Using plasma circRNA_002453 as a novel biomarker in the diagnosis of lupus nephritis

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ABSTRACT

This study aimed to determine the expression of circRNAs in plasma from lupus nephritis (LN) patients to identify novel biomarkers for LN screening. We initially performed microarray screening of circRNA changes in plasma from 5 L N patients, 5 systemic lupus erythematosus (SLE) patients without LN, and 5 healthy controls. We then confirmed the selected circRNA changes in plasma from 59 SLE patients (30 with LN and 29 without LN), 26 rheumatoid arthritis (RA) patients, and 27 age- and sex-matched controls using real-time quantitative reverse transcription-polymerase chain reaction. Spearman's correlation test was performed to assess the correlation of circRNAs and clinical variables. The receiver operating characteristic (ROC) curve was created to evaluate the diagnostic value. We confirmed that plasma circRNA_002453 was significantly elevated in LN patients when compared with SLE patients without LN, RA patients, and healthy controls. Plasma circRNA_002453 was also found to be upregulated in SLE patients when compared with RA patients and healthy controls. Among these LN patients, we detected no significant correlation between plasma circRNA_002453 and disease activity, including complement 3 (C3), complement 4 (C4), and SLE disease activity index 2000 (SLEDAI-2 K) score. However, its expression level was significantly and positively correlated with 24-hour proteinuria (r = 0.571, p = 0.001) and renal SLEDAI score (r = 0.640, p < 0.001). ROC analysis showed that plasma circRNA_002453 had an area under the curve of 0.906 (95% CI 0.838–0.974, p < 0.001) to discriminate LN patients from controls (SLE patients without LN, RA patients, and healthy controls) with sensitivity of 0.900 and specificity of 0.841. The highest Youden index was 0.741 and the corresponding optimal cut-off value was 0.001. This study suggests that upregulated plasma circRNA_002453 level in LN patients is associated with the severity of renal involvement and may also serve as a potential biomarker for LN patient diagnosis.

1. Introduction

Systemic lupus erythematosus (SLE) is an autoimmune disease characterized by the presence of autoantibodies against several self-antigens, which causes serious injury to various organs or systems (Moulton and Tsokos, 2011). Lupus nephritis (LN) is a serious complication of SLE, affecting up to 70% of SLE patients, and about 10%–30% of LN will progress to end-stage renal failure (ESRD) (Thomas et al., 2014). Therefore, early diagnosis and prompt treatment may dramatically modify the course of renal disease and improve the long-term survival.

Circular RNAs (circRNAs), a newly discovered type of noncoding RNAs, which mainly consist of transcripts from the exons that are formed by non-colinear reverse splicing, are widely expressed in mammalian cells and play an important role in the regulation of gene expression at the post-transcriptional level (Memczak et al., 2013). Although circRNAs have been investigated for more than 40 years (Memczak et al., 2013), they have not received significant attention until recently. In contrast to traditional linear RNA, circRNAs do not have free 3′ or 5′ ends, but form covalently closed continuous loops (L. L. Chen and Yang, 2015; Qu et al., 2015). This helps them to avoid the action of RNA exonuclease, allowing them to maintain stable expression, which makes them more suitable as biomarkers than other types of RNA (Petkovic and Muller, 2015; Rybak-Wolf et al., 2015; Shang et al., 2016; Zhong et al., 2016). Studies have demonstrated that circRNAs not only are involved in the development of several diseases, such as cancer (Xie et al., 2016), Alzheimer's disease (Lukiw, 2013), atherosclerotic vascular disease (Wu et al., 2016), and osteoarthritis (Y.

Table 1
Microarray analysis of circRNAs that were up- or downregulated in 5 LN patients compared with 5 SLE patients without LN.

