Proteomic approaches to the study of renal mitochondria

Zdenek Tuma^a, Jitka Kuncova^{a,b}, Jan Mares^{a,c}, Martina Grundmanova^{a,b}, Martin Matejovic^{a,c}

Background and Aims. Dysfunction of kidney mitochondria plays a critical role in the pathogenesis of a number of renal diseases. Proteomics represents an untargeted attempt to reveal the remodeling of mitochondrial proteins during disease. Combination of separation methods and mass spectrometry allows identification and quantitative analysis of mitochondrial proteins including protein complexes. The aim of this review is to summarize the methods and applications of proteomics to renal mitochondria.

Methods. Using keywords “mitochondria”, “kidney”, “proteomics”, scientific databases (PubMed and Web of knowledge) were searched from 2000 to August 2015 for articles describing methods and applications of proteomics to analysis of mitochondrial proteins in kidney. Included were publications on mitochondrial proteins in kidneys of humans and animal model in health and disease.

Results and Conclusion. Proteomics of renal mitochondria has been/is mostly used in diabetes, hypertension, acidosis, nephrotoxicity and renal cancer. Integration of proteomics with other methods for examining protein activity is promising for insight into the role of renal mitochondria in pathological states. Several challenges were identified: selection of appropriate model organism, sensitivity of analytical methods and analysis of mitochondrial proteome in different renal zones/biopsies in the course of various kidney disorders.

Key words: renal mitochondria, proteomics, gel electrophoresis, liquid chromatography-mass spectrometry, diabetes, acidosis, nephrotoxicity, renal cancer

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^aBiomedical Center, Faculty of Medicine in Pilsen, Charles University in Prague, Pilsen, Czech Republic

^bDepartment of Physiology, Faculty of Medicine in Pilsen, Charles University in Prague, Pilsen, Czech Republic

^cDepartment of Internal Medicine I, Faculty of Medicine in Pilsen, Charles University in Prague and Teaching Hospital, Pilsen, Czech Republic
 Corresponding author: Zdenek Tuma, e-mail: Zdenek.Tuma@lfp.cuni.cz

INTRODUCTION

Mitochondria were recognized in the cell in the 19th century. In the first half of the 20th century, biochemical studies were carried out on mitochondrial enzymes and pathways. Electron transport mechanisms and oxidative phosphorylation were then intensively studied¹. Currently, mitochondria are known to be responsible for a variety of functions including regulation of intracellular calcium², generation of reactive oxygen species (ROS) (ref.³), production of nitric oxide⁴ and processes leading to cell death⁵.

Renal mitochondria are important as energy suppliers for active transport processes in nephrons⁶. Dysfunction of mitochondria caused by inherited mitochondrial cytopathies or acquired defects of proteins involved in mitochondrial metabolic pathways contributes to the pathophysiology of acute and chronic renal disorders⁷. Disruption of mitochondrial energetic metabolism⁷⁻⁹ and morphology^{10,11} is recognized as important contributors to tubular dysfunction in acute kidney injury (AKI). Increased production of ROS triggered by hypoxia in mitochondria during sepsis leads to kidney dysfunction and contributes to dysfunction of organs. ROS produced by mitochondria are important in the development of endothelial cell damage during focal segmental glomerulosclerosis (FSGS) (ref.¹²). Specific impairment of renal

mitochondrial metabolism has also been observed in cancer¹³, diabetes^{14,15} and as a consequence of toxic metabolite removal¹⁶. Emerging evidence also suggests that mitochondria represent a promising target for novel treatments¹⁷⁻¹⁹.

Proteomics was initially defined as an effort to identify and describe the complete set of proteins expressed in biological systems. Nowadays, proteomics include the study of protein-protein interactions, subcellular locations, expression levels, and posttranslational modifications of all proteins within cells and tissues. Hence, the proteomics of mitochondria is a powerful tool for understanding the mechanism of mitochondrial response to pathological conditions, evaluating the effects of drugs and for the development of new mitochondria-targeted therapies^{20,21}. Proteomic analysis of renal mitochondria represents a multi-step process that starts with selection of appropriate biological sample and optional reduction of complexity. The next step is a combination of separation methods and mass spectrometry for qualitative and quantitative analysis of proteins and their identification. Data on changes in expression of mitochondrial enzymes, posttranslational modifications, and subunit structure of protein complexes are acquired. The purpose of this review is to provide an update on the methodological progress and potential of the rapidly evolving mitochondrial proteomics approach to facilitate new discoveries in the field of renal pathology.

