Original Paper

A Molecular Signature of Two Long Non-Coding RNAs in Peripheral Blood Predicts Acute Renal Allograft Rejection

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Key Words

Renal transplant • Acute rejection • Long non-coding RNA • Diagnosis • Biomarker

Abstract

Background/Aims: Acute rejection (AR) is a major complication post renal transplantation, with no widely-accepted non-invasive biomarker. This study aimed to explore the expression profiles of long non-coding RNAs (lncRNAs) in the peripheral blood (PB) of renal transplant recipients and their potential diagnostic values. **Methods:** The genome-wide lncRNA expression profiles were analyzed in 150 PB samples from pediatric and adult renal transplant (PRTx and ARTx) cohorts. The diagnostic performance of differentially expressed lncRNA was determined using receiver operator characteristic curve, with area under the curve (AUC) and 95% confidential interval (CI). Finally, a risk score was constructed with logistical regression model. **Results:** A total of 162 lncRNAs were found differentially expressed in PRTx cohort, while 163 in ARTx cohort. Among these identified lncRNAs, 23 deregulated accordingly in both cohorts, and could distinguish AR recipients from those without AR. Finally, a risk score with two most significant lncRNAs (AF264622 and AB209021) was generated and exhibited excellent diagnostic performance in both PRTx (AUC:0.829, 95% CI:0.735-0.922) and ARTx cohorts (AUC: 0.889, 95% CI: 0.817-0.960). **Conclusion:** A molecular signature of two lncRNAs in PB could serve as a novel non-invasive biomarker for the diagnosis of AR in both pediatric and adult renal transplant recipients.

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Introduction

Renal transplantation is the preferred treatment of choice for patients with end stage renal disease (ESRD) [1, 2]. Despite substantial improvements in immunosuppressive therapies and surgical techniques, acute rejection (AR) remains a frequent and serious post-transplantation complication, which contributes to late allograft loss and mortality [3, 4]. To date, the gold standard for diagnosing AR relies on multiple and repeated renal allograft biopsies. Although this invasive procedure has become safer over the years, challenges and complications still exist such as sampling error, inter-observer variability, bleeding, arteriovenous fistula, graft loss, and even death [5]. Hence, development of noninvasive and reliable biomarkers of AR is one of the major objectives in renal transplantation, and various efforts have been made to identify such biomarkers in the peripheral blood (PB) and urine of renal transplant recipients [6-9].

The rapid advances in the depth and quality of transcriptome sequencing have led to the discovery of thousands of long non-coding RNAs (lncRNAs) [10]. As newly discovered members of RNA, lncRNAs are generally defined as transcripts longer than 200 nucleotides with little or no protein-coding potential [11]. A growing body of evidence indicates that lncRNAs could regulate gene expression at the transcriptional and/or post-transcriptional levels, and play fundamental roles in a diverse array of biological processes such as cell cycle control, development, carcinogenesis, and immune response [12-15]. A large number of dysregulated lncRNAs have been identified in the renal cortex tissues of AR recipients [16-18]. Recently, Lorenzen and colleagues have analyzed the lncRNA expression profiles in the urine of renal transplant recipients, and demonstrated that urinary RP11-354P17.15-001 could serve as a novel biomarker of acute T cell-mediated rejection (ATMR) of renal allografts [19]. However, to date, the lncRNA expression patterns in the PB of renal allograft recipients have not yet been investigated.

In the current study, we comprehensively analyzed the lncRNA expression profiles in the PB samples of patients from two cohorts: adult renal transplant (ARTx) and pediatric renal transplant (PRTx) cohorts [6, 7], with the aim to: 1) explore the differentially expressed lncRNAs in PB of renal transplant recipients with and without AR; 2) identify the circulating lncRNAs that could distinguish recipients with AR from those without; 3) generate a lncRNA signature in PB which could serve as a novel diagnostic biomarker of AR in both adult and pediatric renal transplant recipients.

Materials and Methods

Study population

This two-cohort observational study was conducted and reported in accordance with the STROBE (Strengthening the Reporting of Observational studies in Epidemiology) guidelines [20]. In the PRTx cohort, all patients received the renal transplantation at Stanford University and routinely followed up for two years [6]. In the ARTx cohort, the subjects were randomly recruited from five different clinical centers including Scripps Clinic, Cleveland Clinic, St. Vincent Medical Center, University of Colorado, and Mayo Clinic Arizona [7]. The renal biopsies were scored by at least two pathologists blindly and independently according to Banff classification [21]. Finally, a total of 150 biopsy-defined AR and stable (STA) recipients with corresponding PB samples were included (PRTx: n=75, ARTx: n=75), and the detailed selection criteria have been described previously [6, 7]. All subjects were well informed and provided written consent, and the appropriate approvals were obtained from the corresponding institutional review boards [6, 7]. Furthermore, the protocol of current study was approved by the institutional review board of Nanjing First Hospital, Nanjing Medical University.

