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CLINICAL RESEARCH

The Circular RNA *ciRs-126* Predicts Survival in Critically Ill Patients With Acute Kidney Injury

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Introduction: Circular RNAs (circRNAs) have recently been described as novel noncoding regulators of gene expression. They might have an impact on microRNA expression by their sponging activity. The detectability in blood of these RNA transcripts has been demonstrated in patients with cancer and cardiovascular disease. We tested the hypothesis that circulating circRNAs in blood of critically ill patients with acute kidney injury (AKI) at inception of renal replacement therapy may also be dysregulated and associated with patient survival.

Methods: We performed a global circRNA expression analysis using RNA isolated from blood of patients with AKI as well as controls. This global screen revealed several dysregulated circRNAs in patients with AKI. Most highly increased circRNA-array–based transcripts as well as expression of the circRNA target miR-126-5p were confirmed in blood of 109 patients with AKI, 30 age-matched healthy controls, 25 critically ill non-AKI patients, and 20 patients on maintenance hemodialysis by quantitative real-time polymerase chain reaction.

Results: Circulating concentrations of 3 novel circRNAs were amplified in blood of patients with AKI and in controls. *Circular RNA sponge of miR-126* (or *ciRs-126*) was most highly altered compared to healthy controls and disease controls (fold change of 52.1). *ciRs-126* was shown to bioinformatically sponge miR-126-5p, which was found to be highly suppressed in AKI patients and hypoxic endothelial cells. Cox regression and Kaplan–Meier curve analysis revealed *ciRs-126* as an independent predictor of 28-day survival ($P < 0.01$).

Conclusion: Circulating concentrations of circRNAs in patients with AKI are detectable. *ciRs-126* may potentially sponge miR-126-5p and acts as a predictor of mortality in this patient cohort.

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KEYWORDS: acute kidney injury; circulating circular RNAs; mortality; renal replacement therapy

Acute kidney injury is a severe complication in critically ill patients and has been identified as an independent risk factor concerning survival.[1] Mortality of patients with AKI in the intensive care unit setting is highly prevalent and unaltered over recent, years despite significant improvements in supportive care.[2] As revealed by a multicenter study involving a large number of patients in the intensive care unit, the in-hospital mortality of patients with AKI exceeds 60%.[3] Thus, early biomarkers enabling the clinician to detect subclinical AKI identifying patients at particular risk for both death and prolonged kidney failure after AKI in the setting of intensive care medicine and renal replacement therapy (RRT) remains an area of utmost interest. More than 90% of the human genome is transcribed into RNA transcripts without protein-coding potential.[4,5] These so called noncoding RNAs (ncRNAs) are arbitrarily separated into long ncRNAs (lncRNAs, ≥200 nucleotides) and small ncRNAs (≤200 nucleotides), based on their size. Small RNAs such as microRNAs (miRNAs) have been extensively studied over the past years.[6–8] miRNA activity has been shown to be affected by the presence of miRNA sponge transcripts, the so-called competing endogenous RNA. Circular RNAs,

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which have recently been discovered, are likely a part of the aforementioned competing endogenous RNA class. They are endogenously expressed as single-stranded, covalently closed circular molecules.[9] Most circRNAs are produced in a "back-splicing" reaction, in which a splice donor site is joined with an upstream splice acceptor site.[10,11] Circular RNAs have long been described in viroids and viruses[12,13] but have only recently come into focus in the mammalian genome.[14,15] A landmark study has proved the release into the blood of patients and elucidated the biomarker potential of this novel RNA species.[16] Here, it was shown that circRNAs are remarkably stable (probably due to resistance to exonucleases through circularization) and highly expressed compared to their corresponding linear mRNAs. One of the intriguing functions of circRNAs is the sponging of microRNAs, which have been shown to serve as early and reliable biomarkers of AKI by our group.[17] We here present the first study investigating the pattern of circulating circular RNAs in blood of critically ill patients with AKI requiring RRT, and their potential predictive value concerning outcome. Circulating circRNAs as well as potentially regulated miR-126-5p were assessed in RNA isolated from blood of 109 patients with AKI, 30 age-matched healthy controls, 25 non-AKI, critically ill patients and 20 patients on maintenance hemodialysis. *Hsa_circ_0003266* was shown to potentially sponge miR-126-5p and is therefore referred to as *circular RNA sponge of miR-126* (*ciRs-126*).

