Urinary proteomics to decipher molecular pathophysiology of CKD progression

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Introduction

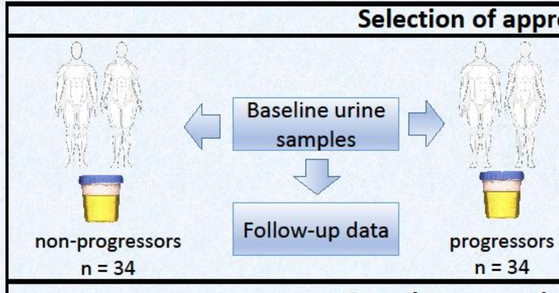
Current diagnostic methods are applicable for the detection of advanced stages of chronic kidney disease (CKD) [1]. However, detection of early stages as well as prognosis of disease progression still remain challenging. As severity of CKD increases with each consecutive stage, so does the social and economical burden associated with it [2]. Therefore, understanding the disease pathophysiology and preventing its progression is an important clinical need. Due to the complex nature of CKD, biomarker discovery studies performed on various molecular levels are required. Such effort for the identification of molecular determinants of CKD progression is performed within the iMODE-CKD research project (www.imodeckd.org).

Aim

The present study involves the urinary proteome analysis of a large sample cohort for the identification of novel biomarkers of CKD progression. Obtained results will be combined with existing –omics data on CKD as well as newly generated –omics findings from iMODE-CKD partners allowing the elucidation of CKD progression.

Materials and Methods

Selection of appropriate clinical samples



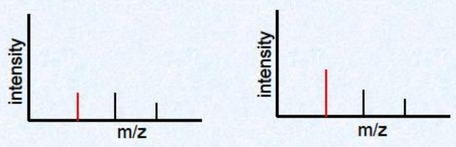
- 500 µL of urine
- CKD stages II – IV
- % annual eGFR slope changes:
  - Non-progressors: between -1.2 and 4.5%
  - Progressors: below -4.3%

Sample preparation and protein digestion


Filter-aided sample preparation

- Samples were processed according to the FASP protocol with minor modifications
- Reproducibility of the approach was confirmed by initial experiments on five technical replicates of stage IV CKD urine samples

LC-MS/MS analysis



Orbitrap Velos

- Chromatography column: C18 reverse-phase (Dionex, Sunnvale, CA, USA)
- Instrument: Orbitrap Velos (FTMS, Thermo Finnigan, Bremen, Germany)
- 8 µg of total protein content loaded onto the column
- 8h gradient run

Data processing, differential expression and bioinformatics analysis


Proteome Discoverer, in-house software, R, Cytoscape

- Peptide identifications present in at least 60% samples (21 our of 34 replicates) in at least one group were used for the analysis
- Normalization based on total-ion-current
- Fold change: ratio progressors/non-progressors
- Differential expression classification:
  - p-Value ≤ 0.05
  - Fold change: below 0.66 or above 1.5

Figure 1: Graphical depiction of the study. 68 baseline urinary samples from CKD patients with at least 2 years of follow-up data were classified as CKD non-progressors or progressors based on the eGFR slope % changes. Samples were processed according to the FASP protocol [3] and analyzed by LC-MS/MS. Protein identification was performed using the SEQUEST search engine (Proteome Discoverer. 1.4., Thermo Scientific) against SwissProt human database. The following stringent criteria were used: peptide confidence: high (FDR<1%), mass peak deviation: 5 ppm and maximum peptide rank: 5. Peptide grouping was enabled. Pathway analysis of differentially expressed proteins was performed using Reactome pathway database by applying the Cytoscape ClueGo plugin.

Literature search

All proteins found to be differentially expressed based on the proteomics analysis were compared to findings reported in the literature. The latter are currently being organized in the form of a CKD proteomics database. So far (up to 04/30/2015) data from 50 manuscripts (carrying the key-words: "kidney" and "proteom*" or "biomarker" and "nephropathy" and "spectro*") have been manually extracted and curated within the iMODE-CKD consortium and were used in the presented study.

