Online Only Information

eMethods

AQP1 ELISA

Thawed urine was vortexed to resuspend urine exosomes and centrifuged (1800*g* for 10 minutes) to remove debris (exosomes remain in suspension at this low speed) before processing for ELISA or Western blot analysis. The urine creatinine concentration was quantified by the Jaffe reaction.[20,21,23] For the ELISA assay, the urine was diluted at least 1 to 10 with a standard ELISA buffer of phosphate buffered saline (PBS) (Fisher BioReagents BP665-1) containing 1% bovine serum albumin (Sigma Chemical either A9647 or A7030) and 0.1% Tween-20 (Sigma Chemical P9416). The ELISA wash buffer consisted of PBS containing 0.05% Tween-20. Human recombinant AQP1 was purchased from Abnova (Taipei City, Taiwan) as the N-terminal glutathione S-transferase conjugate and used to formulate a standard curve. The AQP1 assay is insensitive to human aquaporin-2 up through 1000 pg/ml of aquaporin-2 (Abnova). The assay was performed using Nunc Immuno MaxiSorp 439454 96-well plates with standards and samples in duplicate. A horseradish peroxidase-linked anti-rabbit antibody (R&D Systems HAF008) and 3,3',5,5" tetramethylbenzidine (Sigma-Aldrich, T0440) was used to quantify the AQP1 analyte with the reaction quenched with 0.1 N HCl and absorbance determined at 450nm. The lower limit of AQP1 detection (LLoD) in this assay was 7.1 ± 1.6 pg/ml (mean and standard deviation) over a concentration range up to 100 pg/ml. Inter- and intra-assay variation was 3.9 ± 3.2% and 3.8 ± 3.4%. Assay results were normalized to the urine creatinine concentration. Random reanalysis of samples agreed within 7%.

For the Western blot analysis of PLIN2, urine proteins were precipitated by 15 volumes of acetone/methanol (1:1) and then dissolved in an amount of sodium dodecyl sulfate (SDS) sample

buffer such that the 5 μL of sample applied to the gel reflected the amount of urine containing 10 μg of creatinine.[20,23] The blocked membranes were incubated with a 1:200 dilution of anti-ADFP (PLIN2) (H-80) antibody from Santa Cruz Biotechnology Inc, Santa Cruz, CA in blocking buffer that contained 0.1% Tween-20 overnight. After washing, the membranes were incubated with a 1:2000 dilution of donkey anti-rabbit IgG IRDye 680 (LICOR Biosciences, Lincoln, NE) in blocking buffer with 0.1% Tween-20 for 1 hour. PLIN2 was visualized and quantified using an infrared imager (Odyssey Infrared Imager; LI-COR) and proprietary software. The response of PLIN2 was linear over the range of concentrations found in patient urine. PLIN2 was quantified using relative absorbance units and normalized to urine creatinine excretion. The inter-assay variation from gel to gel was 9%.[2020,23,31] Random reanalysis of samples agreed within 10%. Extracts of two patients, positive for kidney cancer, were included on each gel for quality control for gel-to-gel reproducibility.

eFigure 1. Urine (A) AQP1 (ng/ml urine) and (B) PLIN2 (absorbance/ml urine) concentration in individual patients enrolled between February and December 2012. Concentrations are shown for each of the 720 patients with a history or not of cancer (open circles) and the 19 patients with confirmed kidney cancer (clear cell or papillary subtypes) (filled circles). Three of the 720 screened patients (shaded circles) were subsequently found to have an imaged renal mass based on their CT. The dotted lines represents the cutoff values (52 ng/mg urine creatinine for AQP1 and 9.8 absorbance/mg urine creatinine for PLIN2) derived from the receiver operating characteristic analysis shown in Figure 4.

eFigure 2. Correlation between imaged tumor size and urine biomarkers in the 22 patients with confirmed kidney cancer (clear cell or papillary subtypes). This includes the 19 patients with known RCC (filled circles) and the 3 from the screening population with an incidentally

discovered renal mass (open circles). (A) AQP1 concentrations (Spearman correlation coefficient 0.72, P<0.001) or (B) PLIN2 concentrations (Spearman coefficient 0.74, P<0.001).