METHODS

Patients and Procedures

This study is a *post hoc* measurement of prospectively collected blood samples from the Hannover Dialysis Outcome (HANDOUT) trial.[18] Patients in 7 intensive care units of the tertiary care center at the Hannover Medical School with AKI were evaluated for inclusion. The study protocol was approved by the Hannover Medical School Ethics Committee and was conducted in accordance with the Declaration of Helsinki and German Federal Guidelines. The inclusion criteria were non−post-renal AKI with RRT dependence, indicated by the loss of kidney function of >30% calculated estimated glomerular filtration rate (eGFR) with either the Modification of Diet in Renal Disease (MDRD) or Cockcroft–Gault equation and/or cystatin C−glomerular filtration rate within 48 hours prior to inclusion and oliguria/anuria (<30 ml/h >6 hours prior to inclusion) or hyperkalemia (>6.5 mmol/l) or severe metabolic acidosis (pH <7.15, bicarbonate <12 mmol/l). Exclusion criteria were pre-existing chronic kidney disease as defined by eGFR <60 ml/min or a serum creatinine concentration >1.7 mg/dl more than 10 days prior to initiation of the first RRT. In addition, we considered the presence of an arteriovenous fistula or dialysis catheter as indicative of chronic kidney disease. Further exclusion criteria were participation in another study, consent denial or withdrawal, and need for extracorporeal membrane oxygenation therapy. Enrollment was performed by attending nephrologists after obtaining written informed consent from a patient or his/her legal representatives. If the patient was recovering and able to communicate, he/she was informed of the study purpose, and consent was required to further maintain his/her status as a study participant.

After inclusion, the specific medical condition leading to RRT initiation was documented from a list of 4 possible causes requiring immediate RRT. All patients received a nutritional intake of at least 25 to 30 kcal/kg per day, preferentially delivered as enteral nutrition. The prescribed protein intake was >1.2 g/kg per day. Renal replacement therapy in all patients was performed in a slow extended dialysis mode using the GENIUS dialysis system (Fresenius Medical Care, Bad Homburg, Germany) as described in detail elsewhere.[19] The dose of the RRT was tailored according to the patient's individual need, starting with at least 1 treatment daily. The RRT was discontinued in patients meeting the following criteria for renal recovery: urine output >1000 ml/d and/or increased solute clearance, that is, a decline in pretreatment serum creatinine concentration with eGFR >15 ml/min (by the Modification of Diet in Renal Disease or Cockroft–Gault equation, and/or cystatin C−glomerular filtration rate. Blood was drawn immediately before the start of RRT. The first blood sample was discarded to avoid fibroblasts and activated platelets. Serum creatinine and serum C-reactive protein (CRP) concentrations were determined by an automated analyzer (Beckman Coulter, Brea, CA). The Sequential Organ Failure Assessment (SOFA) score[20] and Acute Physiology and Chronic Health Evaluation II (APACHE II) score[21] were obtained for each patient immediately before initiation of RRT. The presence of sepsis was defined according to the Society of Critical Care Medicine/European Society of Intensive Care Medicine/American College of Chest Physicians/American Thoracic Society/Surgical Infection Society International Sepsis Definitions.[22] Acute kidney injury was classified *post hoc* by means of the Risk, Injury, Failure, Loss of kidney function, and End-stage kidney disease (RIFLE) criteria at initiation of RRT.[23] The Horowitz index was calculated as the ratio of partial pressure of oxygen in blood (PaO_2) and the fraction of oxygen in the inhaled air (FIO_2).

A total of 25 critically ill non-AKI patients (12 male, 13 female; age 52 years, range 42−71 years; 20 patients with acute myocardial infarction, 2 patients with liver disease, 3 patients following surgery) as well as 20 patients on maintenance hemodialysis (11 male, 9 female; age 49

years, range 39–62 years) served as disease controls. Thirty age-matched healthy controls were also included (16 male, 14 female; age 54 years, 46–66 years).

Study Outcomes and Statistical Analysis

The main objective of the study was to analyze the predictive value of circulating circRNAs concerning mortality and renal recovery of critically ill patients with AKI receiving RRT. The study endpoint was defined as survival 4 weeks after initiation of RRT and renal recovery (no RRT requirement) in survivors 4 weeks after initiation of RRT.

A detailed Supplementary Materials and Methods section can be found in the Supplementary Material.

