Kidney tissue proteomics reveals regucalcin downregulation in response to diabetic nephropathy with reflection in urinary exosomes

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Diabetic nephropathy (DN) is a major complication of diabetes mellitus and the most frequent cause of end-stage renal disease. DN progresses silently and without clinical symptoms at early stages. Current noninvasive available markers as albuminuria account with severe limitations (late response, unpredictable prognosis, and limited sensitivity). Thus, it urges the discovery of novel markers to help in diagnosis and outcome prediction. Tissue proteomics allows zooming-in where pathophysiological changes are taking place. We performed a differential analysis of renal tissue proteome in a rat model of early DN by 2-dimensional differential gel electrophoresis and mass spectrometry. Confirmation was performed by Western blot, immunohistochemistry (IHC), and selected reaction monitoring (SRM). Rat urine samples were collected and exosomes were isolated from urine to evaluate if these microvesicles reflect changes directly occurring at tissue level. The protein showing maximum altered expression in rat tissue in response to DN was further analyzed in human kidney tissue and urinary exosomes. Regucalcin protein or senescence marker protein-30 (SMP30) (Swiss-Prot Q03336) was found to be strongly downregulated in DN kidney tissue compared with healthy controls. The same trend was observed in exosomes isolated from urine of control and DN rats. These data were further confirmed in a pilot study with human samples. IHC revealed a significant decrease of regucalcin in human kidney disease tissue vs control kidney tissue, and regucalcin was detected in exosomes isolated from healthy donors' urine but not from kidney disease patients. In conclusion, regucalcin protein expression is reduced in DN kidney tissue and this significant change is reflected in exosomes isolated from urine. Urinary exosomal regucalcin represents a novel tool, which should be explored for early diagnosis and progression monitoring of diabetic kidney disease. (Translational Research 2015;166:474–484)

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Abbreviations: 2D-DIGE = two dimensional differential gel electrophoresis; CHAPS = 3-((3-Cholamidopropyl)dimethylammonio)-1- propanesulfonate; CKD = chronic kidney disease; DN = diabetic nephropathy; FDR = false discovery rate; GFR = glomerular filtration rate; IHC = immunohistochemistry; MALDI = matrix assisted laser desorption ionization; MS = mass spectrometry; PBS = phosphate-buffered saline; SRM = selected reaction monitoring; STZ = streptozotocin; TOF = time of flight; WB = western blot

AT A GLANCE COMMENTARY

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Background

Diabetic nephropathy (DN) progresses silently and accurate diagnosis can only be made by biopsy and usually later, once irreversible damage has already occurred. We performed differential unbiased omic analysis of renal tissue proteome in an experimental model of early DN to find out novel markers of disease. Confirmation of data was performed in human samples.

Translational Significance

Regucalcin was found to be downregulated in diseased kidneys. This change in tissue could be monitored in urinary exosomes, with the same trend. Data suggest that regucalcin might be used in the future for staging and characterization of kidney disease in diabetes.

INTRODUCTION

Diabetic nephropathy (DN) is a progressive kidney disease, a major complication of diabetes mellitus and the most frequent cause of end-stage renal disease, accounting for 40% of patients requiring renal replacement.[1] DN is caused by angiopathy of capillaries in the kidney glomeruli, characterized by nephrotic syndrome and diffuse glomerulosclerosis. Despite the traditional emphasis on glomerular changes, diabetic kidney disease is also characterized by tubular and vascular injury.[2,3] Indeed, high glucose levels directly activate tubular cells to secrete inflammatory mediators and extracellular matrix and may cause tubular cell injury.[4] Inflammatory cytokines from infiltrating inflammatory cells may also injure tubular cells.[5] Moreover, albuminuria as a consequence of glomerular injury is a stressor for proximal tubular cells, promoting also secretion of inflammatory mediators and lethal or sublethal injury.[6] Early DN has no symptoms, it is a silent process, and although it has been investigated for several years, there is a clear need for a better understanding of its pathogenesis. Currently, microalbuminuria (urinary albumin to creatinine ratio >30 mg/g or albumin excretion rate >30 mg/24 hours) is the best noninvasive marker available for assessing risk of developing established DN, characterized by macroalbuminuria (urinary albumin to creatinine ratio >300 mg/g). However, 15%–65% of patients with microalbuminuria regress to normoalbuminuria and do not progress to macroalbuminuria or chronic kidney disease (CKD).[7] Other limitations of albuminuria as a risk marker are that 20%–60% of diabetic patients have decreased glomerular filtration rate (GFR) in the presence of normal urinary albumin excretion, and that histopathologic changes in the kidney structure may be already present in microalbuminuric patients recently diagnosed.[8,9] At the same time, prevention and treatment guidelines focus on risk factors for normoalbuminuric diabetic patients (hypertension, hyperglycemia, smoking, and dyslipidemia) and on preventing progression to macroalbuminuria in microalbuminuric patients.[10] However, these strategies do not succeed for all diabetic patients. Therefore, together with a deeper knowledge of the pathophysiological mechanisms in DN, it is urgent to discover candidate markers to help in better prognosis and outcome prediction.

Tissue proteomics allows "zooming-in" to the local area where pathophysiological changes are taking place. This means investigating, directly, the tissue where original alterations occur. Alterations found out directly in tissue could be, at the end, translated or not into measurable changes in biological fluids.[11,12] In this study, we investigated altered protein expression at the renal tissue level in a streptozotocin (STZ)-induced rat model of early DN by 2-dimensional differential gel electrophoresis (2D-DIGE) in combination with mass spectrometry (MS). Exosomes are membranous vesicles released by cells into different extracellular fluids, which have gained relevance as microvesicles with scavenger and cell-to-cell communication properties.[13,14] Considering that tubular cell exosomes may provide an integrative view of the diverse stressors (high glucose, inflammation, and