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ORIGINAL PAPER

Analysis of HK-2 cells exposed to oxalate and calcium oxalate crystals: proteomic insights into the molecular mechanisms of renal injury and stone formation

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Abstract Exposure to high levels of oxalate and calcium oxalate monohydrate (COM) crystals is injurious to renal epithelial cells and triggers serial responses related to stone formation. Multiple molecules and proteins are involved in this process, but previous studies have generally been limited, without an overall understanding of protein expression alteration after oxalate and/or crystal exposure as well as its role in stone formation. We used proteomic analysis to reveal the changes in the proteome of HK-2 cells induced by oxalate and COM crystals, so as to provide candidate proteins involved in the molecular mechanisms concerning HK-2 cell injury and kidney stone formation. HK-2 cells were exposed to oxalate plus COM crystals at different concentrations in various samples. Cell viability was determined using a Cell Counting Kit-8 assay kit. For proteomic analysis, cells were exposed to oxalate (2 mM) and COM crystals (200 ug/ml) for 12 h. The proteins were separated by two-dimensional electrophoresis and the differentially expressed proteins were identified by liquid chromatography electrospray ionization tandem mass spectrometry (LC–ESI-MS/MS). Validation of protein expression was further performed by Western blot analysis. Oxalate and COM crystals showed concentration-dependent toxicity on HK-2 cells. A total of 12 differentially expressed proteins in HK-2 cells induced by oxalate and COM crystals were identified, which were involved in various aspects of cellular processes. Our study provides a platform for further studying the molecular mechanism of renal epithelial cell injury and kidney stone formation.

Keywords Renal epithelial cell · Oxalate · Calcium oxalate monohydrate · Nephrolithiasis · Proteomics

Introduction

The exact formation mechanism of renal calcium oxalate stones is complex and remains indistinct. Recently, much attention has been paid to the intracellular events possibly related to the initiation of kidney stones. Both animal models of hyperoxaluria and in vitro cell culture studies have demonstrated that exposure to high levels of oxalate [1–4] and/or calcium oxalate monohydrate (COM) crystals [3–6] is injurious to renal epithelial cells and triggers serial responses closely related to stone formation [7]. Oxalate and/or calcium oxalate crystals induce oxidative stress in the kidneys with an increase in free radical production leading to cell damage and even apoptosis or cell death [1, 8–10]. Fragments of dead cells may serve as nidi for crystal nucleation and promote crystal aggregation [7]. Crystal retention may also be promoted with increased exposure of crystal attachment sites as a result of tubular cell detachment or membrane structure alteration after oxalate and/or crystal injury [7, 11, 12]. Besides, cell injury caused by oxalate ions and crystals may elicit alteration of gene expression and protein production of various

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chemoattractants and urinary macromolecules, which either play important roles in inflammatory reaction and cellular signaling pathways contributing to stone formation, or are directly involved in the regulation of crystal nucleation, growth and deposition. Until now, the known crystallization modulators and chemoattractants in renal tubular cells, the expression of which is altered by exposure to oxalate and/or COM crystals mainly include Tamm-Horsfall protein, osteopontin, prothrombin fragment-1, bikunin, fibronectin, matrix gla protein, p38 mitogen-activated protein kinase (MAPK), MCP-1, IL-6, etc. [1, 2, 4, 7, 13–19].

