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Role of Anemia in Progression of Chronic Kidney Disease

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Anemia is a well-known consequence of chronic kidney disease (CKD), and its prevalence progressively increases when the estimated glomerular filtration rate decreases to less than 60 mL/min/1.73m2. However, analyses of the consequences of anemia and of the mechanisms of progression of CKD suggest that anemia also could contribute to the deterioration of kidney function. This hypothesis is based mostly on experimental data that imply that hypoxia of tubular cells plays an important role in tubulointerstitial damage associated with CKD and, thus, in the progression of renal failure. It also is supported by the fact that red blood cells represent a major antioxidant component of blood and that oxidative stress appears to contribute to glomerulosclerosis and tubulointerstitial damage. In humans, post hoc analysis of the Reduction of End points in non insulin-dependent diabetes mellitus (NIDDM) with the Angiotensin II Antagonist Losartan study and analyses of smaller prospective cohorts of CKD patients have shown that anemia is an independent risk factor for progression of CKD. In addition, 3 small randomized studies have suggested that anemia correction could slow the progression of CKD. Thus, the existence of a relationship between anemia and progression of CKD is not only plausible biologically, but also is supported by observational studies and by small intervention studies. However, only a large, randomized, prospective trial will be able to establish if anemia correction can slow the progression of CKD effectively.
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Anemia is a well-known consequence of chronic kidney disease (CKD), and the third National Health and Nutrition Examination Survey has shown that the prevalence of anemia increases in subjects with an estimated glomerular filtration rate (GFR) of less than 60 mL/min/1.73m2.[1] However, analysis of the consequences of anemia and of the mechanisms of progression of CKD suggests that anemia also could contribute to worsening of kidney function, and this hypothesis is supported by limited data derived from clinical studies. In this report we review the mechanisms by which anemia may contribute to the progression of kidney diseases and the clinical data supporting this hypothesis.

Consequences of Anemia

Because there is a linear relationship between hemoglobin concentration and arterial oxygen content of blood (the oxygen binding capacity of hemoglobin is 1.39 mL/g), the primary consequence of anemia is a decrease in the capacity to deliver oxygen to tissues, including kidney. However, anemia also leads to increased oxidative stress because erythrocytes represent a major antioxidant component of blood.[2] Their antioxidant effects are mediated through enzymes such as superoxide dismutase, catalase, glutathione peroxidase, and through cellular proteins that can react with reactive oxygen species, such as low–molecular weight proteins of the erythrocyte membrane, vitamin E, vitamin C, or coenzyme Q. Furthermore, glutathione reductase can regenerate reduced glutathione from its oxidized form, using NADPH produced through the pentose phosphate pathway. Thus, if anemia contributes to the progression of CKD, it should be by enhancing renal hypoxia and/or oxidative stress.

Mechanisms of Progression of CKD

During the course of CKD, nephron destruction is initially caused by direct effects of the underlying disease on glomerular, tubular, or vascular structures. However, once renal

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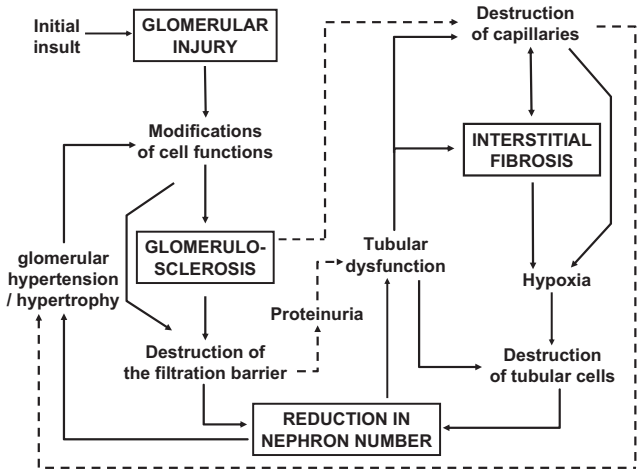

Figure 1 Schematic representation of the vicious circles that accelerate the progression of CKD. The glomerular vicious circle is shown on the left side of the figure, and the tubulointerstitial one on the right side. Links between the two are shown with dotted lines. See text for details.

damage reaches a certain threshold, 2 vicious circles accelerate the progression of CKD, independently of the underlying kidney disease (Fig 1). One links nephron loss with increased glomerular capillary pressure and flow, glomerulosclerosis, and glomerular destruction. The other one links reduction in nephron number with interstitial fibrosis and tubular damage, and appears to be highly dependent on tubulointerstitial hypoxia.

