminar-to-pulsatile flow shift in the arcuate, interlobular and afferent arterioles
		Increased intraglomerular pressure and microalbuminuria
Glomerules	Accumulation of ECM	FSGS
	Glomerular tuft entirely replaced by collagen	Global glomerulosclerosis
	Capsular adhesion and segmental scars	Reduced filtration
Tubules	Cell dilatation and flattening; cell atrophy and loss	Proteinuria
	Epithelial-to-mesenchymal transition	Tubulointerstitial fibrosis and CKD

ECM, extracellular matrix; FSGS, focal segmental glomerulosclerosis; CKD, chronic kidney disease.

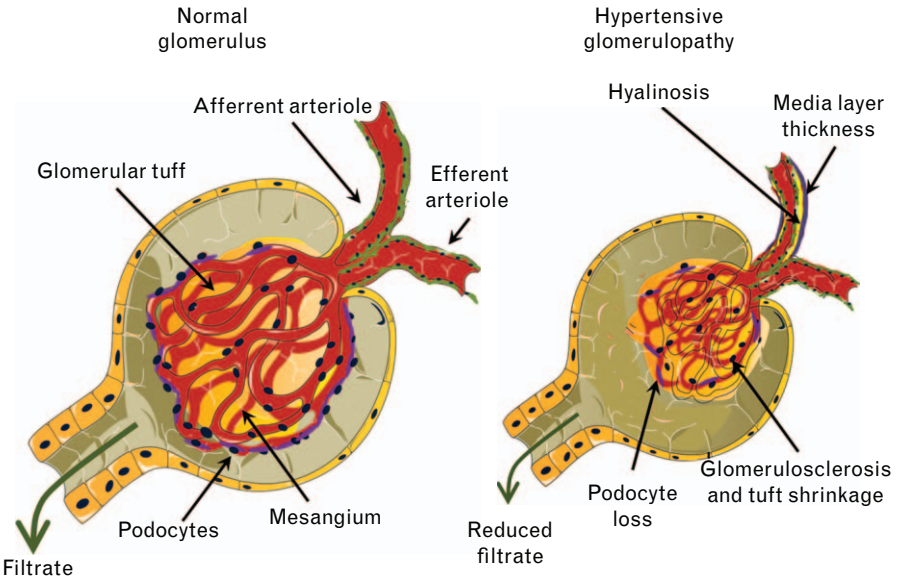

FIGURE 1 Hypertensive glomerulopathy. A large spectrum of glomerular lesions has been reported in hypertension. Herein, the hypertensive glomerulopathy (b) is represented by a glomerulus partially ischaemic and smaller than normal (a). Hyalinosis (*yellow*) and media layer thickness (*violet*) reduce blood flow in the afferent arteriole, favouring shrinkage of the glomerular tuft and podocyte loss. In contrast to diabetes mellitus, no involvement of the efferent arteriole can be observed.

loss and disruption of the filtration barrier ultimately cause proteinuria [20].

In addition to mechanic stress, oxidative stress, inflammation, metabolic and vasoactive factors, as Angiotensin II (Ang II) and high glucose levels, concur to podocyte injury [22,23].

After detachment from GBM, podocytes become free to move through meshes of the glomerular sieve to appear still viable in urine. However, before detachment injured podocytes may release constitutive proteins into urine. Nephrin is the major protein of podocyte filtration slit area and lateral membrane [24]. After binding nephrin and the structurally similar slit diaphragm protein Neph 1, the podocin-nephin-Neph 1 complex acts as a transmembrane receptor for actin-associated proteins to promote their polymerization. Loss of function mutations in the genes coding for these proteins resulted in nephrotic syndrome in humans [25].

Synaptopodin is a prolin-rich actin-associated protein highly expressed in differentiated podocytes [26,27], which, in conjunction with the sialoglycoprotein anion podocalyxin, confers a negative charge barrier that prevents the passage of anionic proteins [28].

Recent studies showed that excretion of podocyte-specific proteins and/or podocyturia precede proteinuria, opening a promising scenario for an early diagnosis and therapeutic approach to renal damage. Unfortunately, the demonstration of viable podocytes in the urine is a complex procedure, not available in all laboratories [29]. However, elevated mRNA levels of podocin and nephrin can be found in the urine from patients with hypertension or proteinuria [24,30], as well as in preeclamptic women [31], suggesting that this approach is feasible for early diagnosing the glomerular damage and hypertensive nephropathy [32]. In addition, hypertensive nephropathy, proved by arteriolar hyalinosis and glomerulosclerosis at biopsy, was associated with blunted gene expression of podocyte markers as well as podocyte number and density. Podocyte changes significantly correlated with glomerular filtration rate but not with proteinuria, suggesting that podocyte injury precedes onset of proteinuria [32]. Although podocytes, the gatekeepers of protein filtration in the glomerulus, are major targets of high BP, prospective studies are needed to prove that podocytes can be markers of hypertensive nephropathy.