However, the interaction between oxalate/COM crystals and renal tubular cells consists of complex processes with multiple molecules and active proteins involved. Since most of the previous studies merely aimed at one or two genes and proteins with isolated results, an overall understanding of the alteration of protein expression after oxalate and/or crystal exposure as well as its role in stone formation is limited. Besides those that have been studied, we speculate that there may be other new candidate proteins that mediate physiological and pathological processes involved in renal epithelial cell injury and kidney stone formation. It was not until recently that studies using comparative proteomic analysis explored the altered proteins in MDCK renal tubular cells (a cell line derived from dog kidney exhibiting distal renal tubule phenotype) in response to calcium oxalate monohydrate crystal at different concentrations [20, 21] or calcium oxalate dihydrate crystals [22]. Although these researchers have found several new candidate proteins, further studies are necessary, since high levels of oxalate are always accompanied with crystalluria under physiological conditions and use of cells from other segments of the nephron may result in different findings. In the present study, we therefore carried out two-dimensional electrophoresis (2-DE) combined with liquid chromatography electrospray ionization tandem mass spectrometry (LC–ESI-MS/MS) to reveal the changes in the proteome of HK-2 cells (a line of human proximal tubular epithelial cells) exposed to oxalate and COM crystals, to provide candidate proteins for new insights into the molecular mechanisms concerning HK-2 cell injury and kidney stone formation. We used HK-2 cells instead of cells from other segments of the nephron, because the proximal tubule is the major site of renal oxalate handling [23]. Moreover, HK-2 cell line has been frequently used in several of previous studies on oxalate or COM crystal-induced renal tubular cell injury. To our knowledge, this study represents the first comparative proteomic analysis of human renal tubular cells injured by both oxalate and COM crystals and we successfully identified proteins that may be involved in the development of stones.

Materials and methods

Preparation of COM crystals

Briefly, equal volumes of 10 mM $CaCl_2$ and 10 mM NaOX were mixed at room temperature. COM crystals were formed immediately and the suspension equilibrated for 3 days at 4°C. The crystals were then washed with deionized water and dried at 60°C. Crystals were confirmed to be COM by Fourier transform infrared (FT-IR) spectroscopy (data not shown). A stock solution of COM (5 mg/ml) was prepared in sterile PBS.

Cell culture and CCK-8 assay

HK-2 cells were kindly provided by Prof. Mei Changlin (Center of Kidney Disease, Second Military Medical University, China). The cells were serially passaged and maintained as subconfluent monolayers on 75 cm^2 Falcon T-flasks in high glucose D-MEM (Gibco, USA) containing 10% fetal calf serum (FBS, Gibco, USA) at 37°C in a 5% carbon dioxide incubator. The medium was replenished two to three times weekly and was changed to serum-free D-MEM before experiments under various conditions conducted to minimize interaction of crystals with serum proteins and other constituents [24].

HK-2 cell cytotoxicity assay

HK-2 cells were grown to confluence on a 96-well plate. For the treatment groups, the medium was then replaced with serum-free D-MEM and the cells were exposed to oxalate (1, 2, 3, 5 or 10 mM) plus COM crystals (200 μg/ml) for various internals (4, 12 and 24 h). We used oxalate plus inorganic COM crystals of 200 μg/ml in the present study because high levels of oxalate are always accompanied by crystalluria under physiological conditions in vivo and studies performed in different cell types had demonstrated that COM crystal binding reaches saturation at concentrations approaching 200 μg/ml [25]. Besides, inorganic COM crystals have been frequently used in many previous studies concerning COM crystals and renal tubular cell injury. Cell viability was determined with a Cell Counting Kit-8 assay kit (CCK-8, Dojindo, Kumamoto, Japan) according to the product protocol. Sham exposure (HK-2 cells in serum-free D-MEM without oxalate and COM crystals) was used as control.

Sample preparation for 2-DE

For the treatment groups, confluent HK-2 cells in serum-free D-MEM were exposed to oxalate (2 mM) and COM crystals (200 μg/ml) for 12 h. The high concentration of