RESULTS

circRNA Expression Analysis in Blood

To assess an impact of AKI on circulating circRNAs, we conducted a genome-wide expression analysis in RNA isolated from whole blood of patients with AKI at inception of RRT (n = 15, 3 pools of 5 patients) and healthy age-matched controls (n = 15, 3 pools of 5 patients). Figure 1 shows that hierarchical clustering analysis clearly distinguished AKI patients from controls, as evidenced by a specific signature of significantly dysregulated circulating circRNAs. Figure 2a shows a scatter plot and Figure 2b a volcano plot analysis of identified circRNAs. circRNAs that were finally assessed in the whole cohort of patients are marked in Figure 2. The clinical characteristics of the whole cohort of AKI patients (n = 109) are shown in Table 1. A total of 4161 upregulated circulating circRNAs were detectable in all groups of the array cohort, showing a signal intensity >9 and differing between patients and healthy controls. Supplementary Table S1 displays the 50 most upregulated circRNAs in blood of AKI patients, and Supplementary Table S2 lists the top 50 downregulated circRNAs.

circRNA Validation in Whole Blood

The detection and amplification of circRNAs in blood is challenging due to their low abundance. To investigate the detectability of dysregulated circulating circRNAs, we assessed the detectability of the most highly dysregulated circRNAs using RNA isolated from whole blood of patients with AKI in agarose gel electrophoresis. Here, we found 3 circRNAs to display specific bands of correct size, including *ciRs-126*, *hsa_circ_0045881*, and *hsa_circ_0001177*. We then performed real-time polymerase chain reaction analysis in a subset of patients with AKI (n = 10). These 3 novel transcripts showed clean amplification curves, showed specific curves in melting curve analysis, and were undetectable in water controls without template. To ascertain that transripts were specifically detected in whole blood, we sequenced transcripts in these patients after polymerase chain reaction amplification, which confirmed that circRNAs were correctly amplified and detectable.

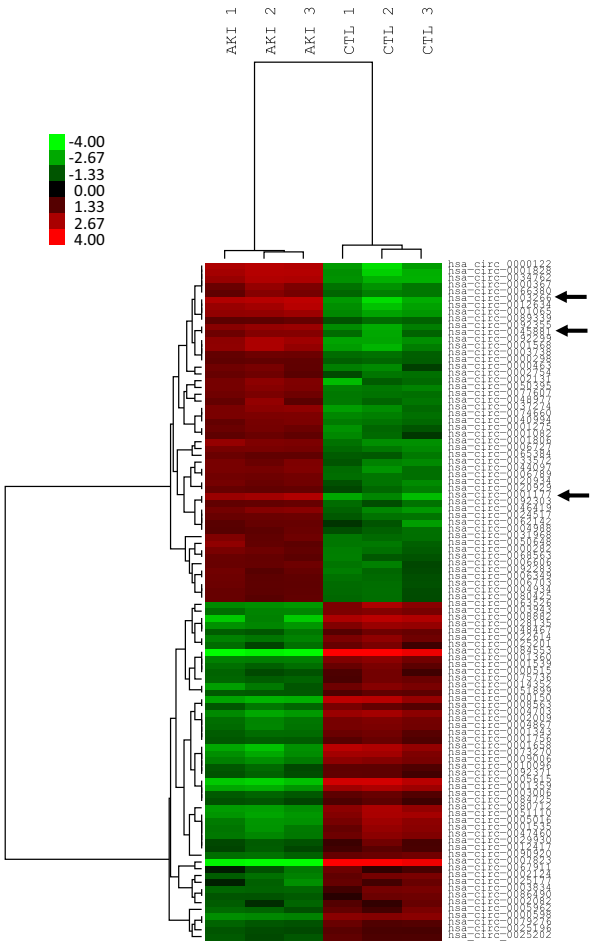

Figure 1. Hierarchical cluster analysis of dysregulated circulating RNAs (circRNAs) in blood of patients with acute kidney injury (AKI) and healthy controls. Red colors represent upregulated circRNAs; green colors represent downregulated circRNAs. *hsa_circ_0003266* (or *ciRS-126*), *hsa_circ_0045881*, and *hsa_circ_00011776* are marked by an arrow.

ciRs-126 Is a Biomarker of AKI

We next analyzed *ciRs-126*, *hsa_circ_0045881*, and *hsa_circ_0001177* in the whole cohort of patients with AKI (n = 109) as well as disease controls (non-AKI, critically ill disease controls, n = 25; patients on maintenance hemodialysis, n = 20) and age-matched healthy individuals (n = 30). As shown in Figure 3a, *ciRs-126* was significantly increased compared to that in healthy controls and disease controls, underlining the specificity as a biomarker in AKI ($P < 0.001$). Both *hsa_circ_0045881* and *hsa_circ_0001177* were significantly increased compared to those in healthy controls, but not compared to those in disease controls (Figure 3b and c). Baseline *ciRs-126* correlated with SOFA score ($r = 0.2$, $P = 0.02$), length of need for respirator therapy ($r = -0.4$, $P = 0.01$), and length of stay on the intensive care unit ($r = -0.5$, $P < 0.01$).