Sample preparation

For studies of renal mitochondrial proteome, tissue samples acquired from animal models or human tissues after resection are used. Renal biopsies are challenging due to small size and therefore limited amount of mitochondrial protein available. Cell cultures as an alternative to laboratory animals are useful for investigation of processes in specific renal cell types²².

Organized distribution of nephrons in renal tissue results in formation of regions with different biochemical and metabolic properties. Differences in the activity of mitochondrial enzymes along the nephron reflect the heterogeneity of the mitochondrial population in nephron segments²³. Therefore, separation of kidney regions (e.g. cortex and medulla) or individual nephron segments is an alternative for reducing sample complexity. Nephron segments dissected from kidney slices²⁴, proximal and distal tubules isolated from renal cortex by collagenase digestion and centrifugation on density gradient^{25,26}, or glomeruli isolated by standard sieving method²⁷ have been used for detailed study of metabolism. These separation methods may also be useful for proteomic studies on renal mitochondria. Using laser capture microdissection, selected cell populations from complex tissue sections can be acquired with high specificity. However, only a part of total cell protein is from mitochondria, and hence sensitive analytical techniques are necessary for using this procedure in the analysis of mitochondria from nephron segments.

Differential centrifugation is often used for separation of mitochondria from cells or tissue homogenates²⁸. Isolation of mitochondria is performed in two centrifugation steps – removing of nuclei, cell membranes and unbroken cells in the first centrifugation step and sedimentation of mitochondria in the second step. A pellet that contains the mitochondrial fraction of the cell can be used for proteomic analysis directly^{29,30} or after purification of mitochondria by centrifugation in density gradients^{31,32}. In purified mitochondria, proteins from other organelles (e.g. endoplasmic reticulum, peroxisomes and cytoskeleton) still may be present due to association of these organelles with mitochondria³³. Analysis of markers of subcellular compartments by Western blotting is used for determination of purity of isolated mitochondrial fractions. Further, subcellular localization of identified proteins can be checked in databases of mitochondrial proteins³⁴ or by systems that predict subcellular localization³⁵. Commercially available kits based on differential centrifugation can simplify and speed up the separation and could be an alternative approach when limited amounts of sample are available or for processing large numbers of samples³⁶.

Alternatives to differential centrifugation are separation of mitochondria using magnetic microbeads of free flow electrophoresis. Using free flow electrophoresis, high purity of mitochondria can be reached³⁷. Low yield and the necessity for specialized equipment are major drawbacks of free flow electrophoresis³⁶. Mitochondria can be isolated with enrichment and purity comparable to ultracentrifugation methods using magnetic microbeads³⁸.

Methods for analysis of mitochondrial proteome

The mammalian mitochondrial proteome comprises approximately 1500 proteins^{39,40}. Most of them are encoded by the nuclear genome and imported into mitochondria. Only 13 protein chains of electron transport system (ETS) subunits are encoded by the mitochondrial DNA.

Mitochondrial proteome contains proteins with a wide range of hydrophobicity. Membrane proteins form a significant part of mitochondrial proteins and are a challenge for separation methods used in proteomics. These proteins are attached to the membrane in hydrophobic regions (e.g. ETS complexes^{41,44}, electron transferring flavoprotein⁴⁵), or embedded in the membrane (e.g. pore forming proteins). Relatively soluble enzymes are present in mitochondrial matrix⁴⁶ and intermembrane space⁴⁷. A significant part of mitochondrial proteins is associated into homo- or heterooligomeric protein complexes whose subunit composition is important for their function³⁰. Analysis subunit composition and stoichiometry of mitochondrial protein complexes requires analysis under native conditions to prevent their dissociation. At present, there is no universal proteomic method that can cover all aspects of mitochondrial proteins. Proteomic workflows based on two-dimensional electrophoresis, blue native electrophoresis and gel-free chromatographic methods have been used for analysis of mitochondria. A short summary of methods used for the analysis of mitochondrial proteome is shown in Table 1.

