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New predictive Biomarkers and novel therapeutic target in Diabetic Nephropathy

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Diabetic nephropathy (DN) is the most important cause of end-stage renal disease (ESRD) and a main factor of diabetes (DM) – related morbidity and mortality. However, its pathophysiological underlying mechanisms remain unclear. Podocyte injury and/or apoptosis is recognized as a hallmark of the renal disease process characterized by failure of the filtration barrier. Hyperglycaemia-mediated podocyte apoptosis and podocyte depletion occurs in animal and human models of both type 1 and type 2 DM. Podocytopenia is present at the early stages of both type 1 and type 2 DM. The coexistence of glomerular basement membrane (GBM) expansion and hyperglycaemia-induced podocyte injury and enhanced apoptosis leads to a marked increase in membranepermeability, thus predisposing to the development of diabetic albuminuria. The diagnosis of DN is traditionally based on the presence of micro-albuminuria (MA). MA has been used indicating the progression of chronic kidney disease (CKD), but it could also cause renal damage in patients with CKD. Further, there is accumulating evidence that proteinuria is an independent risk factor for cardiovascular disease (CVD) as well. Several recent studies have reported these observations suggesting the link between proteinuria, CKD and CVD. However, MA is not an adequate predictor of DN in young or in patients without albuminuria and additional biomarkers of glomerular and tubular injury have been proposed to denude structural lesions of early renal disfunction before the presence of MA. New predictive biomarkers would expose patients at the initial stages of DN, those who will progress to the ESRD and provide preventive and therapeutic interventions of irreversible longterm complications. Biomarkers of glomerular injury, tubular injury, inflammation and oxidative stress precede albuminuria in DN patients, also overlapping each other classification. Glomerular biomarkers include immunoglobulin G (IgG 4, IgG 2 isoforms), ceruloplasmin, collagen type IV (col-IV), laminin, glycosaminoglycans (GAGs), lipocalin-type prostaglandin D synthase (L-PGDS), fibronectin, podocytes-podocalyxin, and vascular endothelial growth factor (VEGF). Podocalyxin and VEGF are essentially considered as podocyte biomarkers. Laminin and col-IV are components of GBM, although the later is also a component of mesangial matrix.

Newer approaches include urinary microRNAs which are short noncoding mRNAs that regulate gene expression and urine proteomics, highlighting a possible role for epigenetic factors in the development of the disease.

Tubular biomarkers have shown that tubular dysfunction can be present early in DN and are early predictors of DN compared to microalbuminuria and other glomerular biomarkers. This category includes neutrophil gelatinase-associated lipocalin (NGAL), α-1-microglobulin, kidney injury molecule 1 (KIM-1), N-acetyl-β-D-glucosaminidase (NAG), cystatin C, and liver-type fatty acid-binding protein (L-FABP).

Biomarkers of inflammation such as TNF-α, IL-1β, IL-6 and IL-18, are involved in the onset and progression with predictive roles in DN. Other biomarkers of inflammation, which are also glomerular markers, include interferon gamma-induced protein (IP-10), monocyte chemoattractant protein 1 (MCP-1), granulocyte colony-stimulating factor (G-CSF), eotaxins, RANTES (regulated on activation, normal T cell expressed and secreted) or Chemokine ligand-5 (CCL-5), and orosomucoid.

Biomarkers of oxidative stress are urinary 8-oxo-7,8-dihydro-2-deoxyguanosine (8oHdG), lipid peroxides, malondialdehyde (MDA) and superoxide dismutase (SOD). The marker of 8oHdG is produced secondary to oxidative DNA damage, and appears in the urine without being metabolized. Biomarkers of fibrosis are col-IV, fibronectin, transforming growth factor β (TGF-β1), matrix metalloproteinase - 2 (MMP-2), tissue inhibitor of metalloprotease-1 (TIMP-1) with pathological accumulation in the glomerulus and tubulointerstitial space significantly associated with renal outcomes in diabetic patients. New therapeutic aspects have shown protective affects against renal fibrosis in a mouse model of type 2 diabetes, including reduction in glomerular col-IV.

Graphical Abstract

Matrix Gelatinases (MMP-2 and -9), TGF-β1, VEGF-A, TIMP-1 and -2, FGF-23, Col-IV in Atherosclerosis – Inflammation – Fibrosis of Diabetic Nephropathy and disease process with Albuminuria: Progress and Challenges.