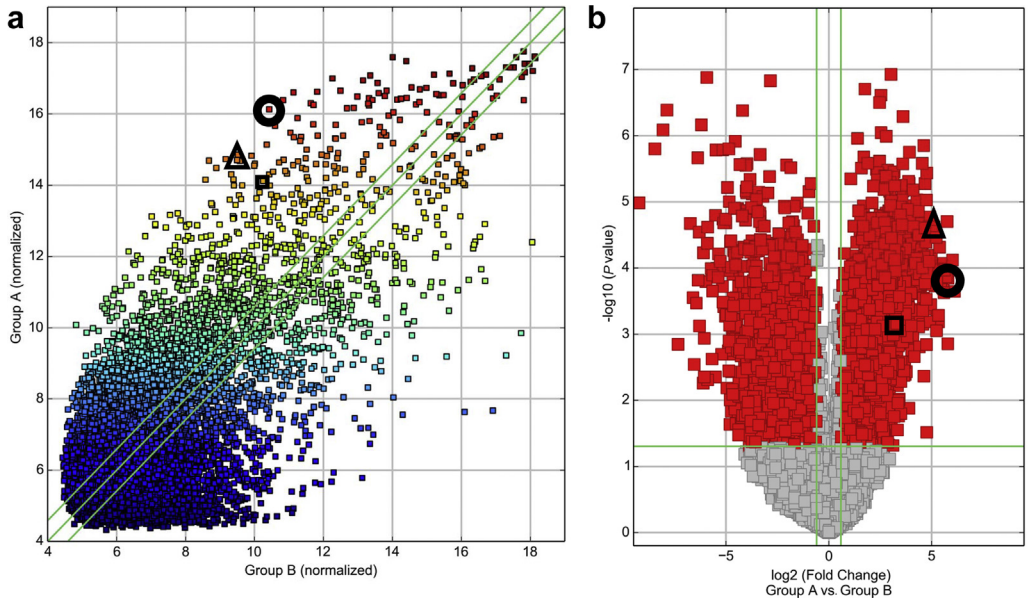

Figure 2. Scatter plot (a) and volcano plot analysis (b) of deregulated circulating RNAs (circRNAs) in blood of patients with acute kidney injury (AKI) and healthy controls. Identified circRNA are further marked: *ciRS-126* (circle), *hsa_circ_0045881* (triangle), and *hsa_circ_0001177* (square).

ciRs-126 Predicts Survival in AKI

Patients were grouped as survivors and nonsurvivors at 4 weeks after initiation of RRT. Patient groups were comparable with respect to baseline demographics and the proportion of RIFLE categories (Table 1). A total of 40 patients died in our cohort. Concentrations of circulating *ciRs-126* were significantly ($P < 0.01$) increased in nonsurvivors. To investigate the prognostic potential of circulating *ciRs-126* concentrations at initiation of RRT with regard to overall mortality, we performed univariate Cox proportional hazards analyses. *ciRs-126* was subjected to natural logarithmic transformation. In our cohort of 109 critically ill patients with AKI, *ciRs-126* concentrations, major surgery, sepsis, shock, APACHE II, SOFA, and Horowitz score showed prognostic significance at a 10% level and were subsequently subjected to multivariate Cox regression analysis (Table 2). Only APACHE II score ($P = 0.06$) and *ciRs-126* concentrations ($P < 0.001$) remained independent predictors of survival in multivariate analysis. In receiver operating characteristic curve analysis *ciRs-126* levels yielded an area under the receiver operating characteristic curve value of 0.92 (SEM: 0.02; 95% confidence interval: 0.88−0.97; $P < 0.0001$). In receiver operating characteristic curve analysis, we defined a cut point of 1.165 relative expression by ΔΔCt analysis with 91% sensitivity and 74% specificity. Figure 3d illustrates the Kaplan−Meier survival curve 4 weeks after initiation of RRT stratified to *ciRs-126* concentrations above and below the median. A log rank test confirmed the statistical significance for circulating *ciRs-126* concentrations ($P < 0.001$). Figure 3e shows receiver operating characteristic curve analysis.

ciRs-126 as a Potential miRNA Sponge

As identified by the Arraystar miRNA target prediction software (Arraystar, Rockville, MD), which is based on the TargetScan and miRanda algorithm, miRNA binding elements were identified regarding *miR-126-5p* in the *ciRs-126* sequence (Supplementary Figure S1). To test the potential interaction between *ciRs-126* and its target miRNA, miR-125-5p was amplified in the whole cohort of patients and was found to be highly suppressed in AKI patients compared to controls, indicating its potential regulation by *ciRs-126* (Figure 3f). We therefore refer to *hsa_circ_0003266* as *circular RNA sponge for miR-126* (*ciRs-126*).

circRNAs and miR-126-5p Are Regulated in Cells

Endothelial cells and proximal tubular epithelial cells are most highly affected by AKI. Accordingly, we assessed the expression of the 3 novel circRNAs in these cells under hypoxic conditions. All 3 transcripts are significantly upregulated in hypoxic endothelial cells, whereas only *ciRs-126* and *hsa_circ_0001177* were induced in hypoxic proximal tubular epithelial cells (Figure 4a–c and Figure 4e–g). miR-126-5p is an endothelial-specific microRNA. Accordingly, miR-126-5p was assessed in endothelial cells *in vitro* and was found to be highly suppressed by hypoxia (Figure 4d).

