Chapter 14
Applications of Urinary Proteomics in Renal Disease Research Using Animal Models

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Abstract Animal models of renal disease are essential tools in research on kidney disease and have provided valuable insights into pathogenesis. Use of animal models minimises inter-individual differences, allows specific pathological changes to be examined, and facilitates collection of tissue samples. Thus, mechanistic research and identification of biomarkers are possible. Various animal models manifesting specific pathological lesions can be used to investigate acute or chronic kidney disease (CKD). Urine, a terminal metabolic product, is produced via glomerular filtration, reabsorption, and excretion in the tubular and collecting ducts, reflecting the functions of glomeruli or tubular tissue stimulated in various ways or subject to disease. Almost 70 % of urinary proteins originate from the kidney (the other 30 % come from plasma), and urinary sampling is important to noninvasively detect renal disease. Proteomics is powerful when used to screen urine components. Increasingly, urine proteomics is used to explore the pathogenesis of kidney disease in animals and to identify novel biomarkers of renal disease. In this section, we will introduce the field of urinary proteomics as applied in different models of animal renal disease and the valuable role played by proteomics in noninvasive diagnosis and rational treatment of human renal disease.

Keywords Urine proteomics · Renal diseases · Animal models

14.1 Progress in Research on Animal Models of Renal Disease

In terms of pathological features, three types of animal model of renal disease may be identified. These are models of acute glomerulonephritis, acute renal tubular injury, and chronic kidney disease (CKD). The principal injury in models of acute

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glomerulonephritis is to the glomeruli; models of acute renal tubular injury exhibit lesions principally of the renal tubules and interstitia; CKD models feature pathological changes in both the glomeruli and tubules.

There are three principal types of acute glomerulonephritis models, the mesangial proliferative glomerulonephritis model, the membranous nephropathy model, and the anti-glomerular basement membrane nephritis model. Proliferative mesangial models, including the rat acute anti-Thy1 nephritis model and mouse snake venom-triggered glomerulonephritis, are characterised by initial mesangiolysis, followed by mesangial cell proliferation and accumulation of mesangial matrix, in turn causing proteinuria. The conditions subsequently resolve, and the histology becomes almost normal [9, 12]. Membranous nephropathy models, such as the passive Heymann nephritis model (PHN), are characterised by the formation of membrane attack complexes inducing injury to podocytes and expansion of the glomerular basement membrane, in turn causing high-level proteinuria [10, 27]. The anti-glomerular basement membrane nephritis model exhibits proliferative glomerulonephritis accompanied by crescent formation and proteinuria and is triggered by the injection of purified anti-GBM immunoglobulin into the tail vein [4, 5, 17]. All of these models find applications in studies of the pathogenesis of glomerular disease and in attempts to identify useful interventions.

Acute renal tubular injury models include the unilateral ureteral obstruction (UUO) model, the ischaemia–reperfusion model, models of drug-induced disease, and crush syndrome (CS) models. The UUO model, featuring unilateral urethral ligation, may trigger renal tubular atrophy and early interstitial fibrosis, but glomerular function remains normal. UUO has been used to induce tubulointerstitial damage and interstitial fibrosis and is important in studies of the mechanisms of renal fibrosis and evaluation of various therapeutic approaches [7, 8]. The rat ischaemia–reperfusion model features renal pedicle occlusion for 45 min, followed by the restoration of blood supply, and is used to study the pathogenesis and mechanisms of acute renal tubule damage [11]. Drug-induced models of renal injury feature obvious injuries to the tubules and interstitia. Nephropathy induced by aristolochic acids and cyclosporine is characterised by the development of tubular lesions, interstitial fibrosis, hyaline degeneration, and damage to small arteries of the kidney [2]. Gentamicin and cisplatin induce acute renal failure characterised by tubular injury and renal tubular epithelial cell death; both apoptosis and necrosis may be in play [6, 21]. Research using such models aids in understanding of the pathogenesis of drug-induced kidney injuries and can be used to develop new approaches to treatment. CS models of acute kidney injury (AKI) simulate the major shock and renal failure developing after crushing of skeletal muscle. The pathogenesis includes rhabdomyolysis, which may be induced by either compression or injection. Akimau et al. [1] and Murata et al. [15] compressed rat hind limbs with weights and found lesions in epithelial cells and the distal tubules, but the glomeruli and proximal tubules were normal. Blachar et al. [3] developed a CS model by infusing 100 mg of muscle protein/kg body weight intravenously in rabbits; renal lesions (including vacuolation of tubular cells and

formation of homogeneous eosinophilic casts) developed. The CS model of Wang et al. [26] features renal tubular cell apoptosis triggered by intramuscular injection of 8 ml/kg body weight of glycerol. In this model, injection of fasudil ameliorated tubular injury by suppressing apoptosis. CS models are used to explore the pathogenesis of AKI induced by rhabdomyolysis and to develop effective therapeutic approaches.

The principal CKD models include the nephrectomy model and the chronic anti-Thy1 nephritis model. Lesions develop in the glomeruli and tubules. In the commonly used 5/6 nephrectomy model, the remnant kidney usually exhibits early compensatory hypertrophy, glomerular perfusion, a high filtration rate, and hyperpressure; these developments are followed by glomerular sclerosis, capillary loop collapse, progressive mesangial expansion, tubular interstitial damage, and, ultimately, progressive renal failure [22]. Also, rats from which two-thirds of kidney tissue has been removed have been used to investigate residual renal destruction [24]. However, this model is not widely employed. Chronic anti-Thy1 nephritis, triggered by a single injection of anti-Thy1 antibody into unilaterally nephrectomised rats, causes development of glomerular necrosis, renal fibrosis, and a decline in renal function 1 month after injection [25]. The various CKD models are used to explore the pathogenesis of renal insufficiency and renal failure.