TUBULOINTERSTITIAL DAMAGE

In hypertensive patients, a large spectrum of lesions in the epithelial tubular cells may develop [6]. Lesions range from cell dilatation and flattening to atrophy and loss (Table 1). Other patterns include flat cells surrounding widely open lumens filled with eosinophilic casts, known as '*thyroid areas*', which are often associated with apolipoprotein *L1* gene renal risk alleles [33]. Inflammatory cells may be interspersed or numerous as in chronic pyelonephritis and may act as a trigger for tubule-interstitial fibrosis (TIF) [6]. Compressed by collagen, peritubular capillaries become atrophic, and distance between tubular cells and capillaries increases, thereby worsening the damage and favouring the development of renal failure [6].

Although the relationship between interstitial damage and renal failure was known since the 1960s, tubulointerstitial lesions remained neglected in the hypertensive disease until 1998 when Fine postulated the '*chronic hypoxia hypothesis*' [34]. The rationale underlying this hypothesis is that injury primarily determined at the glomerular level by hypertension causes changes in postglomerular peritubular capillaries that in turn induce endothelial damage and hypoxia. Microvasculature dysfunction, by inducing hypoxic environment, triggers inflammation, EMT with myofibroblast differentiation and fibrosis [9,35]. Hence,

hypertension-induced hypoxia translates the initial glomerular injury into interstitial damage.

Proximal tubular epithelial cells, which are more susceptible to hypoxic injury than distal cells, activate a complex transcriptional response with expression of genes involved in cell survival and adaptation [34]. The master regulator of this adaptive response is hypoxia-inducible factor α (HIFα) [34], a heterodimeric transcription factor comprising a constitutively expressed β-subunit and an oxygen-regulated α-subunit. Under hypoxia, HIFα protein dimerizes with HIFβ and binds to hypoxia-response elements in the regulatory regions of target genes coding for transforming growth factor β1 (TGFβ1), collagens and other ECM proteins leading to fibrosis [34].

Hypoxia *per se* activates resident fibroblasts favouring the production of interstitial collagen and suppression of ECM degradation. Moreover, the exposure of proximal tubular epithelial cells to hypoxia induces a myofibroblastic phenotype, further enhancing collagen accumulation and mitochondrial damage leading to apoptosis and loss of tubular cells [34]. Hypoxia also induces expression of fibrogenic factors, as TGFβ1 and ET-1, and angiogenic factors, vascular endothelial growth factor and angiopoietin-4, which synergize between them amplifying the fibrogenic response.

Persistent inflammation is an intrinsic component of hypoxia-mediated fibrotic response. Hypoxia is a potent homing signal for inflammatory cells that accumulate at sites of injury and also activates resident immune cells. Moreover, it can alter the function of intrinsic stem cell populations in the interstitium and potentiate IL-1β and IL-1β-induced phospholipase A2 expression and activity in renal mesangial cells [36]. Phospholipase A2 provides fatty acids for production of lipids mediators, as prostaglandins and leukotrienes, and also binds HIF-2α, amplifying the inflammatory and fibrogenic responses.

A great amount of data from animal models provide a compelling argument for the *chronic hypoxia hypothesis* [37], but the key question is whether this may also be applied to hypertension in humans [34,38]. The relationship between rarefaction of peritubular capillaries and tubulointerstitial scarring in human biopsies [39] strongly suggests a role of hypoxia in chronic kidney disease, although a direct demonstration of the *chronic hypoxia hypothesis* in the hypertension nephropathy in humans is still lacking.

Another well known inducer of TIF is Ang II. This peptide, the most powerful vasoconstricting agent of the RAS, is also known to play a key role in cell proliferation, hypertrophy, reactive oxygen species generation, inflammation and ECM production through the induction of cytokines, chemokines and growth factors [40]. Ang II promotes the phenotypic switch of fibroblasts to myofibroblasts, which proliferate in the periglomerular and peritubular spaces, contributing to ECM deposition (reviewed in [41]).

