

About OMICS International

OMICS International through its Open Access Initiative is committed to make genuine and reliable contributions to the scientific community. OMICS International hosts over 700 leading-edge peer-reviewed Open Access Journals and organizes over 1000+ International Conferences annually all over the world. OMICS International journals have over 3 million readers and the fame and success of the same can be attributed to the strong editorial board which contains over 50000 About OMICS International eminent personalities that ensure a rapid, quality and quick review process. OMICS International signed an agreement with more than 1000 International Societies to make healthcare information Open Access. OMICS International Conferences make the perfect platform for global networking as it brings together renowned speakers and scientists across the globe to a most exciting and memorable scientific event filled with much enlightening interactive sessions, world class exhibitions and poster presentations.

www.conferenceseries.com



Are Urinary Biomarkers from Clinical Studies Biomarkers of Disease or Biomarkers of Medicine?

Youhe Gao
高友鹤

Beijing Normal University

2015-9-1 Biomarker 2015 Toronto



Biomarker discovery

Healthy controls

No medications

Patients

Medications

?

Youhe Gao. MOJ Proteomics Bioinform (2014) 1(5): 00028.



Medicine must interfere biomarker study

- **Biomarker are the measurable changes associated with the disease.**
- **Medicine is to help the patient changing from disease state to normal state.**

Youhe Gao MOJ Proteomics Bioinform 2014, 1(5): 00028.



Conclusion

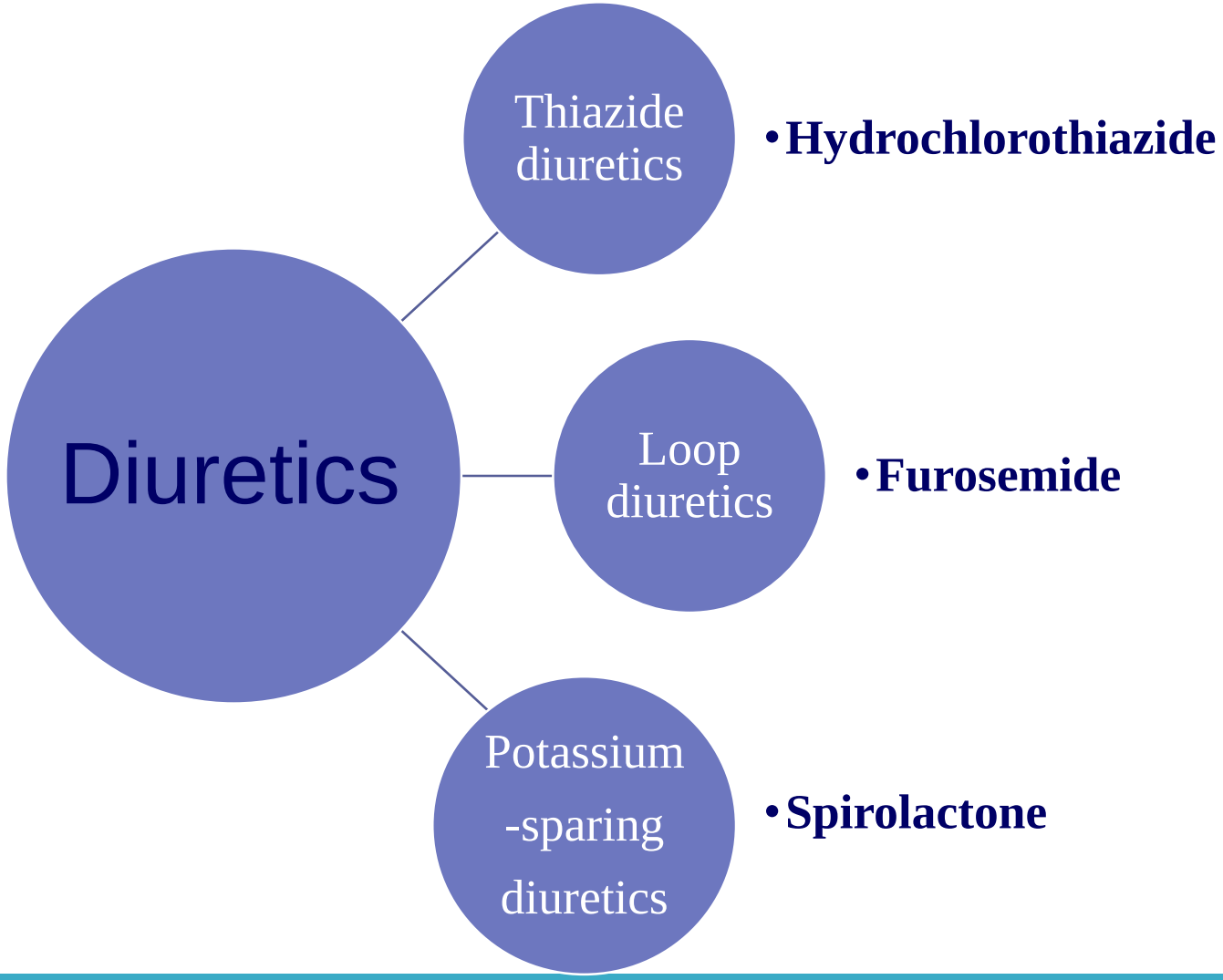
- **Medication must change biomarkers.**