RNA isolation and lncRNA expression profiling

Each PB sample was paired with renal allograft biopsy (in PRTx cohort: within 48 hours; in ARTx cohort: at the time of biopsy), and collected in 2.5 ml PAXgene Blood RNA Tubes (PreAnalytiX, Qiagen,

Valencia, CA; or PreAnalytiX GmbH, Hombrechtikon, Switzerland), and total RNA was extracted using the PAXgene Blood RNA Kit according to the manufacturer's instructions [6, 7]. The well-prepared cDNA was hybridized to Affymetrix Human Genome U133 (HG-U133) Plus 2.0 GeneChips (Affymetrix Inc., Santa Clara, CA), and the raw expression data were deposited in Gene Expression Omnibus database (accession numbers: GSE14346 for PRTx cohort and GSE15296 for ARTx cohort). Even though Affymetrix HG-U133 Plus 2.0 array was initially designed to profile protein-coding mRNAs, it fortuitously contains a large group of lncRNA-specific probes, which makes it possible to explore the lncRNA expression patterns via mining and repurposing the previously published expression microarray data [14, 15]. After quantile normalized and background adjusted with Robust Multichip Average, the microarray data was processed in the R software (version 2.11.1; R Foundation for Statistical Computing, Vienna, Austria) with GATExplorer (file name: ncrnamapperhgu133plus2cdf_3.0) to determine the re-annotated lncRNA expression levels [22]. According to the predesigned selection criteria: 1) with at least three probes mapped uniquely to the RNAdB database [23]; 2) validated in NONCODE: an interactive database with the most complete collection and annotation of lncRNAs [24]; 3) raw expression level more than 1 in at least 10% of all samples; 4) with changes of more than 1.5-fold from the median value in at least 20 % of samples, the eligible lncRNAs were retained for further analysis.

Statistical Analysis

The continuous variables were compared using Student's t test after the normality status confirmed with Kolmogorov-Smirnov and Shapiro-Wilk tests. The differentially expressed lncRNAs in PB between two groups (AR vs. STA) were determined with Significance Analysis of Microarrays (SAM) method embedded in BRB-Array tools (version 4.5.1; National Cancer Institute, Bethesda, MD, USA), which were developed by Dr. Richard Simon and BRB-Array Tools Development Team [25]. To minimize false positives, SAM analysis was performed using stringent statistics variables with a false discovery rate (FDR) less than 0.05, and permutation of 1000. The differentially expressed lncRNAs were defined as those greater than 1.5 in fold change between groups. In addition, the unsupervised hierarchical cluster analysis was performed with one minus correlation and average linkage methods. Receiver operating characteristics (ROC) curves were constructed to assess sensitivity, specificity, and respective area under the curve (AUC) with 95% confidential interval (CI). After cross matched in two cohorts, two most significant lncRNAs (one upregulated and one downregulated) in terms of AUC were selected to generate the Acute Rejection Risk Score (ARRS) with logistical regression model [26]. The value with the highest accuracy (i.e., minimal false-negative and false positive rates) was chosen as the optimal cutoff value. Statistical significance was taken as a two-tailed *P* value <0.05 unless specifically indicated. The statistical analyses were performed using BRB-Array Tools and SPSS (version 21.0; SPSS Institute Inc., Chicago, IL, USA), as appropriate.

Results

Patient characteristics

In this two-cohort observational study, a total of 150 renal transplant recipients were included, with paired PB and renal biopsy samples. In the PRTx cohort (n=75), a summary of 38 recipients experienced AR episodes during the two-year follow up. In the ARTx cohort (n=75), fifty one patients suffered from biopsy-proven AR episodes. No significant difference was observed in the distribution of age, gender, and human leukocyte antigen (HLA) mismatch between AR and STA recipients in both cohorts, and the detailed information has been described previously [6, 7].