DISCUSSION

Our study is the first evaluation of circulating circRNAs in any cohort of patients with kidney disease. We were able to show the following: (i) circulating circRNAs in whole blood of critically ill patients with AKI were detectable; (ii) circulating circRNA concentrations

Table 1. Demographic, clinical, and laboratory characteristics of patients

Characteristic	Total	Survivors	Nonsurvivors	*P* value
Patients, n	109	69	40	0.6
Male, n (%)	64 (59)	40	25	
Female, n (%)	45 (41)	29	15	
Discipline of ICU admission				0.4
Medicine, n (%)	46 (42)	27 (39)	19 (48)	
General surgery, n (%)	27 (25)	16 (23)	11 (28)	
Cardiac surgery, n (%)	36 (33)	26 (38)	10 (24)	
Age, yr	52 (40–63)	52 (44–63)	51 (37–63)	0.8
BMI, kg/m^2	25 (22–28)	25.4 (22–28)	24.7 (22–28)	0.6
Indication for RRT				
eGFR loss >30%	93	59	34	0.9
Oliguria/anuria	71	46	25	0.8
Metabolic acidosis	8	4	4	0.4
Hyperkalemia	6	2	4	0.1
SOFA score	13 (10–15)	14 (11–15.5)	13 (10–15)	0.7
Renal	2 (1.5–3)	2 (1–2.5)	2 (2–3)	0.02[a]
Coagulation	1 (0–2)	2 (0–2)	1 (0–2)	0.6
Cardiovascular	4 (0.5–4)	4 (2–4)	3 (0–4)	0.2
Nervous system	4 (3–4)	4 (3–4)	4 (3–4)	0.9
Respiratory system	2 (1–3)	2 (1–3)	2 (2–3)	0.8
Liver	2 (0–2)	2 (0–3)	2 (0–2)	0.4
RIFLE class				0.1
Risk, n (%)	10 (9)	9 (13)	1 (3)	0.07
Injury, n (%)	15 (14)	9 (13)	6 (15)	0.8
Failure, n (%)	84 (77)	51 (74)	33 (82)	0.3
APACHE II score	34 (27–36)	33 (26–36)	35 (29–39.8)	0.6
CRP (mg/l)	113 (56–197)	82 (46–191)	145.5 (67–211.5)	0.2
MAP (mm Hg)	75.5 (67–90)	77 (70–93)	74 (64–86)	0.3
Heart rate (bpm)	100 (85–110)	99 (84–109)	100 (89–111)	0.3

APACHE II, Acute Physiology and Chronic Health Evaluation II; BMI, body mass index; CRP, C-reactive protein; eGFR, estimated glomerular filtration rate; ICU, intensive care unit; MAP, mean arterial blood pressure; n, number of patients; RIFLE, Risk, Injury, Failure, Loss of kidney function, and End-stage kidney disease; RRT, renal replacement therapy; SOFA, Sequential Organ Failure Assessment.
[a]Significant.

specifically identified patients with AKI; (iii) baseline concentrations of *ciRs-126* correlated with parameters of disease severity; (iv) baseline concentrations of circulating *ciRs-126* were increased in nonsurvivors compared to survivors; (v) *ciRs-126* was identified as a strong independent prognostic factor for 28-day-survival in the multivariate Cox proportional hazards regression analysis and Kaplan–Meier curve analysis; and (vi) circulating levels of miR-126-5p were highly suppressed in AKI patients and endothelial cells.