oxalate and the duration of cell exposure were selected based on earlier reports from others [2, 8, 17, 24, 26, 27] and the results of our previous studies, in which we had determined the effect of oxalate and COM crystals on cell viability and protein expression in HK-2 cells after an exposure for 4, 8, 12 and 24 h. We found that the protein expression was significantly altered after a 12-hour exposure (data not shown). Besides, HK-2 cell cytotoxicity assay showed that the 12-h group had the most linear decrease in cell viability with the least variance (Fig. 1), suggesting that it was stable at the time point between the other two in the analysis. Since proteomic analysis is mainly used for a preliminary screening of altered protein expression profile, we used one time point and a specified concentration in the present study. Sham exposure was used as control. At the end of the experimental period, cells were washed three times with phosphate-buffered saline (PBS; pH 7.4) and incubated on ice with lysis solution (9.5 M urea, 65 mM DTT,4% CHAPS, 0.2% IPG buffer) containing the protease inhibitor PMSF (1 mM) for 30 min. The cell suspension was subsequently sonicated for protein extraction. The protein concentration was determined by the Bradford method.

Two-dimensional electrophoresis and staining

For the first-dimension electrophoresis (isoelectric focusing, IEF), samples containing 100 μg of proteins were diluted with rehydration buffer (8 M urea, 2%CHAPS, 18 mM DTT, 0.5%IPG buffer, bromophenol blue trace) and applied onto a protein IEF cell (Bio-Rad). Samples were then rehydrated with nonlinear gradient IPG strips (13 cm, pH 3–10) at 17°C and at 30 V for 12 h. IEF was performed at 500 V for 1 h, 1,000 V for 1 h, and 8,000 V for 10 h to reach a total of approximately 60–80 kVh. The second-dimensional electrophoresis was carried out by 12.5% SDS–polyacrylamide gels with 40 mA till the bromophenol blue front reached the bottom of the gel. The two-dimensional gels were stained with the silver diamine staining method. For preparative 2-DE, 400 mg of total proteins was separated as described above. Proteins were detected by a modified silver-staining method compatible with MS analysis.

Image analysis

Two-dimensional gels were scanned by a GS-710 imaging densitometer (Bio-Rad). Differences in protein expression levels among samples were analyzed with an Image Master software (Amersham). Following spot detection, a matched set including all three batches of gels was built. A reference gel was selected from the control gels, and unmatched spots were added to the reference gel. Normalization was based on total spot density.

Protein identification by ESI-MS/MS

Differentially expressed protein spots were excised from the preparative gels, destained and digested overnight. Extracted peptides were analyzed by a Finnigan LTQ mass spectrometer (ThermoQuest, San Jose, CA, USA) coupled with a Surveyor HPLC system (ThermoQuest) for protein identification. Briefly, Microcore RP columns (C18 0.15MM*150MM; ThermoHypersil, San Jose, CA, USA) were used to separate the protein digests. Solvent A was 0.1% v/v formic acid, and solvent B was 0.1%v/v formic acid in 100% v/v ACN. The gradient was held at 2% solvent B for 15 min, and increased linearly to 98% solvent B in 90 min. The peptides were eluted from C18 microcapillary column at a flow rate of 120 μl per min and then electrosprayed directly into LCQ-Deca mass spectrometer with the

