Urinary mitochondrial DNA is a biomarker of mitochondrial disruption and renal dysfunction in acute kidney injury

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Recent studies show the importance of mitochondrial dysfunction in the initiation and progression of acute kidney injury (AKI). However, no biomarkers exist linking renal injury to mitochondrial function and integrity. To this end, we evaluated urinary mitochondrial DNA (UmtDNA) as a biomarker of renal injury and function in humans with AKI following cardiac surgery. mtDNA was isolated from the urine of patients following cardiac surgery and quantified by quantitative PCR. Patients were stratified into no AKI, stable AKI, and progressive AKI groups based on Acute Kidney Injury Network (AKIN) staging. UmtDNA was elevated in progressive AKI patients and was associated with progression of patients with AKI at collection to higher AKIN stages. To evaluate the relationship of UmtDNA to measures of renal mitochondrial integrity in AKI, mice were subjected to sham surgery or varying degrees of ischemia followed by 24 h of reperfusion. UmtDNA increased in mice after 10–15 min of ischemia and positively correlated with ischemia time. Furthermore, UmtDNA was predictive of AKI in the mouse model. Finally, UmtDNA levels were negatively correlated with renal cortical mtDNA and mitochondrial gene expression. These translational studies demonstrate that UmtDNA is associated with recovery from AKI following cardiac surgery by serving as an indicator of mitochondrial integrity. Thus UmtDNA may serve as valuable biomarker for the development of mitochondrial-targeted therapies in AKI.

Kidney International advance online publication, 19 August 2015; doi:10.1038/ki.2015.240

KEYWORDS: acute kidney injury; ischemia/reperfusion; mitochondria

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Received 15 December 2014; revised 20 May 2015; accepted 4 June 2015

Acute kidney injury (AKI) is a serious clinical problem, particularly among surgical and critically ill patients.[1,2] Despite advances in diagnosis and patient management, incidence of AKI continues to increase and outcomes continue to be poor. An expanding array of risk factors for AKI and expanded use of nephrotoxic drugs have contributed to its increased incidence and prevalence. AKI is associated with high levels of short- and long-term mortality and increases patient risk of progression to chronic kidney disease and end-stage renal disease.[3–5] Survivors of AKI, particularly those with end-stage renal disease requiring dialysis, are plagued by a variety of chronic sequelae contributing to decreased quality of life and increased utilization of health-care resources.

Cardiac surgery requiring cardiopulmonary bypass (CPB) is a common cause of AKI in humans. Estimates of incidence of AKI in patients following cardiac surgery are as high as 30%.[6,7] Furthermore, AKI is one of the most significant negative predictors of patient outcome in this population.[8,9] Despite the high incidence and severity of CPB-induced AKI, methods for early detection following surgery remain poor. Additionally, there are no effective therapeutic strategies to prevent or promote recovery from AKI in these patients.[10–12] These facts highlight the need to better understand mechanisms of CPB-induced AKI and to develop novel diagnostic tools and therapies.

Renal injury following CPB is multifactorial and poorly understood; however, ischemia–reperfusion (I/R) injury is believed to have a significant role.[13,14] Poor renal perfusion owing to reduced cardiac output, increased systemic inflammation, hemodynamic alterations, volume depletion, and perioperative administration of nephrotoxic drugs contribute to I/R-induced renal injury following cardiac surgery.[15,16] Mitochondrial damage and dysfunction is a key pathophysiological component of renal tubular injury during both the initiation and recovery phases of ischemic AKI.[17,18] Organ reperfusion following ischemia leads to rapid opening of the mitochondrial permeability transition pore causing mitochondrial membrane depolarization, increased production of

reactive oxygen and nitrogen species, and release of apoptotic proteins. Oxidative damage of mitochondrial respiratory complexes and subsequent reduction in mitochondrial function contributes to tissue ATP depletion inhibiting energy-dependent cellular repair mechanisms.[19] Additionally, generation of new, functional mitochondria through the process of mitochondrial biogenesis is persistently suppressed following renal injury in mice.[20,21]

These reports highlight mitochondria as an intriguing diagnostic and therapeutic target for AKI in animals; however, correlative data in humans are limited owing to limited availability of renal tissue for analysis. Current assays for mitochondrial function, including tissue ATP and oxygen consumption measurements, require invasive tissue biopsies.[22] Because of the profound role of mitochondria in tissue repair, biomarkers of mitochondrial dysfunction following CPB may serve as better prognostic measures of AKI progression and recovery compared with existing biomarkers, enabling modification of patient management and development of new mitochondrial-targeted therapies for AKI. To this end, we conducted proof-of-concept experiments that examine the efficacy of urinary mitochondrial DNA (UmtDNA) as a predictive biomarker of AKI development and progression in humans with CPB-induced AKI. Furthermore, we validated UmtDNA as a biomarker of renal mitochondrial integrity in a mouse model of renal I/R injury. Fragments of mtDNA, referred to as mtDNA damage–associated molecular patterns, are released into circulation following injury.[23,24] Released mtDNA can modulate oxidative and inflammatory injury at distant sites through activation of Toll-like receptor signaling.[25] Although numerous studies have evaluated serum and plasma levels of mtDNA as predictors of injury and disease progression, no reports exist of the evaluation of UmtDNA as a specific marker of renal damage/dysfunction.