circRNAs show widespread distribution and diverse functions. They are ~100 nucleotides in length and are highly present in the eukaryotic transcriptome and abundant in exosomes.[24] As stated in the introduction, circRNAs are likely produced in a "back-splicing" reaction, in which a splice donor site is joined with an upstream splice acceptor site, thus forming a circular structure in which the 3′- and 5′-ends are covalently linked.[10,11] These circRNAs are termed "exonic" circRNAs because they arise from known exons at annotated splice sites, as opposed to "intronic" circRNAs, containing a 2′-5′ carbon linkage at the branch point stemming from introns. Owing to their circular structure and the absence of a 5′ cap, it is currently believed that circRNAs are not translated into protein.[25] A characteristic element of circRNAs is the "head-to-tail" splice junction (due to backsplicing), in which exons are organized in reverse order compared to their chromosomal localization. One likely cellular function of circRNAs is the binding and sequestering of miRNAs,[24,26] but this interaction might only be observed in circRNAs with a high number of binding sites for a specific miRNA. For instance, ciRs-7 (circular RNA sponge for miR-7) has been identified in mouse brain and shown to express more than 70 conserved miR-7 target sites.[26] Moreover, it is highly associated with Argonaute (AGO) proteins in a miR-7–dependent manner.[26] It strongly suppresses miR-7 activity, resulting in increased levels of miR-7 targets.[26] circRNAs likely exert further functions, as has been described for lncRNAs. Because circRNAs are less susceptible to exonuclease activity (due to their circular structure), they exhibit significantly longer half-lives than linear RNAs,[24] suggesting their ideal role as stable biomarkers in body fluids of patients.

We identified several putative binding sites of miR-126-5p in the sequence of *ciRs-126*. miR-126 has previously been shown to lead to resolution of AKI in mice.[27] Hematopoietic overexpression of miR-126 increased neovascularization, led to an improvement of kidney function, and increased numbers of bone marrow–derived endothelial cells. The numbers of circulating Lin(−)/Sca-1(+)/cKit(+) hematopoietic stem and progenitor cells were increased.[27] Deactivation of miR-126 was shown to induce a pseudohypoxia state due to increased HIFα expression in kidney cancer. Serpin Family E Member 1 (SERPINE1) was identified as a miR-126-5p target regulating cell motility.[28] Chronic heart failure was significantly associated with lower circulating levels of miR-126-5p.[29] Lack of miR-126-5p reduced endothelial cell proliferation by derepression of the Notch1 inhibitor delta-like 1 homolog (Dlk1).[30] Currently, we are unable to provide definitive proof that *ciR-126* indeed sponges miR-126-5p. Based on bioinformatic sequence homology and level of regulation in our analyses, we hypothesize that it may be regulated by *ciRs-126*. Future studies will address the experimental proof of binding interaction.

There are several distinct possible sources of circulating circRNAs. It was previously shown that circRNAs are physiological components of neutrophils, B-cells, and hematopoietic stem cells, confirming that they may be derived from these circulating cells.[31] It is moreover conceivable that they might also be secreted into the extracellular space and transported in exosomes or small vesicles, as has been shown for microRNAs. This has