eTable 1. Comparison between urine biomarker concentrations in patients with kidney cancer (clear cell and papillary) and other cancers. Comparisons used the Kruskal-Wallis test with least squares difference correction. Results are the p values for between-group comparisons

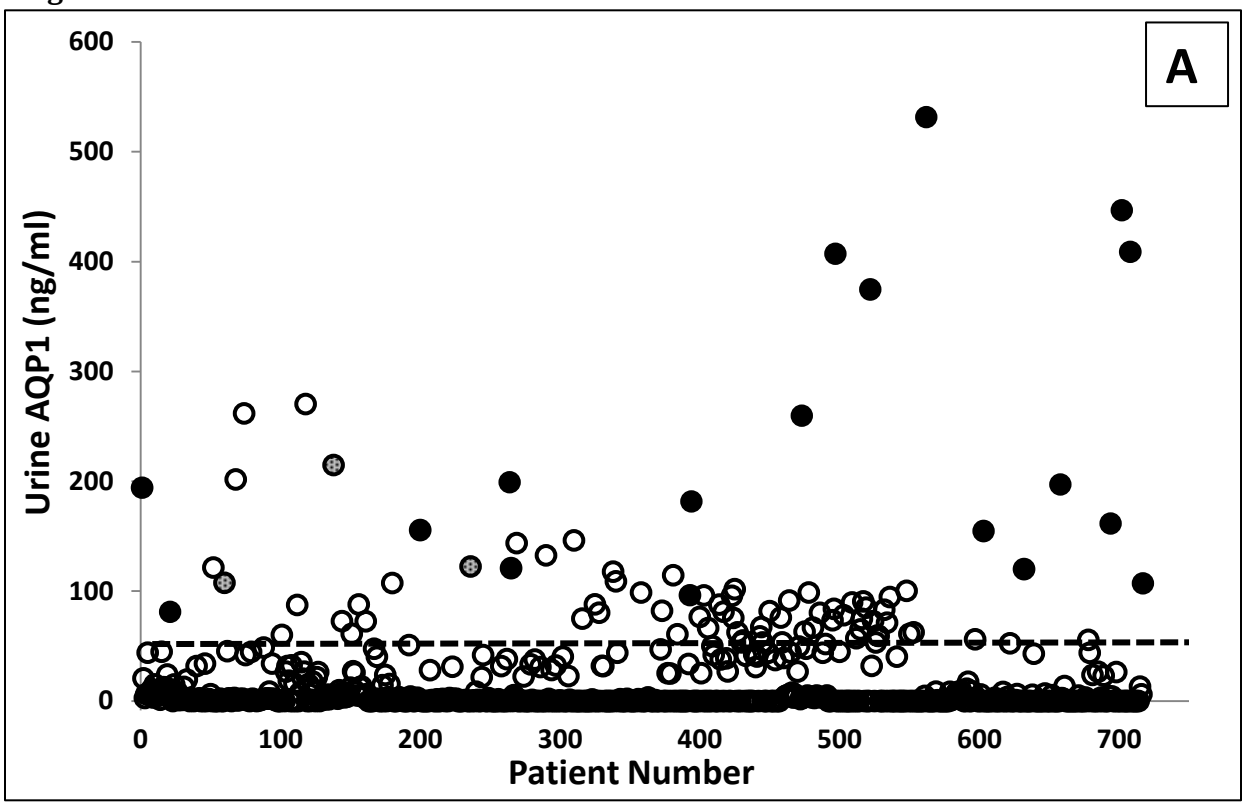
AQP1

	Kidney	Lung	Prostate	Colorectal	Gastrointestinal	Uterine	Ovarian	Pancreatic	Lymphoma	Breast	Other
Kidney											
Lung	<0.001										
Prostate	<0.001	0.329									
Colorectal	<0.001	0.183	0.961								
Gastrointestinal	<0.001	0.048	0.321	0.293							
Uterine	<0.001	0.809	0.343	0.253	0.067						
Ovarian	<0.001	0.899	0.456	0.346	0.089	0.769					
Pancreatic	<0.001	0.112	0.617	0.604	0.585	0.149	0.199				
Lymphoma	<0.001	0.666	0.524	0.396	0.097	0.616	0.837	0.223			
Breast	<0.001	0.413	0.158	0.070	0.022	0.762	0.475	0.048	0.285		
Other	<0.001	0.272	0.115	0.041	0.015	0.649	0.367	0.031	0.191	0.842	