Two-dimensional electrophoresis (2-DE) combines separation of proteins according to their net charge by isoelectric focus in the first step and by molecular weight on polyacrylamide gel in the second dimension. Proteins resolved as spots on gels are then visualized using visible or fluorescent stains and digital images of gels are acquired using a scanner or camera. With 2-DE, fast resolution of soluble proteins with the option of direct evaluation of their isoelectric points and molecular weights can be done. The use of 2-DE for mitochondrial proteomics is limited by resolution of proteins with extreme isoelectric point and molecular weight values, poor separation of hydrophobic membrane proteins, and limited detection of low abundance proteins^{48,49}. Two-dimensional fluorescence difference gel electrophoresis (2D-DIGE) is a variant of 2-DE that improves reproducibility⁵⁰. 2D-DIGE relies on labeling of samples with spectrally resolvable fluorescent cyanine dyes (Cy2, Cy3, and Cy5) and their simultaneous separation on a single polyacrylamide gel. Internal standard prepared by mixing equal amount of each sample is labeled by Cy2; dyes Cy3 and Cy5 are then used for labeling individual samples. Internal standard and two individual samples are mixed together, separated by 2DE, and gels are scanned with the excitation wavelength of each dye used. The use of internal standard in DIGE minimizes inter-gel variability and makes processing of gel images easier⁵⁰. Images are then imported to a specialized 2-DE image analysis software that allows spot detection, and quantification by calculation of the spot volumes⁵¹. Spots of interest are then excised and proteins are identified by combination of in gel digestion and mass spectrometry.

Table 1. Summarization of proteomic methods and their suitability for analysis of mitochondrial proteome.

Method	Principle	Advantages	Disadvantages
Two-dimensional electrophoresis (2DE)	Separation of proteins according to their net charge by isoelectric focusing followed by separation according their masses by polyacrylamide gel electrophoresis.	Widespread, robust, relatively simple method. Compatibility with subsequent analyses (e.g. immunochemic detection, mass spectrometry).	Difficult separation of hydrophobic, extremely acidic or basic proteins. Limited dynamic range. Low throughput, labor-intensiveness.
Two-dimensional fluorescence difference gel electrophoresis (DIGE)	Labeling of samples with fluorescent dyes and separation on one gel with internal standard by 2DE.	Improved reproducibility and less sample demand in comparison with 2DE.	Difficult separation of hydrophobic and extremely acidic or basic proteins. Labor- intensiveness
Blue native polyacrylamide gel electrophoresis (BN-PAGE)	Separation of protein extract on polyacrylamide gel in presence of Coomassie Brilliant Blue.	Analysis of subunit composition of protein complexes. Analysis of hydrophobic complexes. Maintaining activity of protein complexes during separation.	Low throughput and labor-intensiveness. Only protein complexes are detected.
Gel free methods (LC-MS)	Digestion of a complex protein sample, separation of proteolytic peptides by one- or multiple-step of liquid chromatography, on-line mass spectrometric analysis of eluted peptides.	In comparison with gel based methods, less sample requirement and better detection of hydrophobic proteins. Ability to analyze very complex samples by employing multidimensional chromatographic separation.	Incomplete sequence coverage of identified proteins. Low efficiency to detect posttranslationally modified peptides without enrichment.
Microarrays	Detection of proteins captured on surfaces with specific antibodies or surfaces optimized for capture of certain protein types.	High throughput. Analysis of specific molecules by selection of different array types (e.g. posttranslationally modified proteins).	Number of targets is limited by the selected array. Cross reactivity in complex protein mixtures.

Blue native polyacrylamide gel electrophoresis (BN-PAGE) was designed for separation of intact protein complexes. Hydrophobic proteins and complexes are solubilized with a nonionic detergent. Coomassie blue added to sample and cathode buffer binds to hydrophobic proteins and complexes and gives them a negative charge^{52,53}. In two dimensional setup, separation in second dimension in the presence of sodium dodecylsulfate under denaturing conditions allows resolution of subunits of individual complexes⁵⁴. These methods are utilized for investigation of respiratory complexes and their subunit composition in health and disease.