Differentially expressed lncRNAs in PB of renal transplant recipients

After mining Affymetrix HG-U133 Plus 2.0 array with GATExplorer, a total of 5635 lncRNA transcripts were identified with at least three probes mapped uniquely to them, which were further confirmed in NONCODE. In PRTx cohort, a total of 162 lncRNAs were found differentially expressed between two groups (Table 1), among which six lncRNAs were up-regulated in AR recipients' PB. Similarly, a summary of 163 lncRNAs were demonstrated to express differentially between two groups in ARTx cohort (Table 2). However, unlike the PRTx cohort, most lncRNAs (113/163, 69.3%) were upregulated. In addition, the unsuper-

Table 1. Summary of differentially expressed lncRNAs in peripheral blood of pediatric renal transplant recipients

lncRNA	FC	lncRNA	FC	lncRNA	FC	lncRNA	FC
AF264622	2.54	AL117474	0.64	AK022133	0.6	AF126109	0.54
AK024956	1.88	AL713660	0.64	AK023395	0.6	BC040884	0.54
AK026079	1.81	CR613326	0.64	AK021610	0.6	AK000115	0.53
AK055877	1.77	AK093869	0.64	CR625974	0.6	AL050376	0.53
BC026299	1.69	AK025015	0.64	AK021623	0.59	BC016795	0.52
AK024375	1.64	AK022339	0.64	AK027193	0.59	AK027156	0.52
CR620977	0.66	AK095317	0.64	AK094817	0.59	AK098425	0.52
CR597405	0.66	AF339810	0.63	uc003tgp.1	0.59	AK021632	0.52
AK056140	0.66	AK090686	0.63	AF052160	0.59	BC032347	0.51
AK025785	0.66	AK096804	0.63	AF211977	0.59	BC040965	0.51
CR625009	0.66	BC090942	0.63	AK095817	0.59	AK026295	0.51
AK091100	0.66	AK095023	0.63	AK093327	0.59	AF306939	0.51
BC041001	0.66	CR611332	0.63	AF086063	0.59	BC029907	0.51
AK022078	0.66	CR608275	0.63	AK124227	0.59	BC142985	0.51
AJ420595	0.66	AK055719	0.63	AK024981	0.58	AK057167	0.5
CR594753	0.66	CR606559	0.62	AK074610	0.58	AK123904	0.5
U63542	0.66	BC036622	0.62	CR617316	0.58	uc001yfy.1	0.5
AK021636	0.66	AF086270	0.62	BC037916	0.57	AK125443	0.5
AF085998	0.66	BC041937	0.62	AK094078	0.57	BC067863	0.49
AK093907	0.66	AK094724	0.62	AK123567	0.57	CR618720	0.49
AK025546	0.66	AK074440	0.62	AF070592	0.57	AL050082	0.49
AK026716	0.65	AK056007	0.62	AX775927	0.57	BC005232	0.48
BC039426	0.65	X61080	0.62	AK022181	0.57	AL049423	0.48
AK025317	0.65	AK054895	0.62	CR617865	0.57	AK001279	0.48
BC034813	0.65	AK311180	0.62	AK090972	0.57	AK024870	0.46
AK026895	0.65	AK024118	0.62	CR617898	0.57	AK025613	0.46
BC035091	0.65	AL833464	0.62	BC040834	0.56	BC032890	0.44
BC016013	0.65	AK128410	0.62	AK026078	0.56	AF363068	0.44
AL833181	0.65	AK097526	0.62	BC067908	0.56	AB209021	0.43
BC001739	0.65	AK096487	0.62	BC040991	0.56	AK096729	0.43
BC035168	0.65	AK098081	0.62	AK098497	0.56	AK095793	0.43
AK056630	0.65	AK124183	0.62	U79271	0.56	AL157495	0.42
AF400487	0.65	AF086097	0.62	AK123548	0.56	BC043240	0.41
AK022479	0.64	AF075087	0.62	BX537508	0.56	AK055670	0.4
uc002ldy.1	0.64	AK023469	0.62	BC054012	0.56	AK024934	0.4
AF086031	0.64	AK123379	0.61	CR749703	0.55	AK001086	0.4
DQ786290	0.64	AF379627	0.61	AK025702	0.55	AL049951	0.39
AK056744	0.64	AK095250	0.61	BC038181	0.55	AL832284	0.38
AL833737	0.64	AK128638	0.61	BC033248	0.54	AY007155	0.36
AK026900	0.64	CR622106	0.61	AK098491	0.54		
BC067233	0.64	BC041913	0.61	BX648207	0.54		

vised hierarchical clustering with lncRNA expression data could clearly distinguish AR from STA recipients in both cohorts (Fig. 1). Finally, a total of 23 lncRNAs were found dysregulated accordingly in both cohorts (Fig. 2), among which two were upregulated and the remaining 21 were downregulated.