Using a transgenic model of severe hypertension and cardiovascular damage, the TG(mRen2)27 rat, created by the insertion of the mouse renin gene into the rat genome, we found that either angiotensin-converting enzyme (ACE)-inhibition with ramipril [42], or the block of Ang II type 1 (AT1) receptor with the Ang II AT1 receptor blocker irbesartan [43], prevented TIF, supporting the fibrogenic role of Ang II. In this model of Ang II–dependent hypertension, TIF was also prevented by dual inhibition of ACE and neutral endopeptidase [42], an effect that was abolished by the bradykinin B2 receptor antagonist icatibant, supporting a role of B2 subtype receptor in counterbalancing the deleterious effects of Ang II on the tubulointerstitium [42].

A key mechanism for the induction of renal remodelling in hypertension including TIF is the activation of RhoA/Rho kinase pathway downstream the Ang II AT1 receptor stimulation via modulation of the phosphorylation state of the regulatory chain of myosin II (myosin light chain, MLC), mainly through the inhibition of the myosin phosphatase target protein-1 (MYPT-1) [44–46]. Rho kinase, via an inhibitory phosphorylation of MYPT-1, increases the activity of MLC kinase, leading to smooth muscle contraction and cardiovascular and renal remodelling [47]. In these effects, an important role is played by the downregulation of endothelial nitric oxide synthase activity and the existence of an inverse relationship between RhoA/Rho kinase expression and activity with nitric oxide (NO) bioavailability [45]. The importance of the balance between the RhoA/Rho kinase pathway and the NO system is crucial in cardiovascular and renal pathophysiology being involved in vascular remodelling and induction of atherosclerosis, the relationship between inflammation and hypertension, and the relationship between hypertension, glucose metabolism and insulin resistance [47,48], thereby suggesting RhoA/Rho kinase pathway as a target for cardiovascular–renal protection [45].

Indirect support in humans for the proliferative, inflammatory and renal remodelling effects of Ang II via RhoA/Rho kinase may be provided by the results of our extensive studies in Bartter's and Gitelman's syndrome patients, who represent a human model of an endogenous Ang II AT1 receptor antagonism [49,50]. In these rare diseases, patients the increased Ang II levels are not associated with renal disease or endothelial dysfunction, hypertension and microalbuminuria; the NO system is upregulated, and have reduced oxidative stress [45,50]. In Bartter's and Gitelman's patients the activity of RhoA/Rho kinase pathway is blunted, as shown by the reduced expression of the RhoA/Rho kinase activator p63RhoGEF, which is induced by Ang II [50] and the decreased phosphorylation state of MYPT-1 [50]. The blunted activity of RhoA/Rho kinase pathway is associated in these patients with the lack of cardiovascular remodelling in terms of left ventricular mass, end-diastolic volume and mass/volume ratio and carotid intima–media thickness [50] despite their high Ang II levels.

EPITHELIAL-TO-MESENCHYMAL TRANSITION

EMT is a process by which epithelial cells lose their cell polarity and cell–cell adhesion to become mesenchymal cells [51], (Fig. 2). Originally characterized as a key process for organ development under physiologic conditions and for metastasis budding in cancer, in recent yearsEMT has been found to play a crucial role in fibrosis, in particular in the kidney [52,53].

