Opening a treasure chest: glomerular proteome analyses of formalin-fixed paraffin-embedded kidney tissue in the investigation of diabetic nephropathy

Andreas Blutke

Institute of Veterinary Pathology, Centre for Clinical Veterinary Medicine, Ludwig-Maximilians-Universitaet München, München, Germany

Correspondence and offprint requests to: Andreas Blutke; E-mail: blutke@patho.vetmed.uni-muenchen.de

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Worldwide, diabetic nephropathy (DN) persists as a major complication of diabetes mellitus (DM), affecting approximately one-third of DM patients [1]. DN is characterized by development of progressive glomerulosclerotic and tubulointerstitial kidney lesions gradually leading to an increasing loss of functioning kidney parenchyma [2]. As therapeutic intervention is frequently insufficient to prevent development of DN and its progression to end-stage renal
disease (ESRD), the numbers of DN patients requiring dialysis or transplantation are steadily rising. In the past decade, proteomic analyses have arisen as auspicious approaches to investigate the still not completely understood molecular mechanisms involved in the pathophysiology of DN and to identify diagnostic or predicting biomarkers [3,4]. Since glomerular damage denotes the initial event in the pathogenesis of DN and plays a pivotal role for its progression to ESRD [2], the glomerulus represents a particularly relevant analytical target of proteomic investigation of DN. However, compared to the total number of proteomic studies in DN research, only few proteomic investigations of isolated glomeruli of DN patients or experimental animal models of DM-related nephropathy have been published so far. Apart from the obvious biological significance, proteomic analysis of isolated glomeruli principally holds important advantages as compared to investigation of total kidney protein specimen. Using homogenous preparations of isolated glomeruli not only reduces the complexity and inconsistency of the resulting proteomic analysis datasets, as the detected protein abundance profiles can be exclusively assigned to glomerular cells and extracellular glomerular matrix and are thus a priori unbiased by extraglomerular renal tissue components, which might shift the abundance profiles of a considerable set of proteins in an unpredictable, unrecognized and ad hoc variable fashion. As well, detection of statistically significant abundance differences even of low abundant glomerular proteins is facilitated, which most probably would not be surveyed in more complex kidney tissue specimens. Today’s state-of-the-art proteomic analysis provides the most powerful technologies, e.g. ultrasensitive agents for differential protein labelling combined with two-dimensional gel electrophoresis (2-DE) or liquid chromatography (LC) for protein separation, subsequent protein/peptide identification by mass spectrometry (MS) and quantification [3,5,6]. Direct proteomic analysis of thin (frozen) tissue sections by imaging MS allows for spatially divided acquisition of proteomic data and their correlation to distinct morphological tissue structures in the investigated section [7]. Due to essential peculiarities inherent to methodological aspects of different proteomic analysis approaches, any proteomic technology will of course at best only be capable of detecting the presence and, ideally, the abundance of a subset of proteins present in a biological sample. Nevertheless, established proteomic technologies have proven their suitability for both qualitative and quantitative (differential) analyses even of minimal glomerular protein amounts [6,8]. Glomerular proteomic studies conducted on experimental animal models of DM-associated kidney alterations can take full advantage of well-established methods for reproducible isolation of large numbers of fresh glomeruli in high purities, e.g. by sieving or magnetic isolation of glomeruli after embolization with magnetic beads [9], to generate defined homogenous glomerulus preparations, representative for distinct stages of morphological or functional glomerular alteration [10-12]. Performance of glomerular proteomics on human specimens, however, has so far largely been restricted to glomerular samples isolated from fresh or frozen kidney tissue or biopsy material, using sieving/manual microdissection techniques or laser capture microdissection (LCM) for collection of glomerular tissue from frozen sections, respectively [8].

With the availability of these technologies, important factors limiting the performance of meaningful glomerular proteomic studies on human DN remain the acquisition of adequate numbers of homogenous and representative individual specimens, with glomerular protein quantities and qualities sufficient for the scheduled downstream proteomic analyses. Indeed, it is extremely difficult to address these requirements since the availability of fresh/frozen kidney biopsies of DN patients and suitable controls is generally limited, and ordinarily, there is a considerable heterogeneity within the collectives of biopsied DN patients concerning their age, disease history, premedication, co morbidades, etc. Considering the bulk of difficulties affecting a meaningful implementation of glomerular proteomic analyses of human DN, it is most tempting to use the vast archive of standardly formalin-fixed paraffin-embedded (FFPE) kidney tissues as an existing and practically unlimited tenable resource for acquisition of well-defined cohorts of glomerular tissue specimens for proteomic analyses. The technical prerequisites for the implementation of proteomic analysis approaches to FFPE glomerular tissues seem to be given. Recent advances in protein extraction from FFPE tissues, such as heat-mediated retrieval techniques, have particularly surmounted the difficulties associated with protein cross-linking caused by formalin fixation, which has hindered the applicability of proteomic analysis on FFPE samples for years [13]. Combined with highly sensitive methods for protein separation and identification, most commonly based on LC-MS/MS technologies, many studies have already applied proteomic analyses on various types of FFPE tissues [13]. Several reports have also evinced a partially surprisingly high degree of congruence concerning the numbers and identities of proteins detectable in FFPE tissue specimens and comparison samples of fresh/frozen tissue by identical proteomic analysis technologies [13,14]. Recently, performance of high-throughput proteomic analyses by matrix-assisted laser desorption/ionization—imaging MS has as well been made applicable to FFPE tissue sections, representing an interesting alternative to LCM for proteomic analysis of distinct tissue compartments in complex tissue specimens [15]. Despite these technical advances, proteomic analyses of FFPE tissues will yet often be affected adversely by the effects of post-sampling protein degradation and modification processes. Depending on the type of investigated tissue, time and temperature conditions after sample collection, sample processing and storage terms, the first detectable degradative changes of the proteome develop within only a few minutes after tissue sampling [16-18]. Archival FFPE tissues, especially those derived from autopsy cases with prolonged post-mortem intervals and inconsistent fixation conditions, will therefore exhibit variable degrees of proteomic degradation and modification, limiting the representativeness of proteomic data acquired in such specimens, as compared to the in vivo situation.