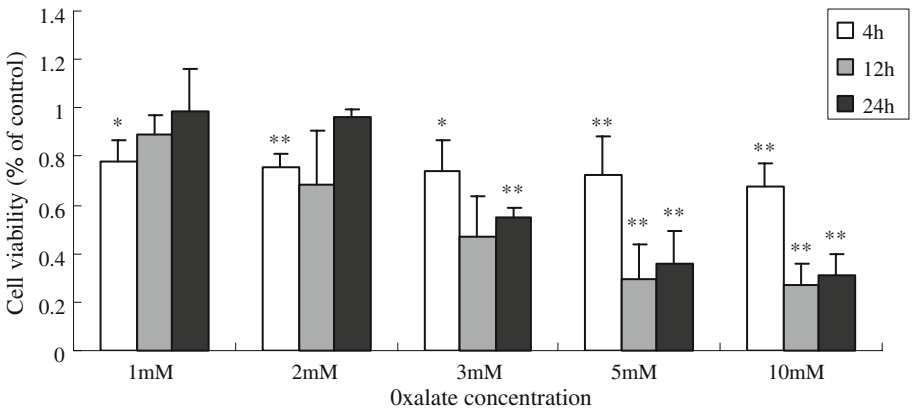

Fig. 1 Cytotoxicity assay by a CCK-8 kit. HK-2 cells were grown to confluence on a 96-well plate. The medium was then replaced with serum-free D-MEM and the cells were exposed to oxalate (1, 2, 3, 5 or 10 mM) plus COM crystals (200 μg/ml) in various samples (4, 12 and 24 h). Cell viability was determined with a Cell Counting Kit-8 assay kit according to the product protocol. $*P < 0.05$ and $**P < 0.01$ as compared with the control group

application of spray voltage of 3.0 kV and with the capillary temperature at 170°C. The full scan ranged from M/Z 400 to 2,000. Protein identification using MS/MS raw data was performed with SEQUEST software based on the database of Swissport. The specie was human. Both b ions and y ions were included in the database search. Protein identification results were filtered with the Xcorr (1 + ≥1.9, 2 + ≥2.2, 3 + ≥3.75) and DelCn(≥0.1). The main function of each identified protein was discussed according to NCBI and Expasy protein database.

Western blot analysis

Western blot analysis of the same protein preparations used in the 2-D gel experiments was used to validate differential expression of two identified candidate proteins (ENO1 and cofilin-1). Protein extracts (50 μg) were separated on 12.5% SDS polyacrylamide gel electrophoresis. Proteins were electroblotted to nitrocellulose membranes, which were then blocked for 30 min at 37°C in 5% (w/v) nonfat dry milk in Tris-buffered saline. The membranes were incubated with mouse anti-human β-actin monoclonal antibody (1:500 in 5% milk/PBS, Sigma, USA), mouse anti-human α-enolas monoclonal antibody (1:500 in 5% milk/PBS, Santa Cruz Biotechnology, USA) and mouse anti-human cofilin-1 monoclonal antibody (1:500 in 5% milk/PBS, Santa Cruz Biotechnology, USA) overnight at 4°C and then incubated for 1 h with goat anti-mouse secondary antibody (1:2,000 dilution). The positive bands were revealed using Western blotting detection reagents and autoradiography films. Relative molecular band intensity was determined by densitometry.

Statistical analysis

One-way ANOVA with LCDs post hoc test was used to compare differences in cell viability among multiple groups. Two-sided Student's *t* tests were used to analyze differences in protein levels between control and the injured cell group. An associated probability (*P*) value of <0.05 was considered significant.

Results

Cytotoxicity assay by CCK-8

Cells exposed to oxalate (1, 2, 3, 5 or 10 mM) plus COM crystals (200 μg/ml) in various samples (4, 12 and 24 h) were determined. As showed in Fig. 1, oxalate and COM crystals showed concentration-dependent toxicity on HK-2 cells, which, however, was not merely increased with action time except in the case of 1 mM oxalate. These results showed that both cell propagation and apoptosis may be included in the complex reactions of HK-2 cells to oxalate and COM crystals with different concentrations and for various exposure times.

Analysis of 2-DE

Changes in protein expression profiles between control HK-2 cells and injured HK-2 cells were examined using 2-DE. Three technical replicates of analytical gels were prepared from one sample in each experiment. We detected 2,204 ± 87 spots in the control maps and 2,347 ± 57 spots in those of the injured HK-2 cells. Comparison of the 2-DE patterns with the Image Master software revealed that exposure to oxalate and COM crystals significantly altered the expression of 12 proteins (a protein was accepted to be differently expressed if there was at least a 1.2-fold difference in the spot intensity associated with a *P* value of <0.05 by Student's *t* test in all matched sets), among which 9 were up-regulated, whereas 3 were down-regulated. A difference of 1.2-fold was considered to be significant, based on our 2-DE results and was supported by literature [28–30]. The representative silver-stained 2-DE gels of proteins are presented in Fig. 2.