Identification of lncRNAs capable of distinguishing AR from STA renal recipients

To assess the diagnostic capabilities of all the 23 lncRNAs, ROC curves were constructed, and the AUC with corresponding 95% CI was generated for each lncRNA in two cohorts (Table 3). The results confirmed that all these lncRNAs could discriminate AR from STA patients with moderate to excellent diagnostic performance. As presented in Table 1 and Fig.

Table 2. Summary of differentially expressed lncRNAs in peripheral blood of adult renal transplant recipients

lncRNA	FC	lncRNA	FC	lncRNA	FC	lncRNA	FC
AK021643	2.74	BC012054	1.65	AK091776	1.55	AK091335	0.63
AF264622	2.55	BC028913	1.65	BC017275	1.54	AK123531	0.63
AK022204	2.29	AK025048	1.64	AK091544	1.54	AK096729	0.63
AF085924	2.16	AK094623	1.64	AK022801	1.54	BC045174	0.63
BC036226	2.12	AF244571	1.64	AK091814	1.54	CR617316	0.62
AF147427	2.08	AK094655	1.63	BC041928	1.54	AK057981	0.62
AK025152	2.01	AK025388	1.63	AF086037	1.54	AF113697	0.62
AK026286	1.95	AK074607	1.63	BC024172	1.54	BC043240	0.62
AK128702	1.94	AF075065	1.63	AK021981	1.53	AK055670	0.62
AK023887	1.89	AK129971	1.63	AF085866	1.53	uc004ebm.1	0.62
AK055572	1.89	AK023733	1.62	AK094167	1.53	AK098051	0.61
AK023302	1.88	AF075111	1.62	BC013931	1.53	U09197	0.61
BC035983	1.87	AF289615	1.61	AK024862	1.53	BX648207	0.61
BC007908	1.86	AK022121	1.61	AK022376	1.53	AK128786	0.6
AB062477	1.86	AK025291	1.61	AK022140	1.53	AF119854	0.6
AK124099	1.86	AK021821	1.6	AK130460	1.52	AL122062	0.59
AK123363	1.84	BC030757	1.59	AK021554	1.52	BC041913	0.59
BC035958	1.8	AJ227898	1.59	BC026239	1.52	BC027967	0.59
AK024517	1.8	AK025552	1.59	AF086356	1.52	BX537762	0.59
AF086051	1.79	BC039388	1.59	AF088069	1.52	AK055612	0.58
AK022162	1.77	AK022346	1.58	AK095787	1.52	AL049951	0.58
BC025999	1.77	AF075072	1.58	BC008462	1.52	BC036622	0.57
AF147324	1.75	BC035063	1.58	AK127827	1.51	CR601532	0.56
BC043356	1.74	AY952882	1.58	AF085972	1.51	CR611332	0.56
AK000942	1.73	BC008631	1.58	AK023865	1.51	X52767	0.56
AF086551	1.73	AK026036	1.57	AK098511	1.51	CR622106	0.55
BC043380	1.73	AK022364	1.57	AK024690	1.51	CR608275	0.55
U43604	1.72	AK022379	1.57	BC035168	1.51	AK021632	0.55
AK094967	1.72	BC017507	1.57	AK074886	1.5	AL157495	0.54
AK025210	1.71	AF085833	1.57	AK021862	1.5	AL833097	0.53
AK124590	1.7	AK024909	1.57	BC017944	1.5	AK026078	0.53
AB051446	1.7	AK001604	1.57	AB075482	0.66	CR618720	0.53
AK025818	1.7	AK055807	1.57	BC009638	0.66	AK090972	0.51
AK024956	1.7	BC035091	1.57	AK097103	0.66	CR749465	0.51
AK055497	1.69	AK022429	1.56	CR606559	0.66	AF147723	0.45
AK091973	1.69	AK025126	1.56	AK001279	0.66	AK023330	0.44
AL833080	1.68	AL833423	1.56	BC035180	0.65	AB209021	0.42
AK054865	1.68	AK025161	1.56	CR592483	0.65	BC015064	0.41
AK022159	1.68	AF143326	1.55	AK098425	0.65	CR617865	0.39
AF086008	1.67	BC009608	1.55	AF093744	0.65	AK124833	0.38
AK093778	1.67	AK025143	1.55	AF116692	0.64		

3, two lncRNAs (AF264622 and AB209021) had the most excellent diagnostic capabilities in both cohorts.