Examples of medication on urine

- Diuretics
- Anesthetics
- Anticoagulants

Diuretics


Diuretics
Thiazide diuretics
•Hydrochlorothiazide
Loop diuretics
•Furosemide
Potassium -sparing diuretics
•Spirolactone



Procedures

- Urine collection:
  - **At therapeutic doses**
  - **Self-control：before and after diuretics or anesthetics oral administration/intraperitoneal injection**
- LC-MS/MS identification (Triple-TOF 5600)
- Label-free quantification
  - $P<0.05$
  - Fold change ≥ 2
  - Spectral count ≥ 5



Results----diuretics

Table 1 Urinary proteins significantly changed after furosemide administration

Accession No.	Protein name	Fold change			Candidate biomarkers
		Rat 1	Rat 2	Rat 3	
P02781	Prostatic steroid-binding protein C2	8.2↑	6.3↑	4.3↑	No
P07647	Submandibular glandular kallikrein-9	3.5↑	6.2↑	5.2↑	Yes
P02782	Prostatic steroid-binding protein C1	7.6↑	5.7↑	5.6↑	No
P02780	Secretoglobin family 2A member 2	9.6↑	5.0↑	6.2↑	Yes
P22283	Cystatin-related protein 2	4.7↑	3.7↑	4.3↑	Yes
P08721	Osteopontin	7.3↓	7.4↓	5.9↓	Yes
Q01177	Plasminogen	2.1↓	2.1↓	3.0↓	Yes

Table 2 Urinary proteins significantly changed after spirolactone administration

Accession No.	Protein name	Fold change			Candidate biomarkers
		Rat 1	Rat 2	Rat 3	
P06866	Haptoglobin	5.0↑	2.1↑	2.2↑	Yes
P81828	Urinary protein 2	3.6↓	3.3↓	3.9↓	Yes
P81827	Urinary protein 1	7.3↓	4.3↓	4.4↓	Yes
P10960	Sulfated glycoprotein 1	4.0↓	3.1↓	2.4↓	Yes
Q09030	Trefoil factor 2	8.5↓	4.7↓	4.2↓	Yes

Table 3 Urinary proteins significantly changed after hydrochlorothiazide administration

Accession	Protein name	Fold change	Biomarkers
P97580	beta-microseminoprotein	4.7↑	No
O35568	EGF-containing fibulin-like extracellular matrix protein 1	3.5↓	No



Results----diuretics Human orthologs

Table 3 Human orthologs of rat proteins significantly changed after diuretic administration

Rat potein ID	Rat protein name	Human protein ID	Human protein name	Human core urinary proteome
Q01177	Plasminogen	P00747[a]	Plasminogen	Yes
Q09030	Trefoil factor 2	Q03403[a]	Trefoil factor 2	Yes
P08721	Osteopontin	P10451[a]	Osteopontin	Yes
O35568	EGF-containing fibulin-like extracellular matrix protein 1	Q12805[a]	EGF-containing fibulin-like extracellular matrix protein 1	Yes
P10960	Sulfated glycoprotein 1	P07602[a]	Sulfated glycoprotein 1	No
P06866	Haptoglobin	P00738[a]	Haptoglobin	Yes
P02781	Prostatic steroid-binding protein C2	P11684[b]	Secretoglobin family 1A member 1	Yes
P07647	Submandibular glandular kallikrein-9	P06870[b]	Kallikrein-1	Yes

Note: [a] Protein present in the 122.R norvegicus.orthologues database; [b] Proteins present in the Ensembl Compare database.