Protein identification by ESI-MS/MS

To identify the differentially expressed proteins, the extracted peptides were subjected to ESI-MS/MS. With this method and subsequent sequence search in the SWISS-PROT database, identities of the 12 spots were determined (Table 1).The identified differentially expressed proteins are involved in various aspects of cellular processes, including energy metabolism, cellular proliferation and apoptosis, stress defense, regulation of Ca^{2+} channel activity, protein synthesis and other functions, indicating the complexity of the interaction between oxalate/COM crystals and HK-2 cells.

Western blot analysis

The expression of ENO1 and cofilin-1 was detected by Western blotting to validate the expression changes observed. Results showed that in the injured HK-2 cells, ENO1 was more highly expressed, while the expression of cofilin-1 was decreased (Fig. 3), which are consistent with the observations in the 2-DE experiment.

Discussion

In the present study, as a first step, we demonstrated that oxalate and COM crystals had concentration-dependent

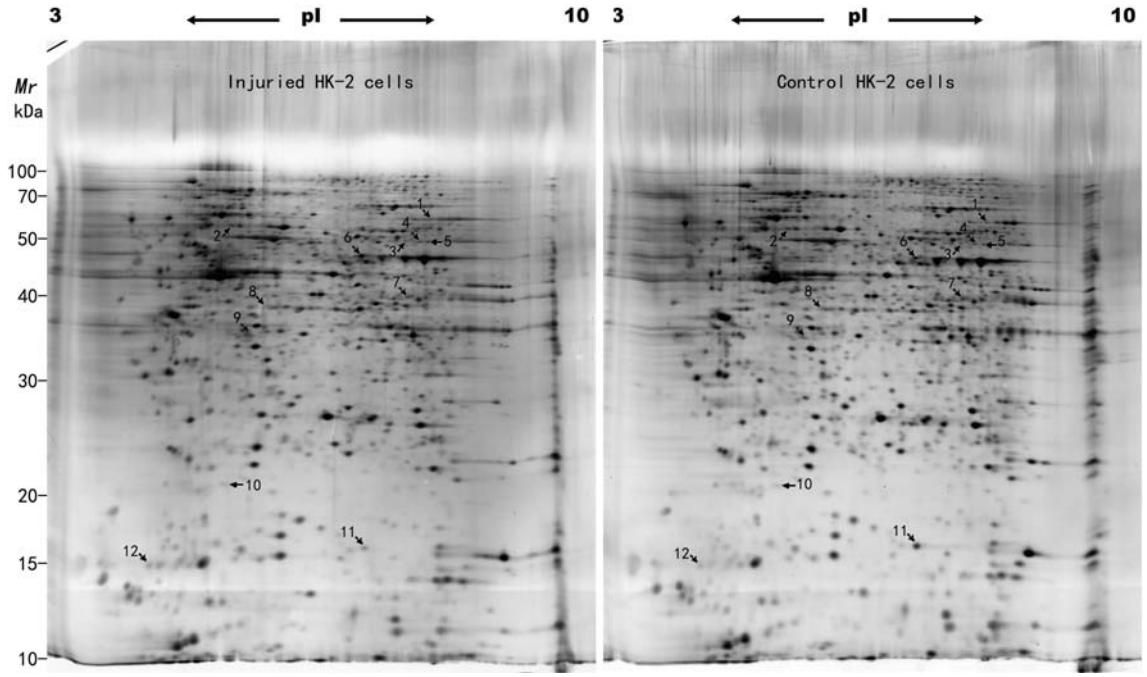

Fig. 2 The 2-DE maps of proteins from the injured HK-2 cells and the control HK-2 cells. Confluent HK-2 cells in serum-free D-MEM were exposed to oxalate (2 mM) and COM crystals (200 ug/ml) for 12 h. HK-2 cells in serum-free D-MEM without oxalate and COM crystals were used as control. At the end of the experimental period, cells were washed and harvested. The cell suspension was subsequently sonicated for protein extraction. Isoelectric focusing performed on pH3-10 IPG strips (13 cm, nonlinear) was followed by the second-dimensional electrophoresis with 12% SDS–polyacrylamide. Gels were stained with the silver diamine staining method