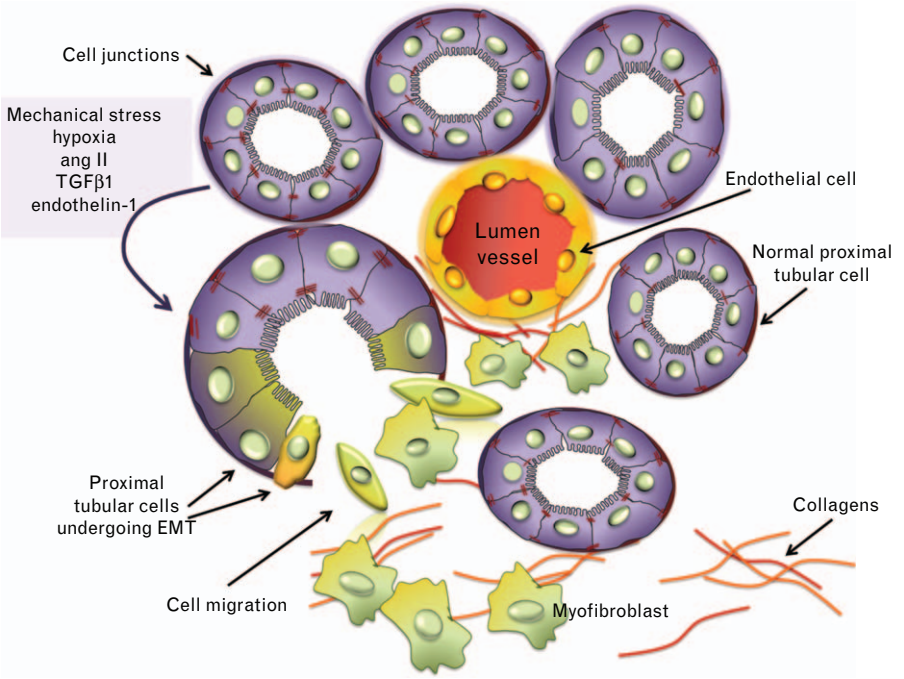

FIGURE 2 Epithelial-to-mesenchymal transition contributes to tubulointerstitial fibrosis. Under physiologic conditions, tubular cells express epithelial markers, as E-cadherin, which is the major component of adherent junctions (*visualised as red dashes*), which tightly connect cells between them. Mechanical stress, hypoxia, angiotensin II, TGFβ1 and endothelin-1 trigger a switch to a mesenchymal phenotype by inducing expression of αSMA and gradual loss of junctions. In the cartoon, the phenotypic switch to myofibroblasts is visualized as a change of colours from bluish-violet to greenish, with bluish and greenish indicating the epithelial and mesenchymal phenotypes, respectively. The coexistence of blue and greenish indicates an intermediate phenotype. The cells undergoing epithelial-to-mesenchymal transition also activate metalloproteinases, which promote basal membrane degradation, favouring movement of cells to the interstitium. Myofibroblasts produce collagens (*red and orange lines*), finally leading to tubulointerstitial fibrosis. (Modified from [55]).

The cell undergoing EMT progressively loses epithelial markers such as E-cadherin, which is the main component of adherent junctions, and acquires markers of mesenchymal phenotype, as a smooth muscle actin (αSMA) [51]. By losing the cell junctions, the epithelial cell becomes free to move towards the interstitial space. After completing the epithelial-to-mesenchymal phenotype switch, the epithelial cell becomes myofibroblast that is able to synthesize αSMA and matrix proteins, including collagen, finally leading to TIF [51].

The main inducers of EMT are TGFβ1 and Ang II, the same factors that classically induce fibrosis, suggesting that EMT is a common mechanism underlying TIF [53,54]. We recently found that also endothelin-1 can induce EMT in the kidney. TIF was prevented in TG(mRen2)27 rats not only by the Ang II AT1 receptor blocker irbesartan, but also by bosentan, an antagonist of endothelin-1 system [43]. However, TIF was not prevented by BMS-182874, a selective ET_A receptor antagonist, suggesting a key role of endothelin ET_B subtype receptors in the development of renal fibrosis [43]. In the same kidney sections of TG(mRen2)27 rats, we found a decrease in the epithelial marker E-cadherin along with an increase in the mesenchymal markers αSMA and S100A4 in the sites of TIF, suggesting EMT as the mechanism underlying fibrosis [55].

In-vitro experiments in our laboratory confirmed that endothelin-1 drives EMT in the renal tubular cells [55]. Exposure of HK2 cells to endothelin-1 caused disruption of cell junctions and synthesis of αSMA, blunting of E-cadherin along with the increase of mesenchymal markers, synthesis of metalloproteinase-9 and cell migration [55]. All The early steps of EMT mainly involved ET_B receptor subtype, which is the the predominant receptor subtype in the kidney tubular cells [55].

MARKERS OF KIDNEY DAMAGE AND THERAPEUTIC IMPLICATIONS

Microalbuminuria is the major marker of hypertensive nephrosclerosis reflecting the loss of the glomerular filter selectivity [56]. Measurement of microalbuminuria is recommended at screening and during treatment in hypertension patients to assess basal renal damage and drug-related protection against damage progression [5,57]. Treatments with either ACE-I or AT1 receptor blockers were effective in lowering microalbuminuria along prevention of progression of renal disease and cardiovascular events, thereby indicating microalbuminuria as a predictive marker of cardiovascular and renal events [58–61].

Whether podocyte damage and loss may reveal glomerular damage earlier than microalbuminuria remains to be established. The available markers of tubular cell injury, as kidney injury molecule-1 or neutrophil gelatinase-associated lipocalin , mostly unveil an acute damage or cell death and, therefore, do not specifically disclose TIF or EMT [62–64].