Establishment of a molecular panel of two lncRNAs predictive of AR

With the two most significant lncRNAs: AF264622 and AB209021, the ARRS was calculated in both cohorts. In the PRTx cohort, ARRS was generated as follows: $ARRS = 1.369 \times \text{expression value of AF264622} - 0.856 \times \text{expression value of AB209021} - 3.368$, and then subjected to ROC curve analysis (Fig. 4). As shown in Fig. 4, the generated ARRS could distinguish AR from STA patients with an AUC of 0.829 (95% CI: 0.735-0.922; $P < 0.001$). Per the ROC curve analysis,

Fig. 1. Unsupervised hierarchical cluster analysis of the differentially expressed lncRNAs. A. 162 differentially expressed lncRNAs in peripheral blood of 75 pediatric renal transplant recipients. B. 163 differentially expressed lncRNAs in 75 adult renal transplant peripheral blood samples.

Fig. 2. Differentially expressed lncRNAs in pediatric and adult renal transplant recipients. Venn diagram of deregulated lncRNAs in both PRTx (pediatric renal transplant) and ARTx (adult renal transplant) cohorts.

an optimal cutoff value (1.886) with selected, and the diagnostic sensitivity and specificity of the two-lncRNA based ARRS for AR detection were 0.658 and 0.946, respectively. In the ARRTx cohort, the same equation was applied to construct ARRS, which was further subjected to ROC curve analysis to determine its diagnostic performance (AUC: 0.889, 95% CI: 0.817-0.960; $P < 0.001$; Fig. 4). Finally, a cohort-specific cutoff value (1.228) was determined, with the corresponding sensitivity and specificity as 0.745 and 0.875.

Discussion

Renal transplantation has processed from a risky experimental therapy to a safe and life-saving treatment for ESRD patients over the past five decades [2]. However, AR still remains a major determining factor which influences the short-term function and long-term outcome of both recipients and allografts [3]. Molecular classification of acute renal allograft rejection independent of invasive biopsy procedure is an unmet need in clinical practice [27-30]. To the best of our knowledge, this is the first study to evaluate the lncRNA profiles in PB of renal transplant recipients. The major findings of the present study are as follows: 1) a variety of circulating lncRNAs were differentially expressed in AR recipients compared with those without AR, among which 23 deregulated accordingly in both PRTx and ARTx cohorts; 2) the 23 differentially expressed lncRNAs could discriminate AR recipients from those without stable renal allograft function in both cohorts, among which AF264622 and AB209021 exhibited the best diagnostic performance; 3) the two-lncRNA (AF264622 and AB209021) based ARRS could serve as a novel and non-invasive biomarker for the early diagnosis of AR in both pediatric and adult renal transplant recipients.

As an endogenous family of noncoding RNA, lncRNAs have been documented to regulate various pathophysiological processes including carcinogenesis and immune response [12,

Table 3. Summary of twenty three lncRNAs with diagnostic values in both pediatric and adult renal transplant recipients

lncRNA	PRTx Cohort			ARTx Cohort		
	AUC	95% CI	P value	AUC	95% CI	P value
Upregulated lncRNAs (AR vs. STA)						
AF264622	0.797	0.696-0.897	<0.001	0.862	0.782-0.942	<0.001
AK024956	0.767	0.657-0.878	<0.001	0.759	0.648-0.870	<0.001
Downregulated lncRNAs (AR vs. STA)						
AB209021	0.807	0.710-0.905	<0.001	0.846	0.758-0.933	<0.001
AK026078	0.747	0.634-0.860	<0.001	0.813	0.715-0.911	<0.001
BC036622	0.753	0.643-0.864	<0.001	0.798	0.694-0.903	<0.001
BX648207	0.779	0.671-0.886	<0.001	0.751	0.641-0.861	<0.001
CR606559	0.743	0.630-0.855	<0.001	0.775	0.664-0.885	<0.001
AK001279	0.768	0.659-0.878	<0.001	0.731	0.621-0.842	0.001
AK021632	0.684	0.559-0.808	0.006	0.779	0.672-0.887	<0.001
AK055670	0.853	0.768-0.938	<0.001	0.681	0.560-0.802	0.012
AK090972	0.718	0.599-0.838	0.001	0.784	0.679-0.889	<0.001
AK096729	0.767	0.659-0.876	<0.001	0.734	0.620-0.848	0.001
AK098425	0.738	0.620-0.855	<0.001	0.722	0.604-0.840	0.002
AL049951	0.742	0.629-0.855	<0.001	0.703	0.584-0.821	0.005
AL157495	0.708	0.589-0.827	0.002	0.803	0.700-0.907	<0.001
BC041913	0.669	0.542-0.795	0.012	0.751	0.632-0.870	<0.001
BC043240	0.751	0.639-0.864	<0.001	0.715	0.599-0.831	0.003
CR608275	0.686	0.563-0.808	0.006	0.832	0.734-0.929	<0.001
CR611332	0.715	0.596-0.834	0.001	0.761	0.655-0.868	<0.001
CR617316	0.718	0.597-0.840	0.001	0.806	0.707-0.905	<0.001
CR617865	0.720	0.602-0.837	0.001	0.833	0.735-0.931	<0.001
CR618720	0.728	0.610-0.846	0.001	0.807	0.708-0.907	<0.001
CR622106	0.692	0.570-0.814	0.004	0.878	0.802-0.954	<0.001