toxicity on HK-2 cells, which, however, was not simply increased with longer action time, indicating potential adaptation reactions in HK-2 cells after injury. Based on the result, we carried out 2-DE coupled with ESI-MS/MS to compare protein expression in control HK-2 cells and HK-2 cells exposed to oxalate plus COM crystals for 12 h, so as to reveal the protein expression pattern for identifying new candidate proteins that mediate physiological and pathological processes involved in HK-2 cell injury and kidney stone formation. With this method, a total of 12 differentially expressed proteins were successfully identified. To better understand the roles of these proteins in HK-2 cell injury and the potential relevance to stone formation, we group and discuss the main proteins according to their functions.

FK506-binding protein 4

One of the up-regulated proteins by the exposure, FK506-binding protein 4 (FKBP4, also called FKBP52, FKBP59), is an immunophilin belonging to the FK506-binding proteins family [31]. The most studied role of FKBP4/FKBP52 is with regard to its involvement in the regulation of androgen receptor-mediated signaling and physiology, by influencing steroid–hormone binding affinity and transcriptional activity [32, 33]. Besides its function as a chaperone, FKBP52 was recently found to have an inhibitory action on the activity of the transient receptor potential (TRP) Ca^{2+} channel TRPV5 to decrease Ca^{2+} reabsorption [34]. In the kidney, the active reabsorption of Ca^{2+} mainly occurs in the distal convoluted and connecting tubules where TRPV5 has been demonstrated to be the gatekeeper in active Ca^{2+} reabsorption, determining the final amount of Ca^{2+} excreted in the urine [35]. Thus, taking into account the close relationship between hypercalciuria and calciferous stones, as well as the interesting findings of up-regulated FKBP4 by exposure to oxalate and COM in the present study, we speculate that in patients with hyperoxaluria, the high concentration of oxalate and/or calcium oxalate may cause high expression of FKBP4 in the renal epithelia, which subsequently inhibits the activity of TRPV5 and decreases Ca^{2+} influx, leading to hypercalciuria and stone formation. Since the mechanism of kidney stone formation remains unclear, such a possible action pathway seems to be of great significance in the prevention and treatment of stone formation and is worthy of further study.

Proteins involved in cellular energy metabolism

In the present study, five proteins involved in cellular energy production were all significantly up-regulated by the exposure. Pyruvate kinase isozymes M1/M2 (PKM2) and L-lactate dehydrogenase B chain (LDHB) are both glycolytic enzymes. Pyruvate kinase catalyzes the transfer