Xundou Li, et al Genomics Proteomics Bioinformatics. (2014) 2(3):120-6



Anesthetics

- Animal experiments
- Surgery
- Pentobarbital sodium and chloral hydrate



Results---anesthetics

Accession	Description	*P* value	Pentobarbital sodium group fold change			Chloral hydrate group fold change			Candidate biomarkers
			Rat 1	Rat 2	Rat 3	Rat 7	Rat 8	Rat 9	
P17475	Alpha-1-antiproteinase	0.034	6.1↑	5.6↑	8.5↑	3.4↑	8↑	3.3↑	Yes
P07154	Cathepsin L1	0.003	2.4↓	3.2↓	4↓	2.4↓	2↓	8.9↓	Yes
P07522	Pro-epidermal growth factor	0.001	2.5↓	2.8↓	3.4↓	5.4↓	3.3↓	2.1↓	Yes
P00758	Kallikrein-1	0.002	2.1↓	3.3↓	3.3↓	7.5↓	2.7↓	2↓	No
Q5XI43	Matrix-remodeling-associated protein 8	0.006	2.8↓	3.1↓	2.6↓	9.3↓	5.3↓	2.8↓	No
P15083	Polymeric immunoglobulin receptor	0.020	2.6↓	2.3↓	2.2↓	3↓	2.7↓	2.8↓	No
P27590	Uromodulin	0.006	3↓	5.2↓	7↓	3.7↓	2↓	2.2↓	Yes
P02770	Serum albumin	0.042	5.5↑	3.1↑	5.4↑	–	–	–	Yes
P12346	Serotransferrin	0.049	6.8↑	2.1↑	4.3↑	–	–	–	Yes
P32038	Complement factor D	0.046	2.2↑	2.4↑	3.9↑	–	–	–	No
P10959	Carboxylesterase 1C	0.034	3.5↑	3.9↑	4.6↑	–	–	–	No
P20761	Ig gamma-2B chain C region	0.030	7.2↑	3.3↑	9.1↑	–	–	–	No
P50123	Glutamyl aminopeptidase	0.044	2.1↓	2.5↓	2.1↓	–	–	–	No
Q62867	Gamma-glutamyl hydrolase	0.046	2.2↓	3↓	3.3↓	–	–	–	Yes
P15684	Aminopeptidase N	0.039	2.4↓	4.4↓	5.7↓	–	–	–	Yes
P26051	CD44 antigen	0.006	2.9↓	2.5↓	2.6↓	–	–	–	No
P36373	Glandular kallikrein-7, submandibular/renal	0.021	2.1↓	2.2↓	3.5↓	–	–	–	Yes
P98158	Low-density lipoprotein receptor-related protein 2	0.004	2.1↓	3.6↓	2.1↓	–	–	–	No
Q64230	Meprin A subunit alpha	0.000	2.7↓	3.6↓	3.4↓	–	–	–	Yes
P28826	Meprin A subunit beta	0.031	3.5↓	4.9↓	10.9↓	–	–	–	No
Q64319	Neutral and basic amino acid transport protein rBAT	0.014	2.5↓	2.7↓	4.5↓	–	–	–	Yes
P29598	Urokinase-type plasminogen activator	0.048	2.5↓	2.2↓	2.7↓	–	–	–	No