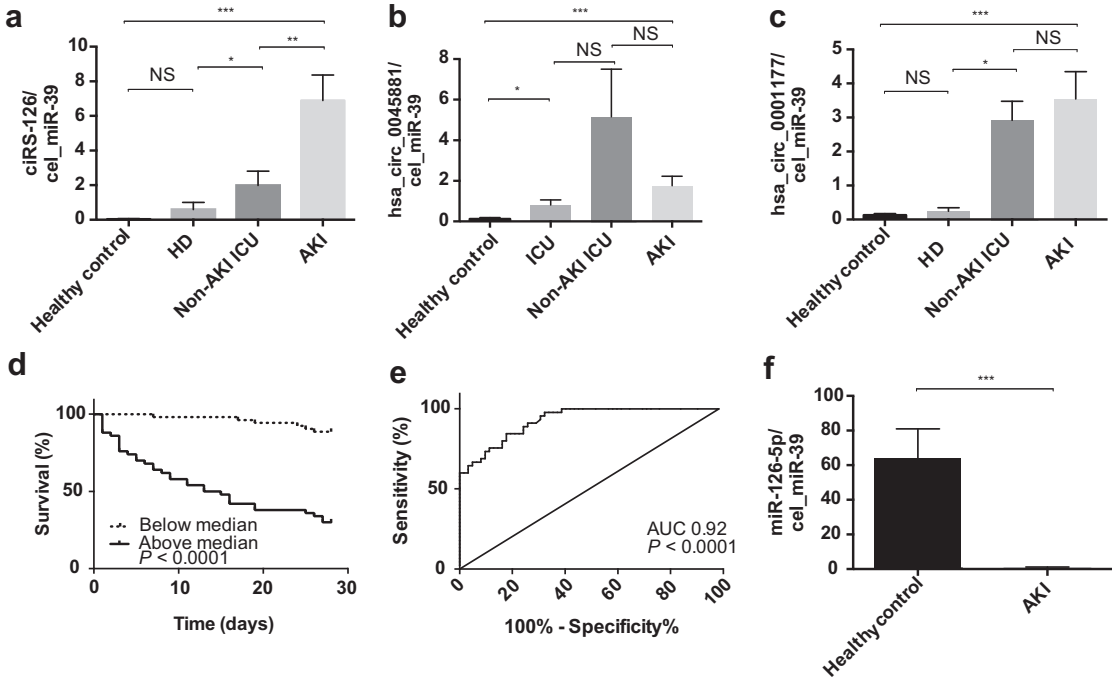

Figure 3. Concentrations of circulating (a) *ciRS-126*, (b) *hsa_circ_0045881*, and (c) *hsa_circ_0001177* in patients with acute kidney injury (AKI) compared to healthy controls and non-AKI, critically ill disease controls and patients on maintenance hemodialysis. (d) Kaplan–Meier curve analysis and log rank testing in AKI patients above and below median during an observation of 4 weeks concerning *ciRS-126*. (e) Receiver operating characteristic curve (ROC) analysis identifies a cut point of 1.165 relative expression with an area under the curve (AUC) of 0.92, a sensitivity of 91%, and a specificity of 74% regarding *ciRS-126*. (f) Circulating levels of miR-126-5p in patients with AKI. ***$P < 0.0001$, **$P < 0.01$, *$P < 0.05$. ICU, intensive care unit; HD, hemodialysis; NS, not significant.

been suggested recently.[32] Platelets may be another important source of circRNAs, as has recently been described.[33,34] CircRNAs have been shown to serve as noninvasive diagnostic tools in patients with atherosclerosis,[15] disorders of the central nervous system,[35] degenerative diseases,[10] and cancers.[32,36]

As has been previously discussed for microRNAs and lncRNAs, a "housekeeping" circRNA has not been identified in bodily fluids of patients, which is why a strategy was chosen to normalize circulating circRNAs to recombinant *Caenorhabditis elegans* miR-39 (cel_miR-39).[6,7,17,37–39] Cel_miR-39 was supplemented to samples during the RNA isolation process.

We focused on the novel circRNA transcript *ciRs-126*, which we identified as a specific biomarker of AKI. A specific role for this circRNA has not yet been described. We herein provide evidence of *ciRs-126* as an independent prognostic survival factor in AKI. Of note, miR-126-5p might be a downstream interaction partner mediating the effects of our newly identified circRNA.

Intriguingly, the linear counterpart *Leucine-rich repeats and Ig-like domains protein 1* (LRIG1) of *ciRs-126* might have an important role in acute kidney injury. It encodes a transmembrane protein that has been shown to interact with receptor tyrosine kinases of the EGFR family,[40] tyrosine-protein kinase Met,[41] and RET proto-oncogene.[42] Met has been shown to be highly important for early cytoprotection and regeneration in ischemic AKI.[43] Activation of EGFR was demonstrated to accelerate recovery from acute nephrotoxic kidney injury.[44]

Table 2. Univariate and multivariate Cox regression analysis for survival

	Univariate			Multivariate		
Variable	HR	95% CI	*P* value	HR	95% CI	*P* value
circRNA_ln	2.678	2.056–3.487	<0.001[a]	2.610	2.012–3.385	<0.001[a]
Sepsis (yes/no)	2.425	1.341–4.388	0.003[a]			
Surgery (yes/no)	2.074	1.088–3.954	0.03[a]			
SOFA score	1.167	1.064–1.280	0.01[a]			
APACHE II score	1.046	1.005–1.088	0.03[a]	1.040	0.999–1.083	0.06[a]
Horowitz score	0.997	0.994–1.000	0.03[a]			

APACHE II, Acute Physiology and Chronic Health Evaluation II; CI, confidence interval; circRNA_ln, log transformed *hsa_circ_0003266*; HR, hazard ratio; SOFA, Sequential Organ Failure Assessment.
[a]$P < 0.1$.