Table 1: Study cohort clinical data

		All n=68	Non-progressors n=34	Progressors n=34	p-Value
Gender [F/M]		21/47	10/24	11/23	0.80
Age [years]		65±15	67±14	67±15	0.29
Body Mass Index [BMI]		29±5	29±4	30±5	0.16
Pulse [min]		68±12	67±11	69±13	0.56
Systolic blood pressure [mmHg]		139±19	134±17	144±19	0.04
Dyastolic blood pressure [mmHg]		79±9	80±7	78±10	0.41
Diabetes [Y/N]		23/45	10/24	13/21	0.45
Baseline SCr [mg/dl]		1.6±0.5	1.6±0.5	1.7±0.6	0.31
Baseline eGFR [ml/min/1.72]		44±16	46±15	42±16	0.26
CKD stage	II	11	7	4	-
	III	43	21	22	-
	IV	14	6	8	-
	All	68	34	34	0.32
Follow up duration [years]		2.6±0.4	2.6±0.4	2.6±0.4	0.52

Table 2: Annual eGFR slope changes

%slope/year	All n=68	Non-progressors n=34	Progressors n=34
minimum	-30	-1,2	-30
maximum	4,5	4,5	-4,3
mean ± SD	-6±9	0.7±1.4	-13±8

Results

Comparison of the differentially expressed proteins with literature data

81 differentially expressed proteins between progressors and non-progressors found by the LC-MS/MS analysis have been previously reported as being differentially expressed in subjects with renal dysfunction compared to controls. The study is ongoing and the presented literature search will be expanded. Nevertheless, the obtained overlaps between the LC-MS/MS analysis and literature data support the validity of our approach.

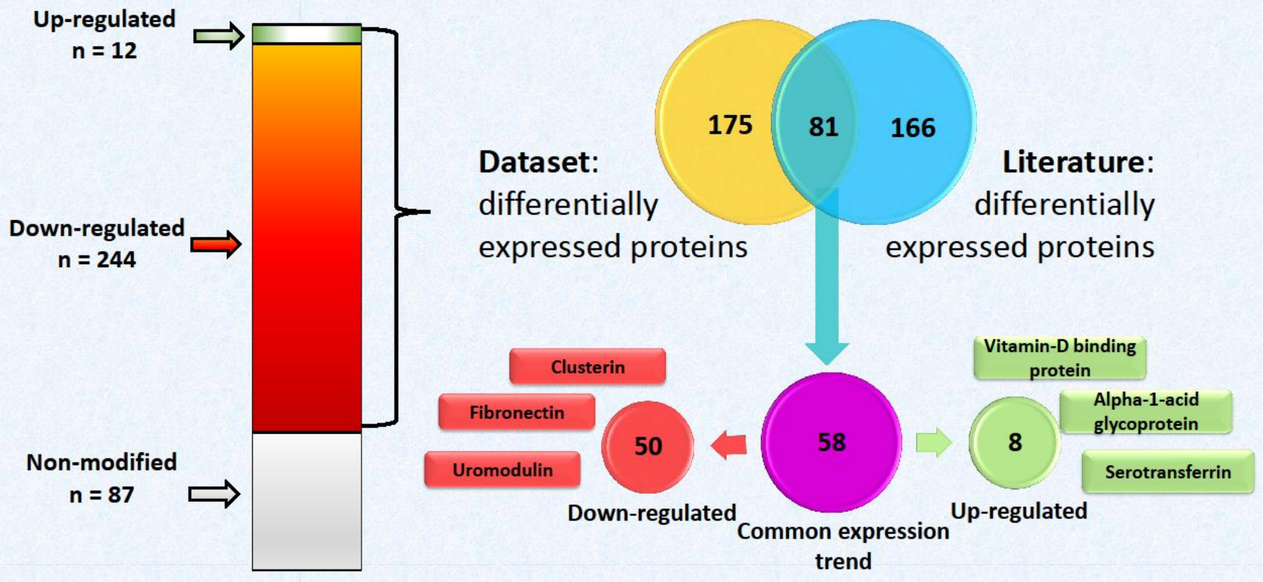


Figure 2: Number of differentially expressed proteins between progressors and non progressors and comparison to literature data.