Mindi Zhao et al (2015) PeerJ 3:e813



Results---anesthetics

Accession	Description	*P* value	Pentobarbital sodium group fold change			Chloral hydrate group fold change			Candidate biomarkers
			Rat 1	Rat 2	Rat 3	Rat 7	Rat 8	Rat 9	
P02650	Apolipoprotein E	0.032	–	–	–	2↓	5.2↓	3.3↓	No
Q9R0T4	Cadherin-1	0.039	–	–	–	2.7↓	3.5↓	2.1↓	Yes
P31211	Corticosteroid-binding globulin	0.038	–	–	–	3.6↓	2.1↓	2.8↓	No
Q9JJ40	Na(+)/H(+) exchange regulatory cofactor NHE-RF3	0.047	–	–	–	3.0↓	2.7↓	3.2↓	Yes
P08460	Nidogen-1 (Fragment)	0.020	–	–	–	4↓	2.5↓	4.1↓	No
Q63083	Nucleobindin-1	0.043	–	–	–	16.7↓	10.8↓	3.7↓	No
P83121	Urinary protein 3	0.033	–	–	–	2.2↓	3.3↓	2.1↓	No
P05371	Clusterin	0.040	–	–	–	5.7↓	5.8↓	2.2↓	No
Q6DGG1	Alpha/beta hydrolase domain-containing protein 14B	0.004	–	–	–	3.4↑	8↑	3.3↑	No
Q6IRK9	Carboxypeptidase Q	0.037	–	–	–	2.9↑	3.6↑	4.8↑	No
P08649	Complement C4	0.028	–	–	–	11.2↑	3.1↑	14↑	No
P61972	Nuclear transport factor 2	0.026	–	–	–	3.1↑	3.1↑	6.5↑	No
P02625	Parvalbumin alpha	0.047	–	–	–	5.9↑	5↑	12.5↑	Yes
Q920A6	Retinoid-inducible serine carboxypeptidase	0.019	–	–	–	4.2↑	4.2↑	4.7↑	No
P82450	Sialate O-acetylesterase	0.016	–	–	–	5↑	9.4↑	2.4↑	No
P07632	Superoxide dismutase [Cu-Zn]	0.019	–	–	–	2.6↑	4.9↑	3.1↑	Yes