Methods utilizing a combination of high performance liquid chromatography with mass spectrometry (LC-MS) are used with increasing tendency in proteomics for qualitative and quantitative analyses of complex protein mixtures extracted from biological samples; they can be also used for identification of proteins in spots from gel electrophoreses. Protein mixture extracted from a biological sample is digested by proteolytic enzyme and resulting peptides are then separated by high performance liquid chromatography. Proteolytic digestion of complex samples results in huge number of proteolytic peptides and multidimensional fractionation of peptides based on combination of two or more chromatographic steps (e.g., reverse phase liquid chromatography, ion exchange chro-

matography) (ref.⁵⁵) offers increased separation capacity and higher resolution. Separated peptides are eluted from the column directly into the mass spectrometer. During the whole chromatographic run, masses of eluting peptides and their fragmentation spectra are recorded by the mass spectrometer. These data are then used for identification of proteins and for their quantification. For quantification of proteins, label free workflows or isotopic labeling are used⁵⁶. Strategies for label free quantification include two distinct groups: measurement based on measurement of area under the curve or signal intensity of precursor ion spectra, and spectral counting in which the number of fragment spectra identifying peptides from a given protein is used to assess relative protein abundance⁵⁷. Quantification by isotopic labeling is based on incorporating of isotopic compound either metabolically by incorporation of specific isotopes, or enzymatically or chemically using reagents that bound to peptides^{58,59}. Labeled samples are combined and subjected to analysis by liquid chromatography and mass spectrometry. Quantitative data are extracted from the intensities of characteristic ions in tandem mass spectra⁵⁹. Advantage of gel free attempts for analysis of mitochondrial proteome is improved detection of membrane proteins that are underrepresented in 2-DE analyses⁶⁰. Microscale techniques that comprise protein extraction, fractionation and pro-

teolytic digestion optimized for the microgram protein range would allow analysis of small samples of mitochondria from biopsies or nephron segments⁶¹.

Protein microarrays allow simultaneous detection of a set of proteins and offer the ability to study multiple samples in an effort to develop protein profile changes across multiple proteins^{62,63}. The ability of microarrays for screening of multiple proteins is advantageous for detection of protein expression changes, protein-protein interactions, and biomarker discovery and validation⁶⁴. Analysis of intact proteins by microarrays instead of proteolytic digests can be advantageous for preservation of specific motifs but conjugation of sample with the tag necessary for subsequent detection or signal amplification may denature, damage, or mask the epitope⁶⁵. Antibody cross-reactivity to non-target proteins can also decrease performance in antibody microarrays^{62,63}. The potential for utilization of protein microarrays in analysis of renal mitochondria could be in high throughput monitoring of selected pathways by analysis of multiple, predefined set of proteins. Arrays based on mass spectrometric detection of proteins bound to affinity surfaces (surface-enhanced laser desorption and ionization, SELDI) have been used for analysis of brain mitochondria^{66,67}. Chips with wide range of surfaces are available for SELDI. The use of chip surface optimized for binding of hydrophobic proteins could be potentially advantageous for analysis of mitochondrial membrane proteins.

CLINICAL AND RESEARCH APPLICATION OF RENAL MITOCHONDRIAL PROTEOMICS

Basic characterization of renal mitochondrial proteome

In renal tissue of various species including mouse, rat and human, mitochondrial proteins have been identified by proteomic methods⁶⁸⁻⁷⁰. Comparison of 2-DE maps of rat kidney cortex and medulla⁶⁸ or human kidney cortex, medulla and glomeruli⁷¹ showed alterations in spots containing mitochondrial proteins. However, this attempt is not suitable for detailed analysis of the mitochondrial proteome due to very low amount of mitochondrial proteins detected. The coverage of mitochondrial proteome is increased when mitochondria prepared by differential centrifugation are used for analysis instead of whole tissue. Analysis of mitochondrial fractions of porcine renal cortex and medulla showed that mitochondrial proteome of the cortex contained enzymes employed in oxygen dependent pathways and mitochondrial proteome in the medulla proteins important for adaptation to low oxygen availability⁷². Further datasets that contain proteins of renal mitochondria are available in experiments that investigated composition of mitochondrial proteome from mouse tissues by 2-DE (ref.⁷³) and rat organs by BN-PAGE (ref.³⁰) and LC-MS (ref.⁷⁴). High throughput proteome analysis done by LC-MS allowed characterization of multiple mitochondrial metabolic pathways⁷⁵. However, translation of animal data to humans is limited by differences in anatomy, metabolism and physiology. On