DN represents an example of the link between progressive glomerulosclerosis and MMP expression. In vitro studies high glucose levels was associated with an increased expression of matrix molecules, whereas the activity of MMPs, namely MMP-2 and -9, was decreased in mesangial cells. In general, down-regulation of MMPs' expression has been associated with the progression of renal dysfunction to CKD in non-inflammatory diseases such as DN. There is a link between intrarenal dysregulation of MMP activity and the development of DN. Pro-inflammatory cytokines have also been associated with DN via theregulation of MMP expression. In details, cytokines such as IL-1, IL-6 and TNF-α stimulate MMP production, whereas factors such as TGF-β, corticoid hormone and insulin-like growth factor (IGF) down-regulate MMP synthesis. A role for glucose and advanced glycation end-products (AGEs) in the regulation of MMP expression have also been demonstrated. Altered MMP expression or activation contributes to DN, and especially to the onset of this characteristic renal hypertrophy, as abnormal extracellular matrix (ECM) deposition is the hallmark of DN. Apart from the direct effect of MMPs on ECM turnover, MMPs may also release and activate several growth factors that have been associated with renal hypertrophy, tubular

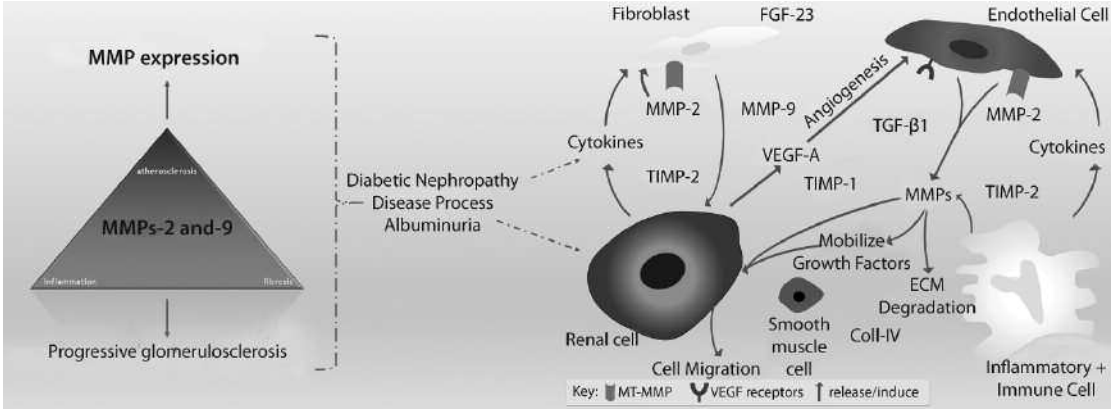

Figure 1

cell proliferation and renal scarring and fibrosis.

Both MMP-2 and -9 are the main enzymes that degrade col-IV, the major collagenous component of the ECM and the architectural structure of BM and GBM. BM is the part of the ECM that is associated with the vascular endothelium. MMP-2 over-expression in transgenic renal proximal tubular epithelium is sufficient to reflect the characteristic pathologic changes of CKD. Data from rodent models suggest a link between MMP-2 dysregulation and DN, but there are also controversial results. In such rodent models of DN the expression and proteolytic activity of MMP-2 in renal tissues was reduced and the activity of TIMP-2 was increased. In contrast, MMP-2 activity was elevated 3.8-6 fold in protein extracts of human diabetic kidney tissue samples. The increased circulating MMP-2 levels in diabetic patients may be explained by the confounding effects of diabetes treatment. For example, insulin can induce MMP-2 activity in rat glomerular mesangial cells (Figure 1).

Dysregulated activity of various growth factors and cytokines may contribute to the development of renal abnormalities in DN. Such growth factors involved in DN are TGF-β, VEGF, connective tissue growth factor (CTGF), IGF, epidermal growth factor (EGF) and platelet derived growth factor (PDGF); TGF-β and VEGF are better known and more widely investigated. TGF-β inhibits MMPs and activates TIMPs. TGF-β also up-regulates integrins, the cell surface receptors for ECM, enhancing cellular ability to interact with specific matrix proteins.

VEGF, a key angiogenic factor, influencing the proliferation of endothelial cells, plays a pivotal role in vascular integrity and pathological angiogenesis and it has been implicated in DN. TGF-β through the signalling pathway of intracellular proteins that transduce extracellular signals, namely small mother against decapentaplegic (SMADs) also regulates VEGF.

New predictive biomarkers uncovering the initial stages of DN, even before MA occurs, would provide an opportunity for preventive and therapeutic interventions preventing or delaying the onset of irreversible long-term complications and improving outcomes, results in a reduction of the severe cardio-renal morbidity and mortality progress in diabetic kidney disease patients.

The future of Preventive Medicine development and drug discovery depends on the selection and validation of rapid, reliable, and quantitative assays of disease biomarkers.

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