Glomerulosclerosis and Progression of CKD

The existence of a glomerular vicious circle was recognized by Brenner et al[3] more than 20 years ago based on analyses of experimental models of renal failure. They showed that reduction in nephron number induces hemodynamic modifications that result in increased glomerular capillary pressure and flow, and postulated that these seemingly adaptative modifications aimed at maintaining GFR are responsible for the injury of glomerular cells and lead to glomerulosclerosis (Fig 1). The major arguments supporting this hypothesis were that interventions that decreased glomerular hydrostatic pressure also protected against glomerulosclerosis.[4-6] In human beings,[7] analyses of sequential renal biopsy specimens obtained in few patients who had an important reduction of their renal mass have shown that a decrease in nephron number also can lead to the development of glomerulosclerosis, supporting the theory of Brenner et al.[2]

It is important to realize that glomerulosclerosis is associated with destruction of glomerular capillaries, and thus with decreased blood flow in downstream peritubular capillaries. Therefore, one of the direct consequences of glomerulosclerosis is a decrease in oxygen delivery to the interstitium.

Tubulointerstitial Lesions and Progression of CKD

Two series of observations derived from careful analyses of renal biopsy specimens have suggested that kidney diseases are responsible for the development of tubulointerstitial lesions, and that in turn these lesions enhance the progression of CKD, thus creating a vicious circle (Fig 1). First, careful analyses of renal biopsy specimens have shown that during the course of many kidney diseases, there is a striking correlation between renal function at the time of biopsy examination and the severity of interstitial fibrosis and tubular atrophy.[8] Remarkably, for most glomerular diseases, the correlation between renal function and tubulointerstitial lesions is much stronger than the one between renal function and glomerular lesions.[9] Second, in longitudinal studies, the extent of interstitial fibrosis is one of the best histologic prognostic markers of most renal diseases, including glomerular and vascular diseases.[10-12]

The link between tubulointerstitial lesions and progression of CKD appears to be a destruction of tubules, with the formation of so-called *atubular glomeruli*[13,14] and, for example, analysis of subtotally nephrectomized rats has shown that, in this model, tubular destruction plays an important role in the progression of renal failure.[15] Twenty-five weeks after subtotal nephrectomy, only 14% of the remaining glomeruli were globally sclerotic, whereas 48% of them were atubular and 26% were connected to an atrophic proximal tubule.[15] Not surprisingly, different studies have shown that tubular hypoxia can induce tubular destruction,[16-21] suggesting that decreased oxygen delivery to tubules and/or increased oxygen consumption by tubular cells could play a role in the ontogeny of tubular lesions, and thus in the progression of CKD.

Hypoxia and Progression of CKD

The role of proteinuria in the pathogenesis of tubulointerstitial lesions, and thus in progression of CKD, has been highlighted repeatedly over the past years.[22] In particular, it has been shown that filtered proteins can damage tubular cells directly, and also can modify the functional characteristics of these cells, leading to the production of proinflammatory and profibrotic molecules.[22,23] However, proteinuria is not the only factor responsible for interstitial fibrosis and tubular damage. As initially pointed out by Fine et al,[24] hypoxia also appears to play an important role in this process.[25]

Evidence for Tubulointerstitial Hypoxia

It may seem paradoxic to discuss the consequences of hypoxia in an organ that has an extremely high blood flow rate and consumes less than 10% of its oxygen supply. However, 2 phenomenons contribute to hypoxia of the juxtamedullary region and outer medulla of the kidney. First, renal blood flow is quite heterogeneous, and although the renal cortex receives up to 80% of the total renal blood flow, outer-medulla and inner-medulla blood flows are only about 1 and 0.5 mL/min/g or less, respectively. In contrast, active transepithelial transports in the proximal straight tubule and thick ascending limb of Henle's loop are responsible for high oxygen consumption in the outer medulla. Second, there are

intrarenal shunts for oxygen between the arterial and venous systems, which decreases oxygen delivery to tubules.[26-29] These shunts appear to be functional and not anatomic, and to result from the fact that branches of the arteries and veins are often in close contact with each other, which allows diffusion of oxygen.

Different experimental studies have provided evidence of tubular hypoxia in animals with kidney disease. For example, perfusion of animals with pimonidazole, a probe that accumulates in hypoxic cells, has shown that tubular hypoxia is an early consequence of glomerular damage or subtotal nephrectomy,[30-32] and these results have been confirmed recently using an elegant transgenic rat model.[33] Nangaku's group generated a transgenic rat that expresses the luciferase reporter gene under the control of a hypoxia-responsive promoter. By using this model, they have been able to identify early diffuse cortical hypoxia in the puromycin aminonucleoside–induced nephrotic syndrome and focal and segmental hypoxia in the remnant kidney model, and to show the existence of a positive correlation between the degree of hypoxia and tubulointerstitial injury.[33]

Mechanisms of Tubulointerstitial Hypoxia

Tubulointerstitial hypoxia appears to be multifactorial owing to (1) decreased diffusion of oxygen from interstitial capillaries to tubular cells, (2) increased oxygen consumption by tubular cells, and (3) a reduction in renal blood flow.