the other hand, several factors make the human research into renal cellular and molecular biology problematic. In particular, difficult access to the human renal tissue and several confounding factors including disease state, the patient's comorbidities and treatment history represent main impediments to the study of human renal mitochondrial proteome. Therefore, the use of clinically relevant animal models remains a cornerstone in the study renal mitochondrial pathology. In this context, pig is highly valuable model organism due to anatomic, physiological and biochemical similarities of porcine and human kidney⁷⁶.

Recent advances in mitochondrial studies: a focus on selected renal pathologies

In the following text, we summarize recent advances from various mitochondrial proteomic studies of the most common pathological states of the kidney. Short summary of utilization of these methods is also available in Table 2. Detailed analysis of all research applications of mitochondrial proteomics falls outside the scope of this work and the reader is referred to recent in-depth reviews^{21,77-80}.

Diabetes

All forms of diabetes are characterized by hyperglycemia, a relative or absolute lack of insulin action, pathway-selective insulin resistance, and the development of diabetes-specific pathology in the retina, renal glomerulus, and peripheral nerves. Oxidative stress is a key component in the development of diabetic nephropathy. Reactive oxygen species (ROS) are produced by cytosolic (such as glycolysis, specific defects in the polyol pathway, uncoupling of nitric oxide synthase, xanthine oxidase, and advanced glycation) and mitochondrial pathways (electron leakage at complex I and at the interface between coenzyme Q and complex III) (ref.⁸¹). Excess amounts of ROS modulate activation of protein kinase C, mitogen-activated protein kinases, and various cytokines and transcription factors which eventually cause increased expression of extracellular matrix genes with progression to fibrosis and end stage renal disease⁸². Impairment of renal mitochondria induced by oxidative stress is an important factor in diabetes; therefore mitochondria represent an important site due to their intensive oxidative metabolism and using proteomics, specific targets for therapy in mitochondria can be revealed.

Proteomic analysis showed upregulation of TCA cycle and fatty acid oxidation proteins by analysis cytosolic compartment of kidney cortex⁸³ and renal mitochondria⁸⁴ of diabetic mice. Increased level of mitochondrial fatty acid metabolism enzymes in renal cortex of diabetic animals supported the hypothesis that insulin resistance can be attributed to increases in intracellular fatty acid metabolites that disrupted insulin signaling⁸³.

Increased expression of mitochondrial 3-hydroxy-3-methylglutaryl-CoA synthase 2 (HMGCS2) was found using 2-DE analysis in kidney of diabetic mice. It was proposed that excess of ketogenic activity resulting from

Table 2. Applications of proteomic methods that were used in studies of the most common pathological states of the kidney.

Pathology	Method	Sample, organism	What was studied
Diabetes	2-DE	Kidney tissue, mouse ^{83,85} Mitochondria, mouse ⁸⁶ Mitochondria, rat ¹⁵	Changes of proteome during diabetes ⁸³ . Proteomic profile of kidney and identification of mitochondrial ketogenic enzyme ⁸⁵ . Posttranslational modification of mitochondrial proteome ^{86,15} .
	LC-MS	Mitochondria, mouse ⁸⁴ Kidney tissue, rat ⁸⁷	Changes of protein expression during diabetes ⁸⁴ . Identification of potential therapy targets ⁸⁷ .
Hypertension	2-DE	Kidney tissue, rat ⁹³	Protein expression profile in a model of hypertension ⁹³ .
	LC-MS	Mitochondria, rat ⁹⁴	Mitochondrial metabolism in mitochondria in a model of hypertension ⁹⁴ .
Acidosis	2D-DIGE	Proximal tubules, rat ⁹⁵	Changes in protein expression during acidosis ⁹⁵ .
	LC-MS	Mitochondria, rat ²⁹	Response of the mitochondrial proteome of renal proximal convoluted tubules to chronic metabolic acidosis ²⁹ .
Acute kidney injury	Western blotting	Kidney tissue, mouse ^{17,96}	Expression of mitochondrial protein after treatment of AKI by antioxidant ¹⁷ . Protective mechanism of mitochondrial sirtuin ⁹⁶ .
Nephrotoxicity	2-DE	Kidney tissue, rat ^{98,99} Cell culture ^{101,102} Mitochondria ²²	Interaction of gentamicin ⁹⁸ and cyclosporine A ⁹⁹ with kidney. Effect of calcium oxalate monohydrate ^{101,22} and dihydrate ¹⁰² .
Renal cancer	2-DE	Tissue, human ¹⁰⁴ Mitochondria, human ¹⁰⁵ Cell culture, human ¹⁰⁸	Protein profiles of tissue and tumor ¹⁰⁴ . Mitochondrial proteomes of different tumors ¹⁰⁵ . Impact of cisplatin administration on protein expression levels ¹⁰⁸ .
	2D-DIGE	Tumor tissue, human ¹⁰⁷	Stage-related changes of proteins in tumors ¹⁰⁷ .
	BN-PAGE	Tumor tissue, human ¹⁰⁶	Composition of ETS complexes in tumors ¹⁰⁶ .