circRNA	P-value	Fold change	Regulation	GeneSymbol
hsa_circRNA_104600	0.004283276	8.012861	up	VDAC3
hsa_circRNA_100332	0.014023518	7.1100883	up	PIP5K1A
hsa_circRNA_102488	0.00813297	7.0877832	up	UBA52
hsa_circRNA_105108	0.005896593	6.9648862	up	FBXO33
hsa_circRNA_103461	0.006244314	6.5324417	up	MGLL
hsa_circRNA_100018	0.008847684	6.4954715	up	GNB1
hsa_circRNA_103637	0.010477059	6.0453621	up	DCUN1D4
hsa_circRNA_000432	0.008803752	5.8252044	up	PARPBP
hsa_circRNA_029965	0.016655623	5.7745598	up	PDS5B
hsa_circRNA_002453	0.005096146	5.6217878	up	RAD18
hsa_circRNA_404022	0.007340132	5.5555571	up	AGPAT6
hsa_circRNA_102543	0.003089939	5.3413235	up	MAP4K1
hsa_circRNA_083776	0.003229951	5.2838472	up	SCARA3
hsa_circRNA_102241	0.00946767	5.2029769	up	FOXK2
hsa_circRNA_104748	0.002537425	4.8055506	up	FOCAD
hsa_circRNA_001177	0.017057701	4.7901846	up	NRIP1
hsa_circRNA_069718	0.017971285	4.788503	up	DCUN1D4
hsa_circRNA_102059	0.02570022	4.6632831	up	MED1
hsa_circRNA_001005	0.024269338	4.5689509	up	ASB3
hsa_circRNA_104743	0.001402712	4.5672106	up	MLLT3
hsa_circRNA_005008	0.007325626	11.5590404	down	HNRNPA1P48
hsa_circRNA_034642	0.003826683	11.300732	down	VPS18
hsa_circRNA_006169	0.009960649	9.2916674	down	HNRNPA3P6
hsa_circRNA_104193	0.025532073	8.7945713	down	AHI1
hsa_circRNA_101085	0.008244689	8.4680748	down	PTGES3
hsa_circRNA_401351	0.012330157	7.8420333	down	SNURF-SNRPN
hsa_circRNA_100395	0.006565194	7.3983919	down	KLHL20
hsa_circRNA_005198	0.020431592	7.3790292	down	PARP4
hsa_circRNA_104757	0.020806711	7.2320712	down	UBAP2
hsa_circRNA_102171	0.022053631	6.8658242	down	SMURF2
hsa_circRNA_100935	0.017638098	6.5713812	down	NOX4
hsa_circRNA_000407	0.030071327	6.0810031	down	SMARCC2
hsa_circRNA_000742	0.018668539	6.0414956	down	RP11-599B13.6
hsa_circRNA_001653	0.042512897	5.8356268	down	DUSP22
hsa_circRNA_404935	0.025872272	5.7440453	down	ZBTB16
hsa_circRNA_007507	0.00230897	5.6584092	down	RASA1
hsa_circRNA_102101	0.022922916	5.4524332	down	CDC27
hsa_circRNA_103137	0.022796117	5.2122425	down	C2CD2
hsa_circRNA_000542	0.042830039	5.1608949	down	ARID4A
hsa_circRNA_100719	0.03556178	5.0438748	down	DOCK1
hsa_circRNA_103852	0.004380202	4.9026976	down	ADAMTS6
hsa_circRNA_407249	0.026153956	4.5978132	down	PRRC2B

Wu et al., 2017), but can also serve as disease biomarkers for both diagnosis and medical treatment, such as for cancer (S. Chen et al., 2017; Huang et al., 2017; Shang et al., 2016), pre-eclampsia (Zhang et al., 2016), and major depressive disorder (Cui et al., 2016). Our previous studies also showed that circRNAs in peripheral blood mononuclear cells from RA patients may serve as potential biomarkers for RA patients diagnosis (Ouyang et al., 2017). However, little is known about these RNAs in human LN.

This study was designed to determine whether circRNAs in the plasma of LN patients could be used as novel biomarkers for the diagnosis of LN. Altered changes of circRNAs may lead to the understanding of potential mechanisms of LN development and therapeutic strategy.

2. Materials and methods

2.1. Patient variables

A total of 127 participants were recruited consecutively for this study: 69 SLE patients (35 with and 34 without LN), 26 RA patients, and 32 healthy controls. All SLE and RA patients were newly diagnosed at the Department of Rheumatology and Immunology at Nanfang Hospital of Southern Medical University between 2015 and 2016. Age- and sex-matched healthy subjects who received a regular physical examination at the Department of Health from the same hospital were recruited as controls. All SLE patients recruited into this study fulfilled at least four

of the American College of Rheumatology 1997 revised criteria for the diagnosis of SLE (Hochberg, 1997), and the diagnosis of LN was defined as SLE patients with proteinuria > 0.5 g /24 h and/or proteinuria > 3+ and/or cellular casts (erythrocyte, granular, tubular, or mixed) (Hochberg, 1997). All RA patients fulfilled the American College of Rheumatology criteria for the classification of RA (Aletaha et al., 2010).

SLE or RA patients with diabetes mellitus, those with malignancies, those with a diagnosis of overlap syndrome (coexistence with other kinds of connective tissue diseases such as Sjogren's syndrome or scleroderma), and those receiving any type of nonsteroidal anti-inflammatory drug, disease modifying antirheumatic drugs, glucocorticoid, immunosuppressor, or biological agent, were excluded. Among the SLE patients with LN, those undergoing hemodialysis or with a history of renal transplantation were also excluded. Among the RA patients, those with proteinuria ≥1+ and/or cellular casts were also excluded. All study protocols were approved by the ethics committee of Nanfang Hospital of Southern Medical University (No. NFEC-2015-102). Written informed consent was obtained from all participants.

2.2. Classification of SLE activity status

The disease activity was assessed using the SLE Disease Activity Index 2000 (SLEDAI-2 K) (Gladman et al., 2002). Renal involvement was assessed using the renal SLEDAI, which consists of the four kidney-related parameters of the SLEDAI-2 K: hematuria, pyuria, proteinuria,