Molecular biology of diabetic glomerulosclerosis

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Abstract. Diabetic nephropathy is one of the leading causes of renal failure in Western countries, where diabetic patients account for nearly half of all patients on haemodialysis. Progressive expansion of the mesangial matrix, and thickening of the glomerular and tubular basement membranes without signs of major cell proliferation are hallmarks of human and experimental diabetic nephropathy. These lesions eventually lead to glomerular fibrosis, a central pathological feature in many human acute and chronic kidney diseases, which progressively destroys the renal filtration unit, and may finally cause renal failure. Indeed, structure–function relationship studies have shown that mesangial matrix expansion is strongly related to the clinical manifestation of diabetic nephropathy.

Key words: angiotensin II; collagen IV; metalloproteinases; transforming growth factor-β; vascular endothelial growth factor

Expansion of mesangial matrix and glomerulosclerosis in diabetic nephropathy

The renal matrix alterations typical of diabetes are the result of increased synthesis and deposition of matrix components, such as collagen III, IV and VI, tenascin and EDA+fibronectin [1,2]; enhanced collagen production, in particular, seems to be a central event in diabetic glomerular matrix expansion, and type IV collagen is one of the earliest matrix proteins involved [3]. Indeed, increased synthesis of type IV collagen, as deduced by the overexpression of its gene, has been demonstrated in diabetic nephropathy by all renal cells, i.e. glomerular mesangial and epithelial cells, and tubular epithelial cells, without any peculiar cell tropism [4]. However, the regulation of mesangial matrix deposition is dynamic, and involves not only synthetic, but also degrading processes. The degradation of extracellular mesangial matrix occurs through the activity of glomerular matrix metalloproteinases (MMPs). The hypothesis of abnormal matrix degradation in diabetic nephropathy has been addressed by experimental studies which demonstrated the inhibition of the degrading wing of matrix turnover; this possibly leads to deranged extracellular matrix (ECM) homeostasis, expansion of the mesangial matrix and glomerulosclerosis [5,9]. Human data in vivo are very similar to experimental findings, as the glomerular MMP2 gene is down-regulated in human diabetes. However, no relationship seems to exist between MMP2 gene expression and the severity of the renal diabetic disease. In fact, MMP2 is down-regulated, while TIMP2 (its specific inhibitor) is still expressed in glomeruli even in patients without any clinical and histological sign of diabetic nephropathy [10]. Thus, the failure of the degrading wing, at least of MMP2, might be a necessary, although not a sufficient condition for the pathogenesis of diabetic nephropathy.

Growth factors and glomerulosclerosis in diabetic nephropathy

Unlike most nephropathies in which renal destruction is associated with progressive renal atrophy, hypertension shows long-term persistence in human diabetes even when an initial reduction in glomerular filtration is observed and microalbuminuria appears. Moreover, diabetic nephropathy is characterized by the expansion of the mesangial matrix, and also by glomerular sclerosis. A role for growth factors in these lesions is generally recognized. In the diabetic kidney, therefore, it is clear that important growth control changes might exist, and be related to the pathogenesis of the disease itself; a role for growth factors may thus be advanced [11].

Shortly following the onset of diabetes in the streptozotocin rat model of diabetes as well as the spontaneous disease in man, glomerular enlargement and hypertrophy, proximal tubular hyperplasia and nephromegaly occur. In the streptozotocin model, other very early events take place in the glomeruli, i.e. platelet-derived growth factor (PDGF), basic fibroblast growth factor (bFGF) and transforming growth factor-β...
nephropathy are well known, and it was suggested that in diabetes mellitus. To date, only two studies have cure diabetic proliferative retinopathy. Endothelial binding of VEGF due to the altered sulfation of be overwhelming, and many experimental approaches molecular species, abnormalities in the expression / growth associated with proliferative retinopathy [17]. VEGF gene, leading to the de novo mitogen for vascular endothelial cells due to the pres- should be noted that this simple scheme does not