increased expression of HMGCS2 contributes to diabetic nephropathy and HMGCS2 may therefore represent a potential therapeutic target⁸⁵.

Proteomic analysis of cytosolic and mitochondrial fractions of diabetic mice kidney provided data about migration of mitochondrial proteins⁸⁶. Increased amount of prohibitin and cytochrome c was detected in cytosolic fraction of diabetic kidney. Mitochondrial prohibitin is involved in cell cycle function and its increased amount in cytoplasmic fraction was attributed to damage of mitochondrial membrane or impaired transport into mitochondrion. The leakage of cytochrome c into cytosol indicated alteration in permeability of mitochondrial membrane.

In diabetes, high blood glucose level results in post-translational modifications of proteins. Proteins modified by methylglyoxal (MGO) were found in renal mitochondria of diabetic rats and identified as enzymes of beta oxidation and subunits of ETS complex I and III. It was shown that activity of complex III was decreased due to its modification by MGO (ref.¹⁵).

Effect of triethylenetetramine (TETA) on mitochondria of diabetic kidney was studied by proteomics⁸⁷. TETA is a copper chelator that prevents or reverses diabetic copper overload and thereby suppress oxidative stress. Analysis of kidney of diabetic animals revealed that treatment by TETA restored decrease of mitochondrial chaperones involved in protein turnover and assembly,

and enzymes involved in oxidative phosphorylation and mitochondrial fatty acid metabolism expression caused by diabetes⁸⁷.

Hypertension

Essential hypertension is a heterogeneous disorder in which both genetics and environmental factors contribute to increased cardiovascular and renal morbidity and mortality⁸⁸. Changes in renal mitochondria in rat models of hypertension include alterations in respiratory functions⁸⁹ and oxidative stress^{90,91} and renal mitochondria represent possible therapeutic target⁹².

Protein expression profiles of the kidney in spontaneously hypertensive rats revealed decreased expression of NADPH dependent mitochondrial isocitrate dehydrogenase⁹³. Decreased defense against mitochondrial oxidative damage in hypertensive rats was suggested due to involvement of this protein in process of glutathione regeneration.

Using salt-sensitive rats as model organism for hypertension, effect of salt-induced hypertension on mitochondria of thick ascending limb of Henle's loop was tested by proteomics⁹⁴. Downregulation of proteins connected with energy metabolism predicted reduction of oxygen utilization in mitochondria in medullary thick ascending limb of Henle's loop of salt sensitive rats⁹⁴.

Acidosis

Metabolic acidosis is caused by overproduction of an acid or reduced recovery of bicarbonate. Adaptive response of renal proximal tubule includes rapid increase of ammoniagenesis and gluconeogenesis. In proximal tubules of acidotic rats, increased levels of mitochondrial proteins associated with catabolism of plasma glutamine were detected by proteomics using 2D-DIGE (ref.⁹⁵). These changes in protein expression levels contributed significantly to the adaptive response to metabolic acidosis and/or renal hypertrophy. In mitochondria of rat proximal convoluted tubules²⁹, abundance of proteins including mitochondrial enzymes involved in glutamine metabolism and acid-base balance was significantly altered in response to metabolic acidosis. During acidosis, increased acetylation of mitochondrial proteins was detected and it was hypothesized that acetylation may prevent protein degradation by blocking sites of ubiquitination.

