The renal archaeologist: digging for clues in archived tissues to understand diabetic kidney disease

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Diabetic nephropathy is the leading cause of end-stage renal failure in the Western world; in the USA, ~40% of adults with diabetes have some degree of chronic kidney disease [1]. Current treatment strategies for diabetic nephropathy include lifestyle improvements (cessation of smoking and dietary modifications), glycaemic control, cholesterol management and reduction of blood pressure [1]. However, there is a clear need for new interventions to be developed; for this goal to be achieved, novel targets that can either predict whether an individual is susceptible to diabetic kidney disease and/or are involved in the pathogenesis of the disease need to be identified. In recent years, technological advances have allowed the discovery of several new candidate molecules that may be involved in the progression of diabetic nephropathy. Woroniecka et al. [2] performed microarray analysis to identify differentially expressed genes in the glomeruli and tubules of human diabetic kidneys versus healthy controls. Other investigators have utilized genome-wide association strategies to identify novel candidate genes which may make an individual susceptible to diabetic kidney disease [3, 4].

In this issue of Nephrology Dialysis Transplantation, Nakatani et al. [5] took an alternative unbiased approach utilizing mass spectrometry-based proteomics (Figure 1). Tissue sections from archived formalin-fixed paraffin-embedded (FFPE) kidneys were obtained from both diabetic patients with nephropathy and non-diabetic controls. The two groups of patients were age- and sex- matched with the diabetic group having elevated fasting plasma glucose, increased glycated haemoglobin levels and a reduction in estimated glomerular filtration rate. Glomeruli were specifically isolated from the tissue sections of ten diabetic and non-diabetic patients using laser-capture microdissection. Extracted proteins were digested in solution with trypsin, labelled using isobaric tags for relative and absolute quantification (iTRAQ) and then analysed using quantitative mass spectrometry. A total of 100 proteins were shown to be differentially expressed in diabetic patients with nephropathy compared to non-diabetics, 55 of which were up-regulated and 45 down-regulated. The majority of proteins found were either extracellular matrix (ECM) components or cell adhesion molecules [5]. Many of the identified proteins in this analysis have been previously implicated in diabetic kidney disease, including apolipoprotein E [6], collagen Type IV [7] fibronectin [7] and transglutaminase 2 [8]. However, several novel proteins were also found, one of which was nephronectin, which was up-regulated in the glomeruli of diabetic patients [5]. The authors confirmed this finding using immunohistochemistry that showed no nephronectin expression in the glomeruli of non-diabetic patients but up-regulation in the ECM of damaged diabetic glomeruli [5]. Moreover, in diabetic individuals, there was a significant positive correlation between nephronectin expression and the level of glomerulosclerosis determined by histological analysis [5].

Nephronectin is an ECM protein, which contains an Arg-Gly-Asp (RGD) motif and was first identified as a ligand for the cell adhesion receptor integrin αβ1 [9]. When ligands interact with integrin receptors, there is subsequent integrin activation, leading to outcomes including cell adhesion to the ECM, cell migration over ECM substrates and cell differentiation or proliferation [10]. Integrin signalling is also bidirectional where changes in the intracellular environment resulting in integrin activation lead to the assembly of their ECM ligands outside the cell [10]. During development, the expression of nephronectin is widespread, with localization observed in the kidney, parathyroid and thyroid glands, developing bone, inner ear and skin [11]. Several functions have been identified for nephronectin including involvement in osteoblast differentiation [12], piloerection [13] and heart development [14]. In the kidney, nephronectin plays a critical role in the early stages of development regulating epithelial–mesenchymal interactions; mice that lack nephronectin display renal agenesis and hyperplasia [15]. However, little is known about the function of nephronectin in the mature kidney or in renal disease; there is some evidence showing that it may have a role in acute tubular necrosis with nephronectin...
up-regulation in both regenerating tubular cells and the urine of mice administered uranyl nitrate [16].