Evaluation of proteins with common expression trends in the dataset and literature

Most of the common protein identifications were found down-regulated in the progressors compared to non-progressors and only a few were up-regulated, suggesting impairment of key biological functions in CKD progressors.

Table 3: List of 30 representative differentially expressed proteins (progressors/non-progressors).

Protein name	p-Value	Fold change	Protein name	p-Value	Fold change
Serotransferrin	4.7E-05	3.19	Vasorin	1.9E-04	0.52
Ceruloplasmin	8.9E-04	1.77	Cadherin-1	2.1E-04	0.41
Alpha-1-antitrypsin	1.2E-03	3.94	Osteopontin	3.6E-04	0.44
Afamin	4.7E-03	2.27	Kininogen-1	5.2E-04	0.40
Alpha-1-acid glycoprotein 2	8.4E-03	1.71	Fibronectin	5.8E-04	0.44
Vitamin D-binding protein	1.0E-02	2.79	Kallikrein-1	7.5E-04	0.22
Alpha-1B-glycoprotein	3.3E-02	1.69	Dipeptidyl peptidase 4	7.9E-04	0.45
Alpha-1-acid glycoprotein 1	3.6E-02	1.62	CD44 antigen	1.4E-03	0.54
Plasma serine protease inhibitor	1.9E-05	0.27	Plasma protease C1 inhibitor	2.6E-03	0.57
Apolipoprotein D	2.6E-05	0.30	Aminopeptidase N	3.0E-03	0.55
Collagen alpha-1(XV) chain	3.8E-05	0.10	Glutathione peroxidase 3	3.2E-03	0.37
CD59 glycoprotein	4.0E-05	0.32	Basement membrane-specific heparan sulfate proteoglycan core protein	5.5E-03	0.53
Pro-epidermal growth factor	7.9E-05	0.23	Uromodulin	7.9E-03	0.56
Cathepsin B	8.9E-05	0.35	Lysosome-associated membrane glycoprotein 2	8.5E-03	0.44
Mannan-binding lectin serine protease 2	1.2E-04	0.35	Clusterin	4.7E-02	0.58

Consistency of results from pathway analysis of data extracted from the literature and newly collected proteomics data

Pathways associated with proteolytic activity (i.e. changes in extracellular matrix) play a substantial role in CKD progression. Common as well as non-overlapping pathways between the present study and literature will be further investigated after expanding the literature search.

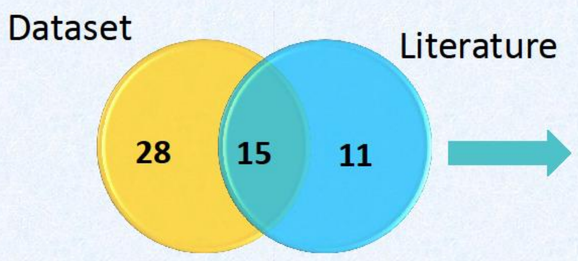


Figure 3. Reactome pathway analysis of datasets obtained from the present study and literature. Common pathways were combined into representative processes.

Conclusions

We have identified a number of differentially expressed proteins between CKD progressors and non-progressors in urine from a large sample cohort. Agreement of the proteomics findings to existing knowledge in the field supports the validity of our approach. As an example, in agreement with the literature, our findings support a significant deregulation of proteolytic activity and extracellular matrix organization in patients with impaired renal functions.

The identification of progression biomarker candidates is still in progress in parallel to a comprehensive literature mining and correlation to clinical data. Promising candidates will be further validated in an independent sample cohort (CKD-Bio prospective study) using multiple reaction monitoring (MRM) and/or ELISA.

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References:

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