First, there is no doubt that accumulation of extracellular matrix, which characterizes interstitial fibrosis, increases the distance between interstitial capillaries and tubules, and thus hampers oxygen transfer from peritubular capillaries to tubular cells.[34]

Oxygen consumption by tubular cells during the course of kidney diseases still needs to be analyzed in detail. However, recent experimental studies have shown that tubular cells display signs of hypoxia during the course of experimental proteinuric nephropathies, suggesting that proteinuria may enhance oxygen consumption and favor hypoxia.[33,35]

Analyses of animals with experimental kidney disease have provided evidence for a decrease in renal blood flow during the course of CKD.[22,31,32,36] It seems that, at early stages, this reduction in renal blood flow is reversible, owing to the combination of an activation of the renin-angiotensin system, an increased production of endothelin and a decreased synthesis of nitric oxide.[22,31,32,36] However, at later stages, it is associated with a destruction of glomerular and peritubular capillaries, and thus becomes irreversible.[30-32,37-39] Similarly, a reduction in the number of interstitial capillaries has been shown consistently by careful analysis of renal biopsy specimens from patients with glomerular, vascular, or interstitial kidney diseases responsible for renal failure, and there is an inverse relationship between renal function and the number of peritubular capillaries.[40,41] As suggested by Johnson's group, the reduction in the number of interstitial capillaries is likely to result from an imbalance between the production of molecules that promote survival of endothelial cells, such as vascular endothelial growth factor, angiopoietin 1, or nitric oxide, and of antiangiogenic factors, such as angiopoietin 2, endostatin, or thrombospondin 1 (TSP-1).[42] Interestingly, TSP1 is not only an antiangiogenic factor, but also a potent activator of latent transforming growth factor (TGF)-β,[43] and it could link the destruction of interstitial capillaries and development of interstitial fibrosis. A decreased production of vascular endothelial growth factor or an increased expression of TSP-1 has been shown in different experimental models of kidney disease, including the remnant kidney model.[44,45] However, it would be important to study systematically the expression of proangiogenic and antiangiogenic factors during the course of kidney diseases.

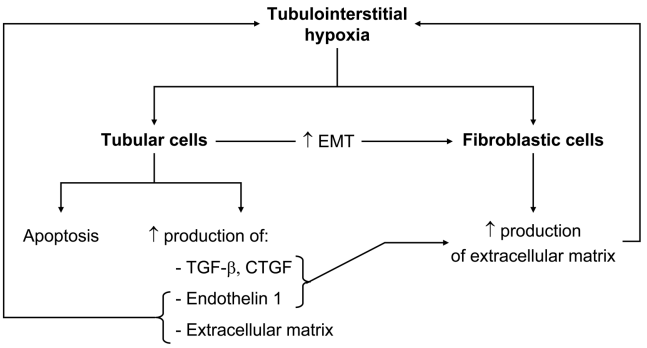

Figure 2 Schematic representation of the consequences of tubulointerstitial hypoxia. See text for details. EMT, epithelial mesenchyme transition.

Consequences of Tubulointerstitial Hypoxia

As expected, various in vitro studies have shown that mild hypoxia or adenosine triphosphate depletion causes apoptosis of tubular cells whereas more severe hypoxia or adenosine triphosphate depletion is responsible for necrosis of these cells.[46-49] Thus, it seems likely that hypoxia of tubular cells can induce tubular damage directly, with formation of nephrons that harbor an intact or almost intact glomerulus not linked to a functional tubule (*atubular glomerulus*),[13,14,16,19-21] The importance of hypoxia-induced tubular damage has been exemplified by careful analysis of heterozygous Oligosyndactylism mice.[19] This radiation-induced mutant strain has reduced glomerular number and increased glomerular size, and it spontaneously develops glomerulosclerosis.[50] Analysis of tubular lesions has shown, first, that tubular ischemia precedes apoptosis, and, second, that apoptotic tubular cells colocalize to hypoxic but not normoxic tubules, strengthening the hypothesis that, in vivo, tubular hypoxia plays an important role in the genesis of tubular damage.[19]

In addition to inducing tubular destruction, hypoxia of tubular and interstitial cells also favors the development of interstitial fibrosis, and thus creates a vicious circle that aggravates hypoxia (Fig 2). In vitro studies have shown that hypoxia can favor interstitial fibrosis by acting at different levels. First, it stimulates the production of profibrotic molecules, such as TGF-β, connective tissue growth factor (CTGF), or endothelin 1 by tubular cells, and the synthesis of

extracellular matrix by these cells.[51,52] Second, it directly enhances the production of interstitial collagens by fibroblastic cells.[53] Third, it also favors the transdifferentiation of tubular cells into fibroblastic cells, both directly and through the overproduction of TGF-β.[54,55] Furthermore, as mentioned earlier, overproduction of TSP-1 could induce both interstitial fibrosis and destruction of interstitial capillaries.