Acute kidney injury

In experimental models of AKI, abnormal mitochondrial biogenesis, fission/fusion, and autophagy have been characterized and recovery of mitochondrial functions was found to be important for function of the kidney¹¹. Ischemia-reperfusion injury in AKI contributes to fragmentation of mitochondria and processes leading to cell death¹⁰. Although proteomic analysis of mitochondria or kidney tissue affected by AKI is not available to date, experimental data suggest that changes in mitochondrial proteins structure and activity could substantially contribute to the onset or progress of renal dysfunction associated with AKI (ref.¹⁷). Recent research showed that mitochondrial proteins could be an important target for therapy^{96,97}. Therefore, proteomic analysis of mitochondria on model systems during AKI could bring important information about mechanisms of mitochondrial damage and reveal potential therapeutic targets.

Detection of nephrotoxic compounds effect

Kidney plays an important role in elimination of xenobiotics, including drugs and toxic environmental agents. During concentration of urine, tubular structures of nephrons are exposed to relative high concentrations of xenobiotic compounds. Knowledge of putative interactions of xenobiotics, drugs or their metabolites with renal mitochondria could help to prevent potential damage to the kidney.

Proteomic analysis of renal cortex was performed to delineate the effects of gentamicin⁹⁸. Gentamicin belongs to aminoglycosides which are known to inhibit sodium and potassium ATPase activity. The analysis revealed that expression of mitochondrial proteins employed in gluconeogenesis and glycolysis, fatty acid utilization and TCA cycle was affected by gentamicin.

Effect of cyclosporine A (CsA) nephrotoxicity was studied on the kidney of normal and salt-depleted rat models by combined strategy employing proteomics and metabolomics⁹⁹. CsA is used after renal transplantation to prevent organ rejection. Mitochondrial proteins of oxidative phosphorylation and fatty acid β -oxidation were

upregulated in low-salt control rats compared to normal-fed animals. Upregulation of these proteins suggested increased energy demand, possibly for retaining normal osmolarity within the cells. In low salt animals, CsA treatment decreased the level of TCA cycle and ETS proteins. CsA more strongly affected the kidneys of rats fed with a low-salt diet due to their higher dependence on the energy production by mitochondrial respiration⁹⁹.

In hyperoxaluria, renal tubular epithelial cells are exposed to oxalate which lead to the activation of intracellular responses, including overproduction of free radicals and reactive oxygen species. Mitochondrial dysfunction is an important event favoring kidney stone formation¹⁰⁰. In renal tubular epithelial cells incubated with calcium oxalate monohydrate (COM) (ref.¹⁰¹) or calcium oxalate dihydrate (COD) (ref.¹⁰²), changes in expression of several mitochondrial proteins were detected. Proteomic analysis of mitochondria²² revealed that COM treatment affected enzymes of cell cycle regulation, carbohydrate, amino acid and energy metabolism. Increased level of oxidatively modified proteins indicated ROS overproduction in COM treated cells. Proteomic approach documented a complex effect of COM crystals on renal cells and showed that mitochondrial pathways of energy metabolism, ROS regulation and oxidative stress response were affected.

Renal cancer

In tumors, energy metabolism is shifted from oxidative phosphorylation to glycolysis. However, synthetic pathways in mitochondria are still important for cancer cells and mitochondria represent important targets for therapy¹⁰³. Increased expression of glycolytic proteins and downregulation of mitochondrial gluconeogenic enzymes that reflect the predominance of glycolysis followed by lactic acid fermentation in the presence of adequate oxygen (Warburg effect) in was detected in renal carcinoma tissue¹⁰⁴. Analysis of mitochondria isolated from renal oncocytoma and chromophobe cell carcinoma was performed by 2-DE (ref.¹⁰⁵). Differences in abundance of ETS subunits, proteins of glycolysis, beta oxidation and antioxidant proteins have been found.