RESULTS

UmtDNA is associated with AKI progression following cardiac surgery

To assess UmtDNA as a biomarker of renal dysfunction in humans, UmtDNA levels were measured by quantitative PCR in urine collected as a component of an NIDDK-funded multicenter trial (DK080234) to determine prognostic biomarkers of AKI following cardiac surgery.[26] Samples were collected through the Southeastern Acute Kidney Injury Network (SAKInet) consortium. The patient cohort included patients who developed AKI and those who did not develop AKI following surgery. Patients were enrolled before surgery for baseline measurements, and urine was collected approximately 1 day following surgery (mean collection time = 1.26 days, median collection time = 1 day). Table 1 contains the demographic and clinical characteristics of the subjects. Renal function was evaluated at collection and during follow-up by serum creatinine, and patients were staged using the Acute Kidney Injury Network (AKIN) criteria.

Table 1 | Patient demographics and clinical characteristics of cardiac surgery patients

	AKI status			
Patient demographics	No AKI	Stable AKI	Progressive AKI	*P*-value
Total patients	17	63	31	
Female	3 (18%)	13 (22%)	15 (48%)	0.02
Black	3 (18%)	16 (27%)	6 (19%)	0.59
Age (years)	65 ± 16	66 ± 11	68 ± 12	0.66
Weight (kg)	81 ± 28	93 ± 20	85 ± 28	0.11
History				
CHF	6 (36%)	14 (24%)	11 (35%)	0.42
Previous cardiac surgery	2 (12%)	9 (15%)	10 (32%)	0.10
Diabetes mellitus	8 (47%)	20 (34%)	14 (45%)	0.45
COPD	2 (12%)	3 (5%)	4 (13%)	0.39
Peripheral vascular disease	0 (0%)	6 (10%)	1 (3%)	0.22
Stroke	0 (0%)	4 (7%)	2 (6%)	0.55
Surgical parameters				
CABG	16 (94%)	42 (71%)	23 (74%)	0.15
Valve replacement	4 (24%)	22 (37%)	15 (48%)	0.23
Bypass	14 (82%)	46 (78%)	27 (87%)	0.57
Bypass time	117 ± 49	139 ± 65	150 ± 90	0.31
Outcomes				
Baseline creatinine	1.3 ± 0.4	1.2 ± 0.3	1.3 ± 0.5	0.41
Collection creatinine	1.4 ± 0.4	2.0 ± 0.7	1.8 ± 0.8	0.0083
Max creatinine	1.4 ± 0.4	2.1 ± 0.8	2.9 ± 1.5	<0.0001
RRT	1 (6%)	1 (2%)	5 (16%)	0.031
Mortality	1 (6%)	1 (2%)	7 (23%)	0.0029
Days to discharge	6.8 ± 2.6	9.3 ± 8.6	15.1 ± 11.4	0.0031
Days to max creatinine	5.6 ± 4.5	2.3 ± 1.7	4.1 ± 2.6	<0.0001

Abbreviations: AKI, acute kidney injury; CABG, coronary artery bypass graft; CHF, congestive heart failure; COPD, chronic obstructive pulmonary disease; RRT, renal replacement therapy. No AKI (Acute Kidney Injury Network (AKIN) 0 throughout follow-up), Stable AKI (AKIN 1+ at collection, collection AKIN = maximum AKIN), Progressive AKI (AKIN 1+ at collection, maximum AKIN > collection AKIN). *P*-values were determined using the chi-square test or analysis of variance, as appropriate.

The urine mtDNA/nuclear DNA (nDNA) ratio was not elevated in patients with AKI (AKIN1+time of collection) (median = 147, IQR 102–610) versus no AKI (AKIN 0) (median = 202, IQR 59–604), and no differences were observed in UmtDNA levels between healthy or any AKIN stage patients based on either AKIN at collection or maximum AKIN stage achieved (Supplementary Figure S1 online).

To assess the efficacy of UmtDNA in predicting worsening of AKI, patients were classified into three groups based on disease progression: no AKI (AKIN 0 throughout follow-up), stable AKI (AKIN 1+ at collection, maximum AKIN = collection AKIN), or progressive AKI (AKIN 1+ at collection, maximum AKIN > collection AKIN). No patients with AKIN 0 at collection who subsequently developed AKI were observed in the cohort. UmtDNA was significantly elevated in patients with progressive AKI (median = 353, IQR 187–1053) versus no AKI (median = 118, IQR 26–517) or stable AKI (median = 104, IQR 50–340) (Figure 1a).

Receiver operator characteristic (ROC) curve analysis was performed to assess UmtDNA as a predictive test for AKI progression. There was no difference between no AKI and stable AKI patients (area under the curve (AUC) = 0.53, $P = 0.73$) (Figure 1b). Elevated UmtDNA was a significant predictor of progressive AKI compared with no AKI