Oxidative Stress and Progression of CKD

Different studies have shown an increased expression of reactive oxygen species during the course of experimental renal diseases and have suggested that oxidative stress plays a role in the progression of CKD.[56] In particular, in vivo, treatment of animals with antioxidant agents can slow the progression of experimental kidney diseases. For example, uninephrectomized rats with diet-induced hypercholesterolemia develop interstitial inflammation and fibrosis. In these animals, antioxidant therapy efficiently prevents the development of interstitial fibrosis.[57] Similarly, treatment with antioxidants can decrease renal damage induced by high salt intake in Dahl salt-sensitive rats or in deoxycorticosterone acetone (DOCA)-salt hypertensive rats.[58,59] Recently, Bottinger's group[60] showed that chronic inhibition of NADPH oxidase can decrease glomerular damage, including podocyte injury in diabetic db/db mice. In rats with anti-Thy 1 glomerulonephritis, treatment with the antioxidant α-lipoic acid also protects against glomerular injury.[61] By contrast, in rats, a prooxidant diet can increase collagen production in the interstitium.[62]

To understand the effects of reactive oxygen species, it is essential to realize that they not only are injurious by-products of cellular metabolism that have the potential to cause damage to lipids, proteins, and DNA, but also essential participants in cell signaling.[63] In particular, reactive oxygen species can induce the release of proinflammatory molecules, such as monocyte chemoattractant protein-1 (MCP-1), and of profibrotic molecules, such as TGF-β1 or plasminogen activator inhibitor-1 (PAI-1),[62,64-66] they also can amplify TGF-β intracellular signaling and favor TGF-β–induced epithelial-mesenchyme transition.[67] They can increase the production of extracellular matrix by fibroblastic cells.[68-70] They also favor both cell proliferation and apoptosis.[71,72] Finally, reactive oxygen species interact with nitric oxide to regulate vascular tone, and thus can aggravate tubulointerstitial hypoxia.[73]

Clinical Studies Focusing on the Links Between Anemia and Progression of Renal Failure

The role of anemia in progression of CKD is supported by 2 kinds of clinical studies: (1) studies of cohorts of patients that show that anemia is an independent risk factor for progression of CKD; and (2) intervention studies that suggest that partial correction of anemia could slow the progression of CKD.

Studies of Cohorts of Patients

The Reduction in End points in Noninsulin-dependent diabetes mellitus with the Angiotensin II Antagonist Losartan (RENAAL) study was a double-blind randomized trial designed to test the renoprotective properties of losartan in patients with type 2 diabetes and overt nephropathy.[74] It included 1,513 patients who were assigned randomly to receive losartan or placebo, in addition to conventional antihypertensive therapy. The mean follow-up period was 3.4 years. Post hoc multivariate analysis of this large cohort of patients showed that, in this population, anemia was an independent risk factor of progression, together with serum creatinine level, proteinuria, and serum albumin level.[75] Interestingly, even a modest degree of anemia was associated with an increased risk for progression. Individuals who had hemoglobin levels greater than 13.8 g/dL showed a Kaplan-Meier event rate for doubling serum creatinine level or end-stage renal disease that was approximately 20%, whereas those with a hemoglobin level of less than 11.2 g/dL showed an event rate that approximated 60%.[75]

Similarly, Parving's group[76] analyzed a cohort of 227 patients with type 2 diabetes and nephropathy who were followed-up at the Steno Diabetes Center and had regular measurements of GFR. The mean follow-up period of this cohort was 6.5 years. Multivariate analysis showed that during follow-up evaluation, albuminuria, systolic blood pressure, hemoglobin A1c level, heavy smoking, presence of diabetic retinopathy, and low hemoglobin level were associated significantly with increased rate of decrease of GFR.[76]

Recently, analysis of a cohort of 131 patients with CKD stages 2 to 5 who were followed-up up at Cremona hospital showed that estimated GFR at inclusion, proteinuria, hemoglobin concentrations, and asymmetric dimethylarginine levels were independent risk factors for progression of CKD or death.[77] In this study, the risk reduction for hemoglobin was 23% per g/dL.

In Okinawa, analysis of a cohort of 71,802 subjects followed-up for 17 years also showed that anemia (defined by hematocrit levels of $<$40% in men and 35% in women) was an independent risk factor for development of end-stage renal disease, both in men and women.[78]

Intervention Studies

Three prospective clinical studies including a limited number of patients have studied the effects of anemia correction with an erythropoiesis-stimulating agent (ESA) on progression of CKD.[79-81]

The first study, published in 1994, included 83 patients with severely impaired renal function (mean measured GFR, 10 mL/min), and severe anemia (mean hematocrit level, 26.8%).[79] After a 2-month stabilization period, 40 patients were assigned randomly not to receive epoetin and 43 to receive epoetin for their hematocrit level to reach 35%. The patients were followed-up for 48 weeks. No beneficial effect

of epoetin could be shown by simply comparing renal survival or rate of GFR decrease. Nevertheless, when the data were analyzed only after the hematocrit levels of the patients included in the epoetin group had reached the target values (ie, after week 16), the rate of GFR decrease was 3 times slower in the treated group than in the control group (-0.13 ± 0.35 mL/min/mo versus -0.39 ± 0.65 mL/min/mo, $P = .05$).