The study by Nakatani et al. [5] provides the first evidence that nephronectin may play a role in the progression of diabetic nephropathy. One could envisage that elevated nephronectin alters the expression of integrin α8β1, which is expressed in glomerular mesangial cells [17]. In turn, this could have direct effects on mesangial cell biology; in vitro studies have shown that integrin α8 promotes cell adhesion but inhibits both migration and proliferation in cultured mesangial cells [18], all of which are important processes in diabetic kidney disease. Alternatively, alterations in integrin activity could lead to changes in ECM components and matrix deposition, which is a common pathological feature of diabetic glomerulopathy. A recent study indicated that lack of integrin α8 enhanced albuminuria and glomerulosclerosis in mice with experimental diabetes induced by streptozotocin [19].

However, many questions regarding the role of nephronectin in diabetic nephropathy remain unanswered. Firstly, Nakatani et al. [5] did not detect any changes in integrin α8β1 in diabetic patients by proteomic analysis and further experiments are needed to address this point. The study did report altered integrin α1 but prior studies have indicated that cells expressing this integrin do not bind to nephronectin, unlike those expressing α8 and αV integrins [9]. Secondly, it is currently unknown whether nephronectin levels are elevated in response to high glucose levels and furthermore, the cellular origins of nephronectin in the diabetic glomerulus are not established. In developing kidneys, nephronectin messenger RNA was localized to the branching epithelium [9]; therefore, it could be hypothesized that nephronectin is synthesized by podocytes in diabetic nephropathy and this could be confirmed by in situ hybridization studies. Finally, the proteomic analysis performed in the manuscript by Nakatani et al. [5] compared patients with diabetic kidney disease and non-diabetics. Therefore, the changes in protein expression observed may not only be induced by diabetes itself but also reflect enhanced renal lesions. To determine the protein changes in diabetic glomerulosclerosis alone, comparisons could be made between patients with diabetic nephropathy and those with diabetes but without kidney disease.

The results from the study by Nakatani et al. [5] indicate that an unbiased proteomic approach to assess FFPE tissue is a useful tool in the hunt for novel molecules involved in diabetic kidney disease; similar strategies have also been undertaken in the context of endometrial and renal carcinoma [20, 21]. The majority of pathologically characterized human archival tissue in hospitals and tissue banks is FFPE and it had been previously thought that the addition of formalin led to protein cross-linking and polymer formation causing the irreversible masking of native proteins preventing the use of this tissue in proteomic applications [22]. This issue has been addressed by the development of commercial kits, which allow the extraction of protein from fixed tissue [22]. Importantly, studies by Hood et al. [23] using mouse livers demonstrated that the quality of protein produced from formalin-fixed and frozen tissues is comparable.

There are several important limitations to performing proteomic analyses on fixed tissue. Firstly, unlike gene expression arrays, a substantial amount of starting material is required for the analysis. This limits the technique to autopsy material meaning further validation of biopsies from living patients is essential. In the study by Nakatani et al., an individual patient did not yield sufficient glomeruli for proteomic analysis and therefore, a more heterogeneous population consisting of ten non-diabetic and ten diabetic samples were pooled together with a total requirement of 1000 laser-capture microdissected glomeruli per group [5]. Although, this approach has the advantage of providing an overview of differentially expressed proteins in the disease condition, important changes may be overlooked due to variation between individuals. In the current study [5], proteomic analysis was also performed using one pooled sample from both the diabetic and non-diabetic groups. Mathematical models were utilized allowing the investigators to produce statistically significant results [24], but the data would have been strengthened if technical or biological replicates had been assessed. The sensitivity of proteomic approaches is also variable and depends on the instruments used for analysis. In the current study, 170 proteins were actually identified with 95% confidence and perhaps important molecular changes could have been missed; this may have been further compounded by the exclusion of initially insoluble material.

Overall, proteomic analysis of FFPE tissue may provide a helpful tool to identify new proteins such as nephronectin that may play a role in renal diseases such as diabetic nephropathy. Following the identification of new components by this unbiased approach, it is important that findings are validated using complimentary techniques and that further studies are designed to provide mechanistic insights.

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References


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