VEGF and diabetic nephropathy

Vascular endothelial growth factor (VEGF) is a potent mitogen for vascular endothelial cells due to the presence of specific receptors found only in these cells. VEGF is a major regulator of physiological angiogenesis, but it is also a crucial mediator of blood vessel growth associated with proliferative retinopathy [17]. Its pathogenetic role in diabetic retinopathy seems to be overwhelming, and many experimental approaches to limit its activity currently are being investigated to cure diabetic proliferative retinopathy. Endothelial dysfunctions which are associated with diabetic nephropathy are well known, and it was suggested that they might have a pathogenetic role. VEGF is an attractive candidate to function as a mediator of endothelial dysfunctions in diabetes. Indeed, a number of similarities exist between diabetic retinopathy and nephropathy, thus supporting a role for VEGF in both. The loss of smooth muscle-like cells (pericytes in the retina, and mesangial cells in the glomerulus) has been reported both in retinopathy and in nephro- pathy [18,19]. Furthermore, mesangiolysis in diabetic glomeruli seems to play a role in the formation of microaneurysms [19], whose presence in retinopathy is typical. Neoangiogenesis is also a common feature of the two long-term complications of diabetes mellitus. Indeed, blood vessel growth has been observed in the kidney in both the experimental streptozotocin model [20] and the human disease [21]. Increased vessel permeability, well known in the diabetic retina, might have its own counterpart in the microalbuminuria of diabetes, at least at the beginning. With this in mind, it is interesting to note that the same protein kinase C (PKC) β-isoform-selective inhibitor is capable of suppressing both the VEGF-induced retinal permeability [22] and early albuminuria in a rat diabetic model [23].

How VEGF might play a role in the pathogenesis of diabetic nephropathy is still a matter of speculation. Under physiological conditions, VEGF is produced in the kidney by glomerular epithelial cells; mesangial and tubular epithelial cells apparently do not normally produce this growth factor, and it was suggested that VEGF expression by mesangial cells in vitro is related to hypoxic culture conditions [24]. However, vascular smooth muscle cells grown in high glucose medium recently were demonstrated to overexpress VEGF through PKC activation, thus raising the possibility that similar cells, such as mesangial cells, might display a similar behaviour [25]. Interestingly, TGF-β, which is overexpressed in the diabetic kidney, also enhances VEGF expression [26]. Thus, there exist conditions in the diabetic kidney that potentially are capable of inducing VEGF overexpression. Hence, glomerular permeabilization by VEGF might induce both albuminuria and an increased mesangial traffic of growth factors from the circulating blood. Growth factors might also originate from activated endothelial cells due to the mitogenic activity of VEGF. Growth factors might then induce matrix synthesis by mesangial cells, leading to glomerulosclerosis. Although this pathogenetic scheme (Figure 1) is purely speculative, it is supported by the observation of similar events in experimental models of vessel wall damage [27,28]. It should be noted that this simple scheme does not consider other aspects of VEGF biology that might also have a special role in diabetic nephropathy, e.g. abnormal regulation in the alternative splicing of the VEGF gene, leading to the de novo appearance or disproportionate abundance in diabetes of some VEGF molecular species, abnormalities in the expression/function of VEGF receptors and abnormal matrix binding of VEGF due to the altered sulfation of extracellular proteoglycans, a well-known phenomenon in diabetes mellitus. To date, only two studies have
In general, conflicting results have been obtained, which are likely to mirror this important homeostatic system's high degree of versatility. The most consistent finding is the down-regulation of angiotensin II receptors in glomeruli and tubules of diabetic rats [32,33], which suggests a chronic, local overproduction of angiotensin II and a negative feedback that decreases receptor expression. While clinical and experimental data support the existence of an unfavourable interaction between diabetes and RAS, the way in which this interaction occurs is not clear. Many observations demonstrate that angiotensin II-mediated signal transduction may be diminished rather than augmented, as expected, in the presence of high glucose [34,35]. However, the finding that angiotensin II stimulates glucose uptake and the transcription of the glucose transporter GLUT-1 in a number of different cells [36,37] raises the intriguing possibility that in diabetic patients at risk for diabetic nephropathy, a primitive increased RAS activity at the level of the kidney, due to a particular genetic background for instance, may lead to much higher intracellular concentrations of glucose compared to diabetics without diabetic nephropathy. The consequence of such a phenomenon is self-evident, as a number of cell abnormalities believed to lead to diabetic nephropathy are related to the intracellular, rather than the extracellular glucose concentrations.

Moreover, an additive mechanism between RAS and high glucose may be proposed; both apply similar or parallel signal transduction pathways in cultured renal cells. Activation of PKC in renal cells is typical of diabetes and high glucose concentration [38], and is important in the high glucose-mediated expression of fibronectin, collagen IV and TGF-β in renal cells [31]. Angiotensin II also activates PKC in mesangial and tubular cells through AT1 receptors [39]. It is therefore possible that hyperglycaemia and angiotensin II exert additive effects on PKC activation, and subsequent target phosphorylations. Indeed, the additive effects of these conditions on the hypertrophy of proximal tubular cells have been demonstrated [40].