The second study, published in 1997, included 73 patients with severe anemia (mean hematocrit level, 27.4%) and renal failure (mean creatinine clearance, 18.2 mL/min).[80] After an 8-week stabilization period, the patients were assigned randomly to receive or not to receive epoetin. Thirty-one patients were left untreated. Forty-two patients received epoetin to increase their hematocrit level to 33% to 35%. The follow-up period was 36 weeks. During this period, creatinine levels doubled in about 52% of patients in the treated group, and in more than 90% of patients in the control group ($P < .0005$). Furthermore, although 64% of patients in the control group required dialysis, only 33% of those in the epoetin group had to start dialysis ($P < .005$).

The third study was published in 2004.[81] Eighty-eight patients with nondiabetic nephropathy, proteinuria of less than 2 g/d, and hemoglobin concentration between 9 and 11.6 g/dL were allocated randomly to early (n = 45) or late (n = 43) treatment with ESA. Patients included in the former group received epoetin to increase their hemoglobin concentration to more than 13 g/dL. Those included in the latter group did not receive epoetin until their hemoglobin concentration decreased to less than 9 g/dL. Treatment with an angiotensin-converting enzyme inhibitor was not permitted during the study. After a median follow-up period of 22.5 months, significantly more patients reached a combined end point of doubling of serum creatinine level, end-stage renal disease, or death in the late treatment group (23 versus 13, $P < .01$). Similar results were observed when the combined end point comprised only end-stage renal disease or death (22 versus 13, $P = .01$).

In conclusion, the hypothesis that correction of anemia with ESA may slow the progression of renal failure is plausible biologically, and results obtained from analyses of cohorts of CKD patients and from small clinical trials suggest that it is worth being tested in a large prospective study. In our opinion, this study should include patients with moderate anemia, treated according to current guidelines, and assigned randomly to receive or not to receive a treatment with ESA to normalize their hemoglobin levels. The effect of early correction of anemia on the progression of chronic kidney disease (ECAP) study, which had a similar design, has shown that such a study is feasible and probably is safe.[82]