The present study by Nakatani et al. now provides the first report of a differential proteomic analysis of glomerular samples isolated from human autopsy FFPE kidney tissue in DN research.
Using LCM, the authors isolated each 100 glomerular cross section profiles from sections of autopsy FFPE kidney tissues of 10 clinically characterized DM patients displaying DN and of 10 non-diabetic individuals as controls. Following heat-induced peptide extraction from pooled glomerular FFPE samples of the DN cohort and of the control cohort, an LC-MS/MS-based differential proteomic analysis was performed using isobaric tags for identification and relative quantification (iTRAQ) of peptides present in any of both investigated samples. In the glomerular specimen, 170 proteins were identified, which seems to be a quite low number and most probably indicates the negative effects of post-mortem proteolysis in autopsy kidney specimens and of formalin fixation and paraffin embedding on the detectability of proteins/peptides with the applied analytical procedure. Among the 170 identified proteins, 100 displayed a statistically significant differential abundance between the DN cohort and the control cohort, which, on the other hand, appears to be a relatively high number. Since no quantitative conformational analyses were performed, this finding has, at least in part, to be considered as a consequence of necessary pooling of the specimen in the case at issue and of the applied statistical comparison of the relative quantities of identified peptides. These aspects will certainly have to be addressed in future proteomic studies investigating glomerular specimen isolated from FFPE kidney tissue.

Despite the investigation of autopsy samples and the given analytical limitations of the present study, approximately one-third of the identified differentially abundant proteins have previously been described in conjunction with renal disease. Further evidence for an assumable plausibility of the proteomic dataset was provided by the immunohistochemical detection of the glomerular presence of identified differentially abundant proteins with a known association with glomerular damage in DN (apolipoprotein E and collagen type IV) in kidney sections of DN patients. The authors focussed their further investigations on nephronectin, which had displayed a 25% increased differential abundance in the pooled glomerular tissue sample of the DN cohort. Nephronectin, a ligand for integrin alpha8-beta1, is an extracellular matrix protein, with an important role during nephrogenesis [19]. Whereas in experimentally induced acute tubular necrosis, increased expression levels of nephronectin have been detected in regenerating tubular cells [20], practically nothing is known concerning the role of nephronectin in glomerular function or disease. Using immunohistochemistry, Nakatani et al. investigated the glomerular presence of nephronectin in paraffin kidney sections of a larger cohort of DN patients, DM patients without DN and non-diabetic individuals. Positive glomerular immunoreactivities of nephronectin were preferentially detectable in glomerulosclerotic lesions in DN patients as well as in the extracellular glomerular matrix in sections from DM patients without DN but were absent in non-diabetic individuals. As a semiquantitative measure for the glomerular nephronectin immunoreactivity, the authors determined the numerical fraction of glomerular cross sections displaying a positive nephronectin immunoreactivity in >10% of the glomerular cross-sectional area and found it to be significantly increased in DN patients as compared to DM patients without DN and non-diabetic individuals. Additionally, this parameter was shown to display a significant positive correlation with the glomerulosclerosis index in DN patients as compared to DM patients without DN. Certainly, further studies are necessary to confirm consistently increased differential glomerular abundance of nephronectin in DN and to validate its possible pathogenetic significance or its suitability as a biomarker for DN.

Apart from that, the true relevance of the present study is rather founded on the demonstration of the principal applicability of proteomic analyses on glomerular samples isolated from FFPE kidney tissue. Now, it is urgently required to impel further glomerular proteomic investigations with a view to integrate the datasets of single analyses into an elucidating overall picture of relevant glomerular proteomic features of DN. Concerning the disposability of large numbers of definable samples for meaningful and representative analyses of alterations of the glomerular proteome, the immense resource of FFPE kidney tissue present in the archives worldwide mirroring almost every conceivable disease condition in DN, represents a buried treasure, which should not be left disregarded.

Conflict of interest statement. None declared.


References

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