Analyses by BN-PAGE can reveal alterations and abundance of ETS protein complexes in renal tumor tissues in comparison with healthy tissue or between tumor types. Using BN-PAGE, differences in patterns of ETS complexes in three types of renal tumors were examined¹⁰⁶. It was found that decreased amount of ETS complexes II, III IV and ATP synthase correlated with aggressiveness of renal cell carcinoma.

Recent evidence showed that mitochondrial proteome is changed during tumor progression¹⁰⁷. Samples of renal cell carcinoma of different stages have been analyzed by 2D-DIGE. Mitochondrial proteins of TCA cycle and ETS system were found to be downregulated according to Warburg hypothesis. Mitochondrial prohibitin and peroxiredoxin-3 showed stage-dependent changes in expression and may be used as potential markers of progression.

Compounds used for anticancer therapy may influence the mitochondrial proteins¹⁰⁸. Cisplatin is an important anticancer drug and its use is frequently limited by

various significant side effects including nephrotoxicity¹⁰⁹. Proteome analysis of renal cells incubated with cisplatin identified changes in several mitochondrial proteins. Upregulation of mitochondrial heat shock protein HSP70 may indicate a defense mechanism against apoptosis. Increase of glycolytic enzyme glyceraldehyde 3-phosphate dehydrogenase was attributed to compensatory effect on disturbed mitochondrial oxidative processes and decrease of mitochondrial superoxide dismutase to decreased activity of free radical defense mechanisms¹⁰⁸.

CONCLUSION AND RESEARCH PERSPECTIVES

Proteomic analyses of renal mitochondria holds great potential for providing information on remodeling of mitochondrial metabolic pathways, protein posttranslational modifications and composition of mitochondrial protein complexes during various renal pathological states. These data can be then used for investigating the pathophysiology of diseases associated with mitochondrial dysfunction and for revealing potential targets for therapy. Nevertheless, many challenges remain. We have developed the following research agenda in relation to the renal mitochondrial proteomics aimed to identify potential research questions to address the existing knowledge gaps.

- Complex structure of kidney tissue is an important factor for analysis of the renal mitochondrial proteome; therefore the important step is the reduction of sample complexity by isolation of renal cortex, medulla, nephron segments and isolation of mitochondria.
- Analysis of mitochondria in renal biopsies represents a significant challenge due to low amount of available protein. Optimization of methods for isolation of mitochondria in high purity and analytical methods for covering wide range of hydrophobicity, abundance and posttranslational modifications of mitochondrial proteome is important.
- Combination of proteomics with methods that reflect metabolic activity (e.g. metabolomics, high resolution respirometry) can provide more detailed understanding of renal mitochondrial physiology and pathology.
- There is a pressing need to better understand the nature, time course and magnitude of mitochondrial proteome changes in different renal zones in the course of various kidney disorders.
- Further proteomic studies are also required to better understand how multiple comorbidities (such as diabetes, heart failure) as well as aging alter the renal mitochondrial proteome, enhance the intrinsic susceptibility of mitochondrial system to insults and how they affect the effectiveness of novel therapies.
- Due to the inaccessibility of the kidney tissue under clinical conditions, future research will need better preclinical models of AKI and CKD that recapitulate the complex nature of human disease.

ABBREVIATIONS

ATP, Adenosine triphosphate; AKI, Acute kidney injury; BN-PAGE, Blue native polyacrylamide gel electrophoresis; CKD, Chronic kidney disease; COD, Calcium oxalate dihydrate; COM, Calcium oxalate monohydrate; CsA, Cyclosporin A; ETS, Electron transport system; HMGCS2, 3-hydroxy-3-methylglutaryl-CoA synthase 2; HPLC, High performance liquid chromatography; IMM, Inner mitochondrial membrane; LC-MS, Liquid chromatography - mass spectrometry; MGO, Methylglyoxal; MPT, Mitochondrial permeability transition pore; MW, Molecular weight; OMM, Outer mitochondrial membrane; pI, Isoelectric point; ROS, Reactive oxygen species; SELDI, Surface-enhanced laser desorption and ionization; TCA, Tricarboxylic acid cycle; TETA, Triethylenetetramine; VDAC1, Voltage-dependent anion-selective channel 1; VDAC2, Voltage-dependent anion-selective channel 2; 2-DE, Two-dimensional electrophoresis; 2D-DIGE, Two-dimensional fluorescence difference gel electrophoresis.

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