References

1. Astor BC, Muntner P, Levin A, et al: Association of kidney function with anemia: The Third National Health and Nutrition Examination Survey (1988-1994). Arch Intern Med 162:1401-1408, 2002
2. Grune T, Sommerburg O, Siems WG: Oxidative stress in anemia. Clin Nephrol 53:S18-S22, 2000
3. Brenner BM, Meyer TW, Hostetter TH: Dietary protein intake and the progressive nature of kidney disease: The role of hemodynamically mediated glomerular injury in the pathogenesis of progressive glomerular sclerosis in aging, renal ablation, and intrinsic renal disease. N Engl J Med 307:652-659, 1982
4. Dworkin LD, Hostetter TH, Rennke HG, et al: Hemodynamic basis for glomerular injury in rats with desoxycorticosterone-salt hypertension. J Clin Invest 73:1448-1461, 1984
5. Anderson S, Meyer TW, Rennke HG, et al: Control of glomerular hypertension limits glomerular injury in rats with reduced renal mass. J Clin Invest 76:612-619, 1985
6. Zatz R, Meyer TW, Rennke HG, et al: Predominance of hemodynamic rather than metabolic factors in the pathogenesis of diabetic glomerulopathy. Proc Natl Acad Sci U S A 82:5963-5967, 1985
7. Remuzzi A, Mazerska M, Gephardt GN, et al: Three-dimensional analysis of glomerular morphology in patients with subtotal nephrectomy. Kidney Int 48:155-162, 1995
8. Nath KA: Tubulointerstitial changes as a major determinant in the progression of renal damage. Am J Kidney Dis 20:1-17, 1992
9. Mackensen-Haen S, Eissele R, Bohle A: Contribution on the correlation between morphometric parameters gained from the renal cortex and renal function in IgA nephritis. Lab Invest 59:239-244, 1988
10. Wehrmann M, Bohle A, Bogenschutz O, et al: Long-term prognosis of chronic idiopathic membranous glomerulonephritis. An analysis of 334 cases with particular regard to tubulo-interstitial changes. Clin Nephrol 31:67-76, 1989
11. Bogenschutz O, Bohle A, Batz C, et al: IgA nephritis: On the importance of morphological and clinical parameters in the long-term prognosis of 239 patients. Am J Nephrol 10:137-147, 1990
12. Wehrmann M, Bohle A, Held H, et al: Long-term prognosis of focal sclerosing glomerulonephritis. An analysis of 250 cases with particular regard to tubulointerstitial changes. Clin Nephrol 33:115-122, 1990
13. Mackensen-Haen S, Bader R, Grund KE, et al: Correlations between renal cortical interstitial fibrosis, atrophy of the proximal tubules and impairment of the glomerular filtration rate. Clin Nephrol 15:167-171, 1981
14. Marcussen N: Atubular glomeruli and the structural basis for chronic renal failure. Lab Invest 66:265-284, 1992
15. Gandhi M, Olson JL, Meyer TW: Contribution of tubular injury to loss of remnant kidney function. Kidney Int 54:1157-1165, 1998
16. Yamamoto K, Tomita N, Yoshimura S, et al: Hypoxia-induced renal epithelial cell death through caspase-dependent pathway: Role of Bcl-2, Bcl-xL and Bax in tubular injury. Int J Mol Med 14:633-640, 2004
17. Hotter G, Palacios L, Sola A: Low O2 and high CO2 in LLC-PK1 cells culture mimics renal ischemia-induced apoptosis. Lab Invest 84:213-220, 2004
18. Cachat F, Lange-Sperandio B, Chang AY, et al: Ureteral obstruction in neonatal mice elicits segment-specific tubular cell responses leading to nephron loss. Kidney Int 63:564-575, 2003
19. Khan S, Cleveland RP, Koch CJ, et al: Hypoxia induces renal tubular epithelial cell apoptosis in chronic renal disease. Lab Invest 79:1089-1099, 1999
20. Beeri R, Symon Z, Brezis M, et al: Rapid DNA fragmentation from hypoxia along the thick ascending limb of rat kidneys. Kidney Int 47:1806-1810, 1995
21. Allen J, Winterford C, Axelsen RA, et al: Effects of hypoxia on morphological and biochemical characteristics of renal epithelial cell and tubule cultures. Ren Fail 14:453-460, 1992
22. Remuzzi G, Bertani T: Pathophysiology of progressive nephropathies. N Engl J Med 339:1448-1456, 1998
23. Eddy AA, Giachelli CM: Renal expression of genes that promote interstitial inflammation and fibrosis in rats with protein-overload proteinuria. Kidney Int 47:1546-1557, 1995
24. Fine LG, Ong AC, Norman JT: Mechanisms of tubulo-interstitial injury in progressive renal diseases. Eur J Clin Invest 23:259-265, 1993
25. Eckardt K-U, Bernhardt WM, Weidemann A, et al: Role of hypoxia in the pathogenesis of renal disease. Kidney Int 68:S46-S51, 2005
26. Welch WJ, Baumgartl H, Lubbers D, et al: Nephron pO2 and renal oxygen usage in the hypertensive rat kidney. Kidney Int 59:230-237, 2001
27. Lubbers DW, Baumgartl H: Heterogeneities and profiles of oxygen

