Identifying biomarkers of fibrosis from the urinary proteome of renal fibrosis patients using DIA mass spectrometry David R. Spiciarich, Solange Moll, Marco Prunotto, Sumedh R. Sankhe, W. Rodney Mathews, Veronica G. Anania 1. Department of OMNI Biomarker Development, Genentech, Inc., South San Francisco, CA 2. Division of Clinical Pathology, Department of Pathology and Immunology, Geneva University

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Background and objectives

Renal fibrosis is a hallmark of chronic kidney disease, entailing an excess accumulation of extracellular matrix components, primarily collagen species, disrupting organ architecture and leading to renal function loss. Histological assessment of renal tissue obtained by biopsy is the gold standard for determining the extent of fibrosis; however, the procedure is invasive. Urine is proximal to the site of disease activity and can be collected non-invasively, thereby making it an ideal matrix for biomarker discovery. Here, the results of a study to characterize the urinary proteome are presented focusing in particular on investigating collagen fragments as potential biomarkers for fibrosis in urine longitudinally sampled from renal transplant patients.

Cohort

Renal transplant patients (n=19) with 2-4 longitudinal biopsies with matched urine (n=56) were collected at Geneva University Hospital with informed consent.





Methods

Urine samples were prepared with a 10 kDA filter-assisted preparation (FASP) followed by trypsinolysis and LC-MS/MS analysis. A spectral library was built from twelve pooled urine samples processed on a Q-Exactive-HF with a 120 min gradient using a Top 15 data-dependent acquisition (DDA) method. The resultant raw files were searched for both tryptic and semi-tryptic peptides using SpectroMine (Biognosys AG, Switzerland). Individual samples were processed using a data-independent acquisition method (DIA) with one full range MS1 and 22 DIA variable width windows and analyzed with Spectronaut. Biopsies were fixed and stained with trichrome stain and the extent of fibrosis was determined by a trained pathologist.

	Proteins	Peptides (with PTMs)
Renal fibrosis library (Tryptic & semi-tryptic)	3,481	54,114
Collagens	28	4,012
Collagen peptides with hydroxyproline		3,145
Collagen neoepitope peptides		3,657

Evaluation of three sample preparation methods: 10kDA FASP effectively captured collagen proteins & peptides

		HVA
Prep	aration Method description	600
1.	 FASP 10 kDa MWCO filters 	500 -
	 Protein extraction 	400 -
2.	• FASP 30 kDa MWCO filters	300 -
	 Protein extraction 	200 -
3.	 Norgen ProteoSpin[™] Urine Protein 	100-
All	• Tryptic digest	Col
	 C18 cleanup for mass spectrometry 	- 10

In this retrospective study, 56 urine samples from 19 renal transplant patients who had undergone >3 sequential kidney biopsies with progressive fibrosis score (0 to 60%) were analyzed by DIA.

	Proteins	Peptides	Collagen proteins
Average	1,888	21,301	18
Avg Lib Recovery (%)	54.2	52.9	
Total	3,034	49,580	22

Fibrosis score moderately tracks with collagen protein family members, but interestingly several collagens were correlated with other collagens.

	Fibrosis score	COLIAI	COLIAZ	COL3A1	COL4A1	COL4A2	COL5A1
Fibrosis score		-0. 23	- <mark>0.</mark> 21	-0.29	-0.31	-0.43	- 0. 14
COL1A1			0.74	0.72	0.30	0.29	0.74
COL1A2		and the second		0.81	0.53	0.49	0.61
COL3A1		Contraction of the second			0.59	0.60	0.66
COL4A1						0.61	0.19
COL4A2		10	· · · · · · · · · · · · · · · · · · ·				0.35
COL5A1	1.						
	10 40	25 26 27	23 24 25 2	4 25 26	21 22 23	18 21 1	5 17 19

Findings from this study suggest that changes in collagen peptides measured in urine may reflect ongoing fibrogenesis, degradation of existing fibrotic tissue, or tissue remodeling. This approach potentially offers a noninvasive view into fibrotic status and repeat sampling can be easily implemented in the context of a clinical trial. Urinary collagen peptides are being further investigated in independent cohorts, and results from this study will inform biomarker strategies in future clinical studies of novel antifibrotic agents.

References

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Results

rrelated					-								-
	Tryptic	Semi- yptic	-tr	(Pears p-valı	son r Je <.(>.4,)5)			ŞC	Fib	c.	serum	proteir
COL1A1	143	3 4	414			2				rosis	JEGF	inine	Correl
COL1A2	118	8 2	210			6		Fibr			0.45	0.50	
COL3A1	195		514			11		score	e -		-0.45	0.52	0.45
COL4A1	6)	7			2						0.07	0.40
COL4A2	20)	10			1		uΕ	GF			-0.87	-0.49
LOL5A1	()	3			1	S	erum	-				
LOL6A1	45		13				C	reatin	ine 🔒	1	· · · · · · · · · · · · · · · · · · ·		0.44
	32	ŀ	/			1				•		•	
	13	5	3			1	Pro	oteinu	ıria i		· · · · · · · · · · · · · · · · · · ·		•
rrelate	urinary with fib	colla rosis ເດີ	gen sco Pro	pept ore (F	ides Pears ດິ	from	n bot orre	th th latio	e m on p လ	ature <0.05	and). Pro	pro-o	domains
	with fib score	collag rosis collag	gen SCC Pro-CCT	pept pre (F	ides Pears	from on c	orre	th th latio	e m n p colaa1-M	ature <0.05 COLAA1-M	and). pro-COLAA2	pro-0	domains Correlation
Fibrosis score	score	collago rosis Coller Coller M 0.37 -0.	gen sco Pro-Con	pept ore (F OCLAR 0.35	ides Pears	from on c Pro-Cor 0.52	orre 0.35	th th latio	e ma on po COLAA1-N -0.55	ature <0.05 COLAA1-M -0.52	and). Pro-COLAA2 -0.48	CO5A1-M -0.64	Correlation
Fibrosis score uEGF	vrinary with fib	collago rosis Collago Collago 0.37 -0.	gen sco Pro-Co 26	pept ore (F ore (P 0.35 -0.58	ides ears 0.50	from on c Pro-C 0.552 (0	0.35 0.18	th th latio	e ma on po COLAA1-M -0.55	ature <0.05 Col AP1-1 -0.52	and). Pro-COLAA2 -0.48	pro-c 0.64	Correlation 1.0 0.8 0.6 0.4 0.2 0.0 -0.2
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Conclusions

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