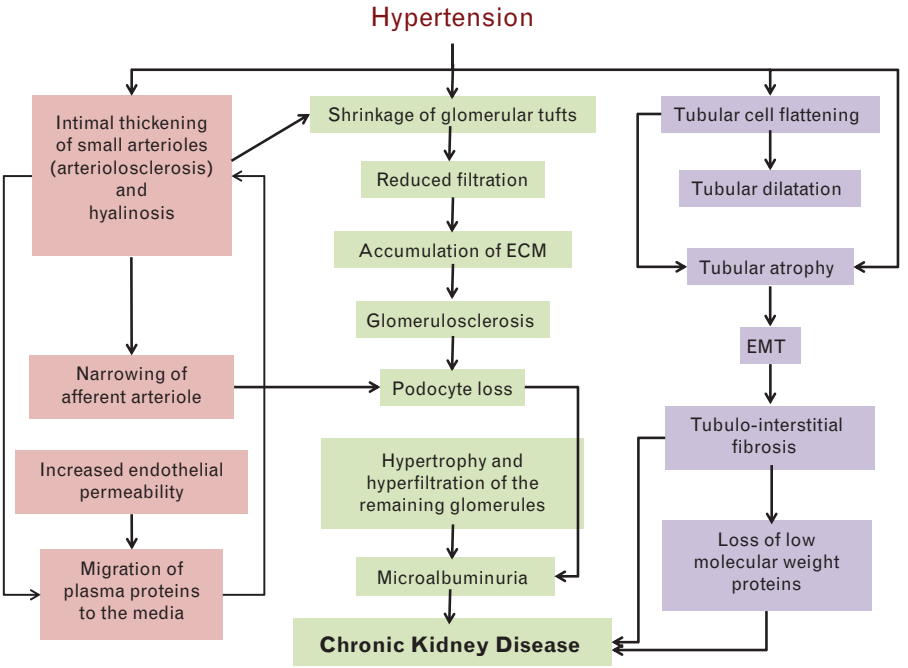

FIGURE 3 Changes associated with the hypertensive nephropathy. Hypertension affects the small vessels (*pink*), mostly the afferent arteriole, inducing intimal thickness and hyalinosis, which cause partial ischaemia of the glomeruli favouring sclerosis, shrinkage of the tufts and podocyte loss (*green*). The remaining glomeruli become hypertrophic maintaining filtration but favouring microalbuminuria. Hypertension also affects the tubuli (*violet*), inducing cell flattening and lumen enlargement. Mechanical stress and/or activation of the renin–angiotensin system and/or the endothelin system trigger epithelial-to-mesenchymal transition, finally leading to tubulointerstitial fibrosis. Changes in the vascular (*pink*), glomerular (*green*) and tubular (*violet*) compartments concur to development of chronic kidney disease.

CONCLUSION

Hypertensive nephrosclerosis classically entails hyalinization and sclerosis of interlobular and afferent arterioles, along with patchy fibrosis in the glomerular and tubulointerstitial compartments (Fig. 3). Mechanical stress caused by high BP, stimulation of RAS and activation of resident fibroblasts were deemed for years to be the major pathogenic mechanisms underlying kidney damage in hypertension. More recently, podocyte damage/loss, which leads to blunting of selectivity in the glomerular filtration barrier, and EMT, that replenishes the myofibroblast pool with tubular epithelial cells, have been identified as key processes that favour glomerular and tubulointerstitial damage in hypertension. Solid evidences indicate EMT and podocyte loss as emerging fields for research and as promising tools for the development of novel therapeutic strategies to prevent the deterioration of renal function in hypertension.

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Conflicts of interest

There are no conflicts of interest.

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Reviewers' Summary Evaluations

Reviewer 1

Strength: comprehensive overview of the literature.

Weakness: no formal search strategy.

Reviewer 2

Nephroangiosclerosis, that is the hypertension-dependent kidney disease, is a classic of medicine and hypertension is considered a common cause of renal failure. Hypertensive kidney disease is characterized by slow progression toward end-stage renal disease at least in controlled hypertensives. The presence of hypertensive kidney disease, however, is an unfavourable prognostic factor also for non-renal complications of hypertension. For years, hypertensive kidney disease has been considered as a disorder limited to afferent arterioles and glomeruli. This review highlights the need to update this concept because hypertensive kidney disease includes also alterations of podocytes, glomerular basement membrane, Bowman's capsule, and peri-tubular interstitium.