Mindi Zhao et al (2015) PeerJ 3:e813

Western blot

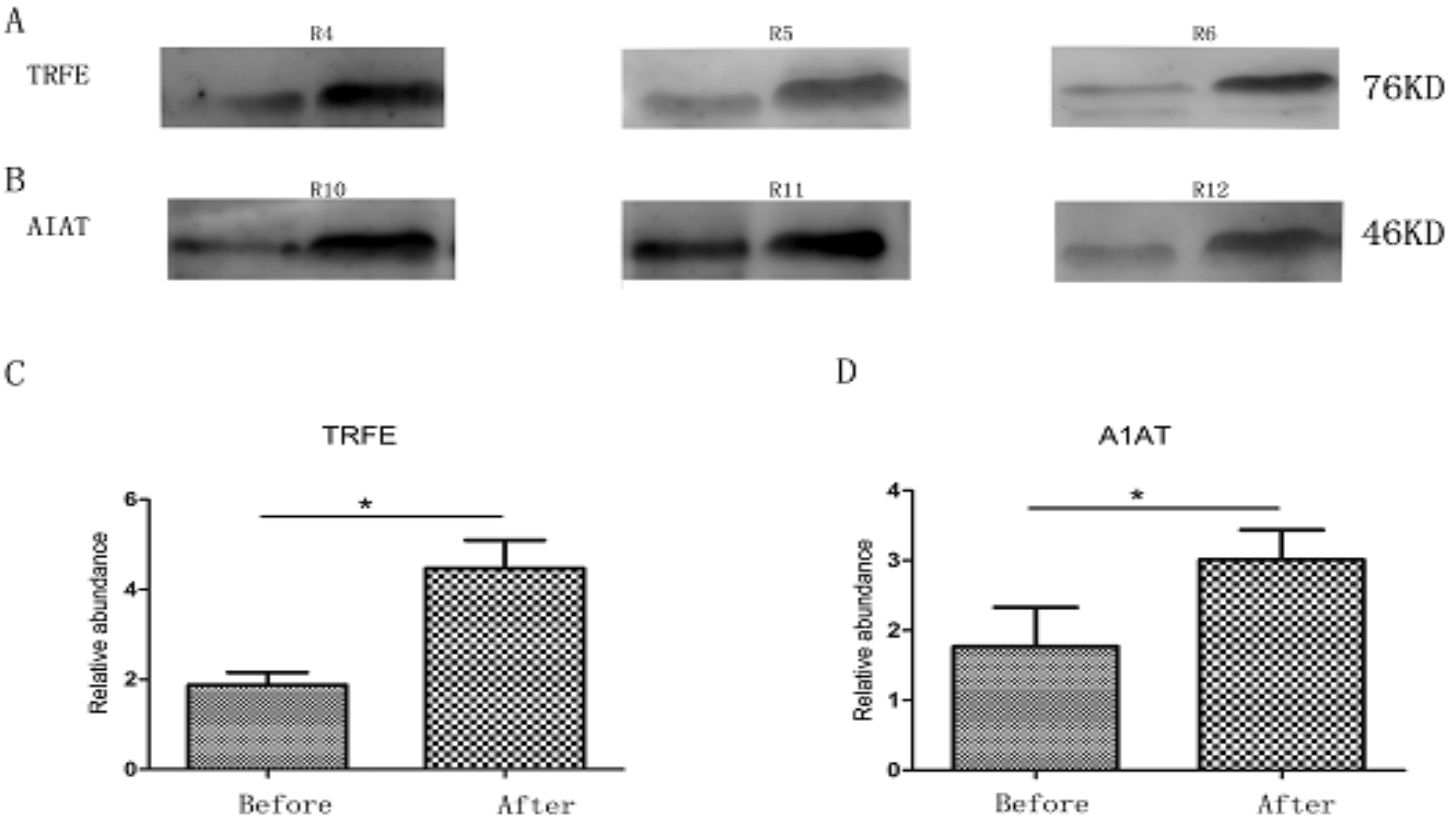


Figure 2 Semi-quantitative western blot analysis of two proteins. (A) Levels of urinary transferrin before and after pentobarbital sodium anesthesia. (B) Levels of urinary alpha-1-antiproteinase before and after chloral hydrate anesthesia. (C) Quantitation of the transferrin by western blot analysis from 3 independent biological replicates. (D) Quantitation of the alpha-1-antiproteinase by western blot analysis from 3 independent biological replicates * indicates $p < 0.05$ (data were analyzed by t test).

Mindi Zhao et al (2015) PeerJ 3:e813

Company Logo



Results---anesthetics Human orthologs

Group	Uniprot (rat)	Human ensembl gene ID	Uniprot (human)	Protein name	Related-disease
Both group	P27590	ENSG00000169344	P07911	Uromodulin	Fanconi Syndrome (*Cutillas et al., 2004*)
	P00758	ENSG00000167748	P06870	Kallikrein-1	None
Pentobarbital sodium group	Q64319	ENSG00000091513	P02787	Serotransferrin	Diabetic Nephropathy (*Narita et al., 2004*)
	Q628 67	ENSG00000163631	P02768	Serum albumin	Nephrotoxicity (*Nordberg et al., 2005*)
	P12346	ENSG00000137563	Q92820	Gamma-glutamyl hydrolase	Uranium Nephrotoxicity (*Malard et al., 2009*)
	P02770	ENSG00000138079	Q07837	Neutral and basic amino acid transport protein rBAT	Sodium Loading (*Thongboonkerd et al., 2003*)
Chloral hydrate group	P07632	ENSG00000142168	P00441	Superoxide dismutase (Cu–Zn)	Nephritis (*Curtis et al., 1989*)
	Q6IRK9	ENSG00000104324	Q9Y646	Putative uncharacterized protein	None
	P02625	ENSG00000100362	P20472	Parvalbumin alpha	Skeletal Muscle Toxicity (*Dare et al., 2002*)
	P31211	ENSG00000170099	P08185	Corticosteroid-binding globulin	None
	Q9R0T4	ENSG00000039068	P12830	E-cadherin	Diabetic Nephropathy (*Jiang et al., 2009*)
	Q6DGG1	ENSG00000114779	Q96IU4	Alpha/beta hydrolase domain-containing protein 14B	None
	Q920A6	ENSG00000121064	Q9HB40	Retinoid-inducible serine carboxypeptidase	None
	P02650	ENSG00000130203	P02649	Apolipoprotein E	Bladder Cancer (*Linden et al., 2012*)
	Q9JJ40	ENSG00000174827	Q5T2W1	Na(+)/H(+) exchange regulatory cofactor NHE-RF3	Aldosteronism (*van der Lubbe et al., 2012*)
	Q63083	ENSG00000104805	Q02818	Nucleobindin-1	None