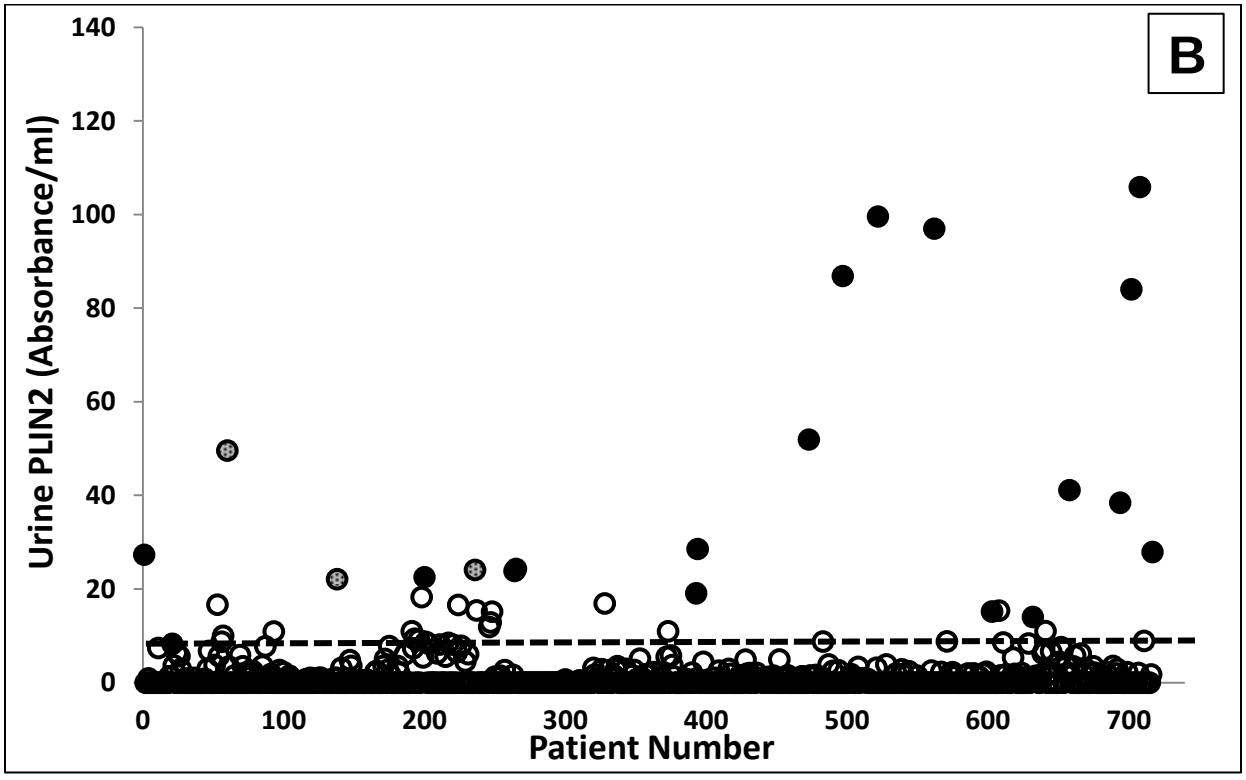
PLIN2

	Kidney	Lung	Prostate	Colorectal	Gastrointestinal	Uterine	Ovarian	Pancreatic	Lymphoma	Breast	Other
Kidney											
Lung	<0.001										
Prostate	0.002	0.001									
Colorectal	<0.001	0.031	0.082								
Gastrointestinal	<0.001	0.316	0.001	0.033							
Uterine	<0.001	0.576	0.013	0.299	0.227						