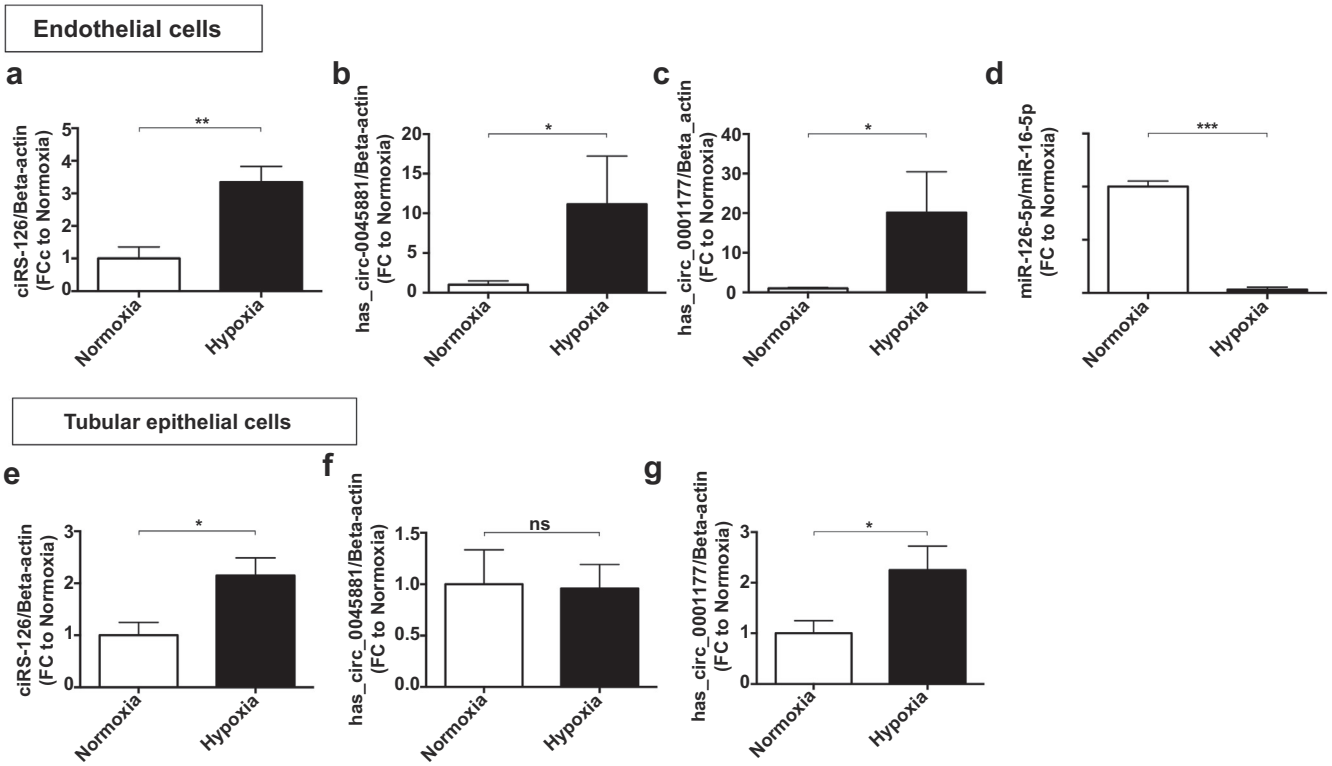

Figure 4. (a–c) Levels of *ciRS-126, hsa_circ_0045881, and hsa_circ_0001177* in cultured endothelial cells and (e–g) proximal tubular epithelial cells subjected to hypoxia. (d) miR-126-5p in endothelial cells subjected to hypoxia. ***$P < 0.0001$, **$P < 0.01$, *$P < 0.05$. FC, fold change; NS, not significant.

Because miR-126 has been shown to control endothelial cell homeostasis[30] as well as hypoxia signaling[28] and has also been shown to be an important protective microRNA in AKI,[27] we hypothesize that *ciRs-126* may be part of signaling cascade involving LRIG and miR-126, which may have implications as a cytoprotective mechanism in AKI, which is released into the circulation to counteract progressive kidney injury.

Important limitations to our study should be mentioned. First, we do not outline insights into associated molecular mechanisms of *ciRs-126* release. In-depth basic science studies are warranted to assess the specific function of *ciRs-126* in the kidney. Second, our study represents merely a single-center experience with a limited number of patients. Larger independent cohorts are highly desirable to validate our findings. Finally, most patients recruited in our study were patients with severe kidney injury (RIFLE stage 3). It would be desirable to include more patients with mild and moderate kidney injury (RIFLE stages 1 and 2) in future studies.

In conclusion, our study is the first to provide insights into the concentrations of circulating circRNAs in critically ill patients with AKI. A large number of circulating circRNAs can be reliably detected in blood of AKI patients. We identified the novel circRNA transcript *ciRs-126* as a strong and independent predictor of mortality in critically ill patients with AKI. Circulating *ciRs-126* may therefore be an intriguing RNA-based biomarker that might reflect miRNA dysregulation noninvasively.

DISCLOSURE

All the authors declared no competing interests.

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SUPPLEMENTARY MATERIAL

Supplementary Methods.

Table S1. Top 50 upregulated circRNAs in blood of AKI patients versus healthy controls, analyzed circRNAs in the whole cohort are marked in red.

Table S2. Top 50 downregulated circRNAs in blood of AKI patients versus healthy controls.

Table S3. Primer pairs.

Figure S1. Sequence base pairing between *ciRs-126* and miR-126-5p (B). Bp = base pairs.

Supplementary material is linked to the online version of the paper at www.kireports.org.

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