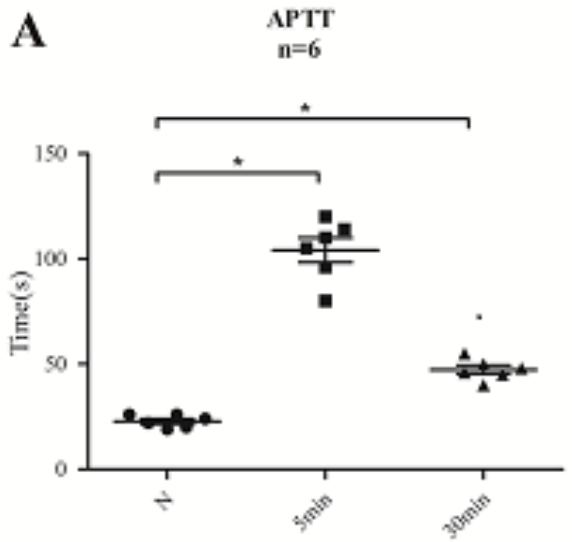
Mindi Zhao et al (2015) PeerJ 3:e813



Changes of proteins induced by anticoagulants can be more sensitively detected in urine rather than plasma

Menglin Li, et al. Sci China Life Sci, (2014) 57(7): 649-656.

Changes of protein induced by anticoagulants

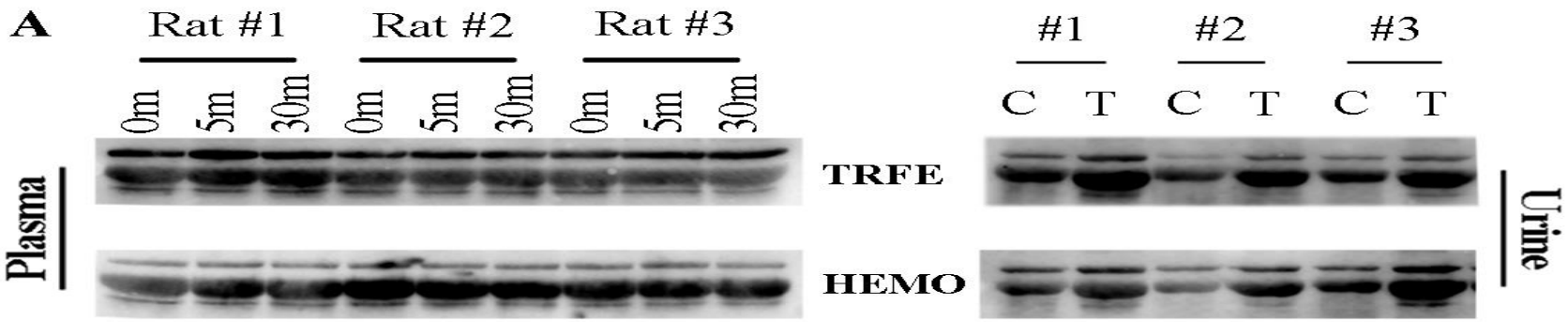


Clotting times increased

Changes

- Heparin
- 27 proteins in urine
- 3 proteins in plasma

- Argatrabon
- 61 proteins in urine
- 1 proteins in plasma



Validation of changes in protein levels

Menglin Li, et al. Sci China Life Sci, (2014) 57(7): 649-656.



Summary

1. Diuretics, anesthetics and anticoagulants can all affect the urinary proteins
2. These effects deserve attention in protein biomarker studies in urine and blood
3. When saving urinary samples, medication information of patients must be recorded.



Roadmap

- Minimizing confounding factors by using animal models (of disease) to study the effects in urine
- Validate in human samples

Company Logo



Workshop in the afternoon

- **Why should we save urine samples?**
  - **Biomarker is change.**
  - **Urine accumulates lots of changes.**
  - **Urine is a better biomarker source.**
- **How to save it easily, economically at room temperature for a long time?**



Acknowledgement

- Xudou Li
- Mindi Zhao
- Menglin Li

- Funding from Ministry of Sci & Tech of China



Team Biomarkers welcomes you all to the next chapter – 7th International Conference on Biomarkers & Clinical Research scheduled for Nov 28-30, 2016 in Baltimore, USA

Please Visit:

www.conferenceseries.com

http://www.omicsonline.org/

http://biomarkers.conferenceseries.com/

Let Us Meet Again in Baltimore, USA