Ovarian	<0.001	0.219	0.022	0.491	0.106	0.678					
Pancreatic	<0.001	0.214	0.073	0.735	0.098	0.562	0.813				
Lymphoma	<0.001	0.693	0.001	0.027	0.450	0.441	0.164	0.163			
Breast	<0.001	0.352	<0.001	0.008	0.623	0.265	0.073	0.082	0.651		
Other	<0.001	0.588	0.001	0.112	0.218	0.847	0.450	0.383	0.419	0.196	

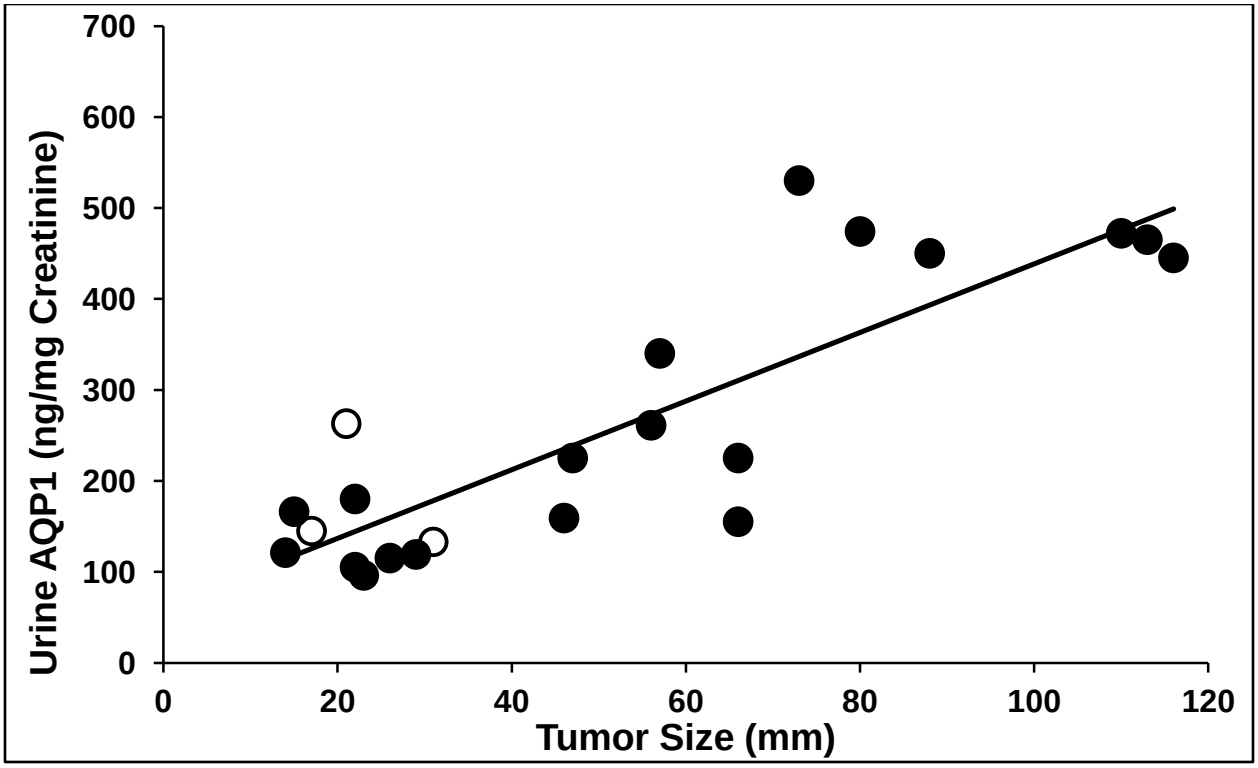
eFigure 1A



eFigure 1B



eFigure 2A



eFigure 2B