**The renin–angiotensin system (RAS) and diabetic nephropathy**

The angiotensin-converting enzyme inhibitors decrease the progression of diabetic nephropathy and reduce proteinuria by mechanisms that can be attributed only in part to an effective control of systemic hypertension. This has raised the hypothesis that an increased activity of the RAS and synthesis of angiotensin II might play a role in the initiation and progression of diabetic nephropathy by non-haemodynamic mechanisms. Angiotensin II has many non-haemodynamic effects on renal cells that may contribute to the progression of diabetic nephropathy. Angiotensin II may truly act as a growth factor and a profibrogenic peptide, and it induces hypertrophy and/or proliferation of glomerular and tubular cells, stimulates the synthesis of collagen and fibronectin, inhibits the synthesis of perlecan, and lastly reduces ECM turnover [31]. However, although a consistent number of investigations have evaluated the systemic and renal expression of RAS components in diabetes, the effects of diabetes on the expression of each single component are still not fully understood.
glomerulosclerosis [41]. The recent demonstration that angiotensin II up-regulates the expression of TGF-β receptors is a further intriguing part of this story [43]. By increasing the sensitivity of mesangial cells to TGF-β, angiotensin II might be involved in the initiation of the TGF-β autoinduction loop and, thus, might play an outstanding role in the derangement of the switch-off mechanism which putatively is involved in the healing process of renal lesions. Such a derangement would promote lesion progression toward a chronic, fibrotic disease (glomerulosclerosis), rather than remodelling to the original anatomo-functional structure [44].

The extension of the hypothesis that TGF-β is the missing link between RAS and glomerulosclerosis, with PKC occupying the convergence step between angiotensin II and high glucose-dependent pathways to diabetic nephropathy, is a logical development (Figure 2). However, it is not known whether intracellular high glucose and angiotensin II share the same intracellular signalling cascade (i.e. similar or different PKC isoforms) in TGF-β gene induction, although it was demonstrated in glomerular epithelial cells that at least some effects are distinct, thus suggesting different pathways [45]. Furthermore, there is no perfect overlap between the biological effects of angiotensin II and TGF-β on mesangial cells in vitro, as clearly shown by differential effects on perlecan production [46].

The proposed model for the interaction between high glucose, angiotensin II and TGF-β is, therefore, somewhat simplistic, but the relevance of its comprehension is not merely speculative. It may offer the opportunity to discern additional pharmacological targets for the treatment of diabetic nephropathy. As clearly shown by the previous exceptions to the proposed model [45,46], full intervention in the different events triggered by high glucose, angiotensin II and TGF-β in diabetes mellitus most likely requires drugs that act on different pathogenetic pathways. Therefore, the search for ancillary therapy [4] for diabetic nephropathy that could be added to metabolic control and RAS modulation seems warranted.

**Functional interplay between growth factors**

The interaction between angiotensin II and TGF-β is a clear example of the complexity and integration of the pathogenetic network causing diabetic glomerulosclerosis. Different pathogenetic levels may be recognized: metabolic irregularity; genetic susceptibility; haemodynamic components; the autocrine–paracrine network, etc. Furthermore, each of these levels may be even more complex. Considering the autocrine–paracrine network for example, a pivotal role in diabetic nephropathy most likely can be ascribed to TGF-β, but perhaps also to VEGF. However, the levels of expression or activities of the single growth factors, and consequently the biological effects, mutually interfere. In other words, we have to see the autocrine–paracrine pathogenic level as an integrated network. No well-designed investigation has addressed this problem in diabetes or in the “high glucose” milieu but, in different experimental settings, it has been demonstrated that the hypertrophic vs hyperplastic potential of angiotensin II depends on the level of TGF-β in proximal tubular cells [47], and that the mitogenic activity of angiotensin II on smooth muscle cells is partly dependent on the induction of PDGF and TGF-β synthesis [48]. As a further example, in experimental mesangio-proliferative glomerulonephritis induced by anti-Thy 1.1 serum, glomerular sclerosis is prevented by anti-TGF-β [49], anti-PDGF [50] and anti-bFGF [51] antibodies, along with heparin [52]. Thus, it is possible to prevent sclerosis by acting on different sections of the same, complex pathogenetic network. The recognized genetic background of diabetic nephropathy possibly also constitutes another level of this complex network.

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