14.2 Urine Proteomics of Normal Animals

The protein profiles of normal urine have been described. Thongboonkerd et al. [23] analysed urine proteins of SD mice using 2D-PAGE, followed by MALDI-TOF-mediated protein identification. A total of 350 protein spots were found and 111 identified. The proteins included transporters, transport regulators, enzymes, signalling proteins, cytoskeletal proteins, signal-binding proteins, and receptors. The cited authors also [23] investigated the urine proteins of rats acutely overloaded with sodium. The levels of all of neutral endopeptidase, proteins of solute carrier family 3, meprin 1α, diphor-1, heat-shock protein 72, vacuolar H^+ATPase, ezrin, ezrin/radixin/moesin-binding protein, glutamine synthetase, and guanine nucleotide-binding protein fell, whereas those of albumin and α-2u globulin rose, compared to controls. Such changes were suggested to be associated with tubular transport of sodium.

14.3 Urinary Proteomics in Renal Disease Animal Models

Currently, research focus is on the use of the renal AKI model (featuring tubular lesions). For example, Rucevic et al. [20] used proteomics to explore the effects of 4 days of aristolochic acid treatment on the urine proteins of DBA and C57BL mice. The levels of various proteins, including those of the cytoskeleton and those

involved in kidney development and inflammation, changed in response to induced renal injury. The results aid the diagnosis and treatment of human aristolochic acid nephropathy. Rouse et al. [18] examined urinary proteins in a model of gentamicin-induced kidney disease. The levels of collagen type I and III fragments were elevated, in agreement with histopathological data. Also, m- and a-glutathione S-transferases (mGst and aGst), renal papillary antigen-1 (Rpa-1), kidney injury molecule-1 (Kim-1), lipocalin-2 (Lcn-2), osteopontin (Opn), and clusterin (Clu) were present at notably higher levels in urine 1–3 days after gentamicin treatment, but the levels of mGst, aGst, and Rpa-1 recovered to normality at day 10 and those of Kim-1, Lcn-2, Clu, and Opn recovered at day 15 [19]. More importantly, the level of Rpa-1 reflected repair and recovery of the tubular and collecting ducts, and Rpa-1 may serve as a biomarker of tubular regeneration. Zhou et al. [29] examined urine proteins in a cisplatin-induced AKI model. Urinary exosomes isolated by differential centrifugation were analysed by 2D differential gel electrophoresis and the proteins identified using MALDI-TOF-TOF or LC-MS/MS. A total of 18 proteins were upregulated and 9 downregulated 8 h after cisplatin injection. The fetuin-A level increased 52.5-fold by day 2 (1 day before the rise in serum creatinine) and remained elevated to day 5 (the peak of renal injury) after cisplatin injection. The urinary fetuin-A level increased 31.6-fold in the early phase (2–8 h) of reperfusion after ischaemia and was elevated in three ICU patients with AKI compared to those without AKI. Thus, the urinary fetuin-A level may serve as a biomarker for early diagnosis of AKI and may predict the extent of renal injury. Maddens et al. [13] induced AKI by the inoculation of *Escherichia coli* into aged mice in which uterine ligation had been performed. Urinary chitinase-3-like proteins 1 and 3 were detected only in septic mice with severe AKI. Also, the human homologue, chitinase 3-like protein 1, was present at higher levels in urine of septic patients with AKI than without, supporting the notion that urinary chitinase 3-like protein 1 may play a role in human infection-induced AKI. Such work may identify biomarkers reflecting the level of tubule damage, and this will aid in the noninvasive diagnosis of AKI patients.

Proteomics has been used in the context of other models of renal disease. Wu et al. [28] compared urine proteins in mice with moderate and severe immune-mediated nephritis triggered by the injection of anti-GBM antibody and found that severely injured mice expressed significantly higher urinary levels of vascular cell adhesion molecule-1 (VCAM-1), P-selectin, tumour necrosis factor receptor I (TNFRI), and CXCL16, suggesting that these proteins were associated with the development of spontaneous immune nephritis. Nabity et al. [16] analysed the urine proteins of dogs with X-linked hereditary nephropathy (XLHN). Retinol-conjugated protein (RBP) was first detected in urine approximately 2 months before azotaemia development, and the RBP level increased as disease progressed, suggesting that urinary RBP might serve as a biomarker for the early detection of tubulointerstitial damage. Moreno et al. [14] used SELDI-TOF to define serum and urinary biomarker signatures associated with a rapid therapeutic response to a cyclin-dependent

kinase (CDK) inhibitor in the jck mouse model of PKD and found that 20 urinary and 21 serum biomarkers might aid further assessment of CDK inhibitors as therapeutic agents for the condition.

14.4 Conclusion

Urinary proteomics will undoubtedly contribute to future research on kidney disease. Many studies have shown that urinary proteomics aids in the understanding of pathophysiological mechanisms and the discovery of novel biomarkers and therapeutic targets. However, the scope of urine proteomics is limited, and some commonly used renal disease models, including the glomerulonephritis and CKD model, are not currently amenable to further analysis using urine proteomics. Therefore, future developments in urine proteomics are required for detailed investigation of the molecular mechanisms of various animal disease models and identification of novel biomarkers, eventually guiding noninvasive diagnosis of and effective therapy for human renal diseases.

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