pressure in brain and kidney as examples of the pO2 distribution in the living tissue. Kidney Int 51:372-380, 1997
28. Zhang W, Edwards A: Oxygen transport across vasa recta in the renal medulla. Am J Physiol 283:H1042-H1055, 2002
29. Brezis M, Rosen S: Hypoxia of the renal medulla—its implications for disease. N Engl J Med 332:647-655, 1995
30. Matsumoto M, Tanaka T, Yamamoto T, et al: Hypoperfusion of peritubular capillaries induces chronic hypoxia before progression of tubulointerstitial injury in a progressive model of rat glomerulonephritis. J Am Soc Nephrol 15:1574-1581, 2004
31. Manotham K, Tanaka T, Matsumoto M, et al: Evidence of tubular hypoxia in the early phase in the remnant kidney model. J Am Soc Nephrol 15:1277-1288, 2004
32. Izuhara Y, Nangaku M, Inagi R, et al: Renoprotective properties of angiotensin receptor blockers beyond blood pressure lowering. J Am Soc Nephrol 16:3631-3641, 2005
33. Tanaka T, Miyata T, Inagi R, et al: Hypoxia in renal disease with proteinuria and/or glomerular hypertension. Am J Pathol 165:1979-1992, 2004
34. Tsai AG, Johnson PC, Intaglietta M: Oxygen gradients in the microcirculation. Physiol Rev 83:933-963, 2003
35. Ries M, Basseau F, Tyndal B, et al: Renal diffusion and BOLD MRI in experimental diabetic nephropathy. Blood oxygen level-dependent. J Magn Reson Imaging 17:104-113, 2003
36. Aiello S, Remuzzi G, Noris M: Nitric oxide/endothelin balance after nephron reduction. Kidney Int Suppl 65:S63-S67, 1998
37. Ohashi R, Shimizu A, Masuda Y, et al: Peritubular capillary regression during the progression of experimental obstructive nephropathy. J Am Soc Nephrol 13:1795-1805, 2002
38. Ohashi R, Kitamura H, Yamanaka N: Peritubular capillary injury during the progression of experimental glomerulonephritis in rats. J Am Soc Nephrol 11:47-56, 2000
39. Kang DH, Hughes J, Mazzali M, et al: Impaired angiogenesis in the remnant kidney model: II. Vascular endothelial growth factor administration reduces renal fibrosis and stabilizes renal function. J Am Soc Nephrol 12:1448-1457, 2001
40. Bohle A, Mackensen-Haen S, Wehrmann M: Significance of postglomerular capillaries in the pathogenesis of chronic renal failure. Kidney Blood Press Res 19:191-195, 1996
41. Choi YJ, Chakraborty S, Nguyen V, et al: Peritubular capillary loss is associated with chronic tubulointerstitial injury in human kidney: Altered expression of vascular endothelial growth factor. Hum Pathol 31:1491-1497, 2000
42. Kang DH, Kanellis J, Hugo C, et al: Role of the microvascular endothelium in progressive renal disease. J Am Soc Nephrol 13:806-816, 2002
43. Crawford SE, Stellmach V, Murphy-Ullrich JE, et al: Thrombospondin-1 is a major activator of TGF-beta1 in vivo. Cell 93:1159-1170, 1998
44. Kang DH, Joly AH, Oh SW, et al: Impaired angiogenesis in the remnant kidney model: I. Potential role of vascular endothelial growth factor and thrombospondin-1. J Am Soc Nephrol 12:1434-1447, 2001
45. Hugo C, Shankland SJ, Pichler RH, et al: Thrombospondin 1 precedes and predicts the development of tubulointerstitial fibrosis in glomerular disease in the rat. Kidney Int 53:302-311, 1998
46. Tanaka T, Hanafusa N, Ingelfinger JR, et al: Hypoxia induces apoptosis in SV40-immortalized rat proximal tubular cells through the mitochondrial pathways, devoid of HIF1-mediated upregulation of Bax. Biochem Biophys Res Commun 309:222-231, 2003
47. Lieberthal W, Menza SA, Levine JS: Graded ATP depletion can cause necrosis or apoptosis of cultured mouse proximal tubular cells. Am J Physiol 274:F315-F327, 1998
48. Feldenberg LR, Thevananther S, del Rio M, et al: Partial ATP depletion induces Fas- and caspase-mediated apoptosis in MDCK cells. Am J Physiol 276:F837-F846, 1999
49. Lieberthal W, Levine JS: Mechanisms of apoptosis and its potential role in renal tubular epithelial cell injury. Am J Physiol 271:F477-F488, 1996
50. He C, Zalups RK, Henderson DA, et al: Molecular analysis of spontaneous glomerulosclerosis in Os/+ mice, a model with reduced nephron mass. Am J Physiol 269:F266-F273, 1995
51. Nir A, Clavell AL, Heublein D, et al: Acute hypoxia and endogenous renal endothelin. J Am Soc Nephrol 4:1920-1924, 1994
52. Higgins DF, Biju MP, Akai Y, et al: Hypoxic induction of Ctgf is directly mediated by Hif-1. Am J Physiol 287:F1223-F1232, 2004
53. Orphanides C, Fine LG, Norman JT: Hypoxia stimulates proximal tubular cell matrix production via a TGF-beta1-independent mechanism. Kidney Int 52:637-647, 1997
54. Manotham K, Tanaka T, Matsumoto M, et al: Transdifferentiation of cultured tubular cells induced by hypoxia. Kidney Int 65:871-880, 2004
55. Zavadil J, Cermak L, Soto-Nieves N, et al: Integration of TGF-beta/Smad and Jagged1/Notch signalling in epithelial-to-mesenchymal transition. EMBO J 23:1155-1165, 2004
56. Modlinger PS, Wilcox CS, Aslam S: Nitric oxide, oxidative stress, and progression of chronic renal failure. Semin Nephrol 24:354-365, 2004
57. Eddy AA: Interstitial fibrosis in hypercholesterolemic rats: Role of oxidation, matrix synthesis, and proteolytic cascades. Kidney Int 53:1182-1189, 1998
58. Meng S, Cason GW, Gannon AW, et al: Oxidative stress in Dahl salt-sensitive hypertension. Hypertension 41:1346-1352, 2003
59. Beswick RA, Zhang H, Marable D, et al: Long-term antioxidant administration attenuates mineralocorticoid hypertension and renal inflammatory response. Hypertension 37:781-786, 2001
60. Susztak K, Raff AC, Schiffer M, et al: Glucose-induced reactive oxygen species cause apoptosis of podocytes and podocyte depletion at the onset of diabetic nephropathy. Diabetes 55:225-233, 2006
61. Budisavljevic MN, Hodge L, Barber K, et al: Oxidative stress in the pathogenesis of experimental mesangial proliferative glomerulonephritis. Am J Physiol 285:F1138-F1148, 2003
62. Nath KA, Grande J, Croatt A, et al: Redox regulation of renal DNA synthesis, transforming growth factor-beta1 and collagen gene expression. Kidney Int 53:367-381, 1998
63. Thannickal VJ, Fanburg BL: Reactive oxygen species in cell signaling. Am J Physiol 279:L1005-L1028, 2000
64. Sung FL, Zhu TY, Au-Yeung KK, et al: Enhanced MCP-1 expression during ischemia/reperfusion injury is mediated by oxidative stress and NF-kappaB. Kidney Int 62:1160-1170, 2002
65. Satriano JA, Shuldiner M, Hora K, et al: Oxygen radicals as second messengers for expression of the monocyte chemoattractant protein, JE/MCP-1, and the monocyte colony-stimulating factor, CSF-1, in response to tumor necrosis factor-alpha and immunoglobulin G. Evidence for involvement of reduced nicotinamide adenine dinucleotide phosphate (NADPH)-dependent oxidase. J Clin Invest 92:1564-1571, 1993
66. Iglesias-De La Cruz MC, Ruiz-Torres P, Alcami J, et al: Hydrogen peroxide increases extracellular matrix mRNA through TGF-beta in human mesangial cells. Kidney Int 59:87-95, 2001
67. Rhyu DY, Yang Y, Ha H, et al: Role of reactive oxygen species in TGF-beta1-induced mitogen-activated protein kinase activation and epithelial-mesenchymal transition in renal tubular epithelial cells. J Am Soc Nephrol 16:667-675, 2005
68. Geesin JC, Hendricks LJ, Gordon JS, et al: Modulation of collagen synthesis by growth factors: The role of ascorbate-stimulated lipid peroxidation. Arch Biochem Biophys 289:6-11, 1991
69. Geesin JC, Hendricks LJ, Falkenstein PA, et al: Regulation of collagen synthesis by ascorbic acid: Characterization of the role of ascorbate-stimulated lipid peroxidation. Arch Biochem Biophys 290:127-132, 1991
70. Houglum K, Brenner DA, Chojkier M: d-alpha-tocopherol inhibits collagen alpha 1(I) gene expression in cultured human fibroblasts. Modulation of constitutive collagen gene expression by lipid peroxidation. J Clin Invest 87:2230-2235, 1991
71. Allen DA, Harwood S, Varagunam M, et al: High glucose-induced oxidative stress causes apoptosis in proximal tubular epithelial cells and is mediated by multiple caspases. FASEB J 17:908-910, 2003
72. Martindale JL, Holbrook NJ: Cellular response to oxidative stress: Signaling for suicide and survival. J Cell Physiol 192:1-15, 2002