Table 1 Identified proteins in HK-2 cells altered by oxalate and COM crystals

Spot	SWISS- PROT accession	Name of protein	Intensity levels (Mean ± SD)		Degree of change (fold)	No. of identified peptides	Cover percentage (%)	Theoretical Mw (kDa)/ pI	Measured Mw (kDa)/pI	Function
			Control	Oxlate + COM						
Up-regulated proteins										
1	P14618	Isoform M1 of Pyruvate kinase isozymes M1/M2	0.0146 ± 0.0006	0.0329 ± 0.0140	1.24	9	10.17	57.9/7.96	55/7.5	Energy metabolism
2	Q02790	FK506-binding protein 4	0.0335 ± 0.0022	0.0508 ± 0.0076	1.21	16	19.39	51.8/5.35	52/5.5	Ca^{2+} channel activity regulation
3	P28838	Isoform 1 of Cytosol aminopeptidase	0.0114 ± 0.0009	0.0276 ± 0.0091	1.50	6	9.22	52.77/6.3	50/7.2	Protein synthesis
4	Q16658	Fascin	0.0220 ± 0.0009	0.0410 ± 0.0116	1.29	12	16.84	54.5/6.84	50/7.4	Cell movement
5	P25705	ATP synthase subunit alpha	0.0180 ± 0.0028	0.0412 ± 0.0110	1.45	5	7.78	59.8/9.61	50/7.6	Energy metabolism
6	P06733	Isoform alpha-enolase of Alpha-enolase	0.0617 ± 0.0032	0.1185 ± 0.0267	1.41	56	36.41	47.2/7.01	45/6.7	Energy metabolism
8	O95861	Isoform 1 of 3'(2'),5'-bisphosphate nucleotidase 1	0.0215 ± 0.0017	0.0327 ± 0.0042	1.23	3	6.82	33.4/5.46	38/6	Energy metabolism
9	P07195	L-lactate dehydrogenase B chain	0.0409 ± 0.0069	0.0859 ± 0.0270	1.23	7	16.47	36.6/5.71	34/6	Energy metabolism
12	P08708	40S ribosomal protein S17	0.0438 ± 0.0058	0.0713 ± 0.0066	1.29	8	29.63	15.6/9.85	15/4.5	Protein synthesis
Down-regulated proteins										
7	O43684	Mitotic checkpoint protein BUB3	0.0641 ± 0.0066	0.0409 ± 0.0061	1.23	12	23.93	37.2/6.36	39/7.2	Cell propagation and apoptosis
10	P06748	Isoform 2 of Nucleophosmin	0.0565 ± 0.0034	0.0280 ± 0.0040	1.66	4	9.06	29.5/4.47	23/5.5	Cell propagation and apoptosis
11	P23528	Cofilin-1	0.1345 ± 0.0024	0.0569 ± 0.0068	2.08	10	34.34	18.5/8.22	16/6.7	Cell propagation and apoptosis

MW/pI molecular weight/isoelectric point

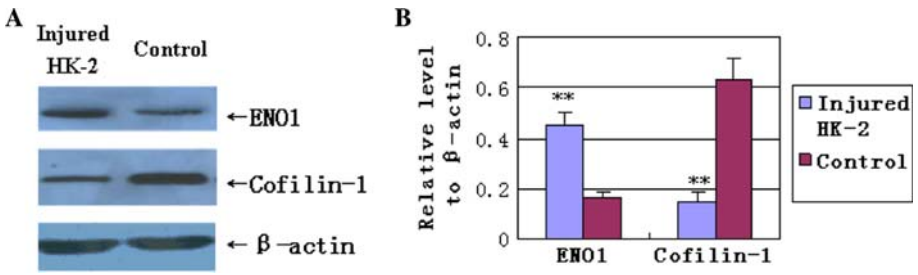

Fig. 3 Western blot analysis of the same protein preparations used in the 2-D gel experiments was used to validate differential expression of two identified candidate proteins (ENO1 and cofilin-1). **a** Representative Western blots for the expression analyses of ENO1 and Cofilin-1. β-actin was used as the loading control. **b** The ENO1 and Cofilin-1 protein levels relative to β-actin protein levels were assessed by densitometric analysis $^{**}P < 0.01$, compared with the respective controls

of a phosphoryl group from phosphoenolpyruvate (PEP) to ADP, generating ATP. Pyruvate is reduced to lactate in a reversible reaction catalyzed by lactate dehydrogenase when there is lack of O_2 [36]. Perhaps, the increase in the two proteins in response to oxalate and COM exposure is an adaptive and protective response of HK-2 cells to increase energy production. This is further supported by the observed up-regulation of ATP synthase subunit alpha (ATP5A1). Within the mitochondrion, ATP is produced from ADP by mitochondrial membrane ATP synthase in a process known as oxidative phosphorylation.

Another piece of evidence supporting the adaptive response of HK-2 cells to the injury caused by oxalate and COM crystals is the increased expression of alpha-enolase (ENO1), an enzyme that plays multiple roles in various processes, including glycolysis, growth control, hypoxia tolerance and allergic responses [37]. Studies with endothelial cells have proved that in a hypoxic situation, upregulation of α-enolase may provide protection to the cells by increasing anaerobic metabolism [38]. We presume that a similar response may occur in HK-2 cells in adapting to injury induced by oxalate/COM crystals.

Proteins involved in cell propagation and apoptosis

Although COM crystals alone may increase DNA synthesis and proliferation of renal epithelial cells [39], more researchers have demonstrated that oxalate inhibits the proliferation of several types of renal cells [1, 8–10, 40, 41]. In our study, high levels of oxalate plus COM crystals were observed to have a negative effect on the growth rate of HK-2 cells measured by CCK-8. This may also be reflected by the down-regulation of mitotic checkpoint protein Bub3 (BUB3). During cell division, Bub3 localizes to kinetochores before chromosome alignment and is necessary for kinetochore localization of Bub1 and BubR1, two important proteins of the BUB/MAD signaling pathway, subsequently activating the checkpoint in response to unattached kinetochores [42].

Conversely, another protein related to cell viability, cofilin-1, was down-regulated in our study. Cofilin-1 controls reversible actin polymerization and depolymerization induced by TGF [43], and is thus involved in the dynamics of actin cytoskeleton, which is directly related to apoptosis and cell motility. Reduction of cofilin protein levels with small-interfering RNA resulted in inhibition of both cytochrome c release and apoptosis [44]. In our study, the observed down-regulation of cofilin-1 in HK-2 cells remains unexplained, but perhaps it is a counteraction to cell apoptosis caused by oxalate and COM crystal injury. This is supported by studies in which oxalate at certain low concentrations or COM crystals were shown to promote renal tubular cell proliferation [45, 46], though high levels of oxalate and COM crystal injury have shown to induce renal tubular cell apoptosis. Whether the cell attempts to counter the oxalate apoptosis by decreasing proteins along the apoptotic pathway would have much biological sense in the understanding of tubular cell injury and is worthy of further investigations.

Proteins involved in protein synthesis

Two proteins up-regulated by oxalate/COM crystal injury, isoform 1 of cytosol aminopeptidase (LAP3) and 40S ribosomal protein S17 (RPS17), are both involved in protein synthesis. LAP3 is also responsible for the processing and regular turnover of intracellular proteins. The up-regulation of the two proteins may reflect abnormally expressed proteins or increased protein turnover in HK-2 cells as a result of oxalate/COM injury.

Proteomic analysis has become one of the most promising tools for investigating pathophysiology at the molecular level by measuring the relative differences in protein expression between samples under different experimental conditions. However, like any other new technique, limitations of proteomics due to technical variation and biological variation are also ineluctable. For example, spots in 2D gels are notorious for containing 1–22

proteins, and variations in phosphorylation and glycosylation can change the MW by up to 40 kD, resulting in omission of some interesting proteins. The degree of technical variation from the process of 2-DE has been defined as up to 20–30% coefficient of variation [47, 48]. These disadvantages of proteomics, as well as different experimental conditions used, may be the reasons that several previously described proteins dysregulated in stone formers were not observed in our study, as well as in the similar studies of Thongboonkerd V et al. [20] and Semangoen T et al. [21, 22]. Despite all this, proteomic analysis still present as an unbiased approach that gives an overall screening of altered protein expression profile.

In conclusion, the interaction between oxalate/COM crystals and renal tubular cells consists of complicated processes with multiple molecules and active proteins involved. High levels of oxalate and COM crystals may cause cell damage, apoptosis or cell death. However, the cells have adaptive responses as self-protection from damage and express various proteins promoting or inhibiting stone formation. Our initial characterization of this proteomic profiling provides a platform for further investigations of functional roles of the identified proteins in the molecular mechanism concerning renal epithelial cell injury and kidney stone formation.

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