73. Zou A-P, Li N, Cowley AW Jr: Production and actions of superoxide in the renal medulla. Hypertension 37:547-553, 2001
74. Brenner BM, Cooper ME, de Zeeuw D, et al: Effects of losartan on renal and cardiovascular outcomes in patients with type 2 diabetes and nephropathy. N Engl J Med 345:861-869, 2001
75. Keane WF, Brenner BM, de Zeeuw D, et al: The risk of developing end-stage renal disease in patients with type 2 diabetes and nephropathy: The RENAAL study. Kidney Int 63:1499-1507, 2003
76. Rossing K, Christensen PK, Hovind P, et al: Progression of nephropathy in type 2 diabetic patients. Kidney Int 66:1596-1605, 2004
77. Ravani P, Tripepi G, Malberti F, et al: Asymmetrical dimethylarginine predicts progression to dialysis and death in patients with chronic kidney disease: A competing risks modeling approach. J Am Soc Nephrol 16:2449-2455, 2005
78. Iseki K, Ikemiya Y, Iseki C, et al: Haematocrit and the risk of developing end-stage renal disease. Nephrol Dial Transplant 18:899-905, 2003
79. Roth D, Smith RD, Schulman G, et al: Effects of recombinant human erythropoietin on renal function in chronic renal failure predialysis patients. Am J Kidney Dis 24:777-784, 1994
80. Kuriyama S, Tomonari H, Yoshida H, et al: Reversal of anemia by erythropoietin therapy retards the progression of chronic renal failure, especially in nondiabetic patients. Nephron 77:176-185, 1997
81. Gouva C, Nikolopoulos P, Ioannidis JP, et al: Treating anemia early in renal failure patients slows the decline of renal function: A randomized controlled trial. Kidney Int 66:753-760, 2004
82. Rossert J, Levin A, Roger SD, et al: Effect of early correction of anemia on the progression of CKD. Am J Kidney Dis 47:738-750, 2006