Fig. 3. Expression levels and diagnostic characteristics of circulating lncRNAs in pediatric and adult renal transplant recipients. The expression levels of each lncRNA between groups were compared with Student's t test. AR, acute rejection; STA, stable function; AUC, area under the curve; 95% CI, 95% confidential interval.

31]. Mounting evidence indicated lncRNAs in blood could serve as novel biomarkers for the early diagnosis and prognosis prediction of human diseases [32-34]. In renal transplantation, the global lncRNA expression levels have been profiled in three AR renal biopsy tissues and control tissues from three renal tumor patients, and a total of 5339 lncRNAs were proven

Fig. 4. The molecular signature of two lncRNAs in peripheral blood for the diagnosis of acute rejection. A and D. lncRNA risk score distribution; B and E. Heatmap of the lncRNA expression profiles; rows represent lncRNAs, and columns represent patients. The red dotted line represents the lncRNA signature cutoff value. C and F. Receiver operating characteristics curve analysis of the lncRNA signature for the diagnosis of acute rejection in renal transplant recipients.

to differentially express between groups, among which five lncRNAs (AF113674, uc003wbj, uc010ftb, uc001fty, and AK129917) were selected for further validation and confirmed by quantificational real-time polymerase chain reaction (qRT-PCR) [16, 17]. In a recent clinical study, Lorenzen et al. comprehensively analyzed the genome-wide lncRNA expression profiles in the urine samples (9 ATMR vs. 9 STA recipients), and found a summary of 4189 differentially expressed lncRNAs. During the further validation in a cohort of 93 recipients (62 ATMR vs. 31 STA) with qRT-PCR, urinary RP11-354P17.15-001 was proven as a reliable biomarker for the diagnosis of ATMR [19]. However, to date, the lncRNA expression profiles in PB of renal transplant recipients have never been studied.

In this two-cohort observational study with 150 renal transplant recipients, we comprehensively analyzed the genome-wide lncRNA expression profiles in PB samples via mining Affymetrix HG-U133 Plus 2.0 microarray data. Among the 5635 re-annotated lncRNA transcripts, 162 were aberrantly expressed in PRTx cohort and 163 in ARTx cohort. Of all these differentially expressed lncRNAs, a total of 23 dysregulated accordingly in both cohorts, and could discriminate AR recipients from those without AR. Finally, with the two lncRNAs (AF264622 and AB209021), an ARRS was generated and proven to distinguish AR patients with excellent diagnostic performance (PRTx cohort: AUC:0.829, 95% CI:0.735-0.922; and ARTx cohort: AUC: 0.889, 95% CI: 0.817-0.960).

Of these two AR-related lncRNAs: AF264622 and AB209021, none has been studied previously. AF264622 is a 484 bp lncRNA located with the intron of solute carrier family 8, member 1 (SLC8A1), which is located on chromosome 2p22.1. The SLC family consists of over 300 membrane-bound proteins that facilitate transporting of various substrates across biological membranes, which in turn exert fundamental functions in pathophysiological processes including acute renal allograft rejection [35, 36]. However, as one member of SLC family, the biological roles of SLC8A1 in renal transplant remain yet to investigate. AB209021 is located on chromosome 3q27.3, which spans 5267 bp and consists of three exons. Structurally, AB209021 shares the exon sense-overlapping relationship with the gene eukaryotic translation initiation factor 4A2, which is a highly conserved gene for one

of the protein-synthesis initiation factors involved in the binding of mRNA to the ribosome [37]. Overall, these two lncRNAs might play substantial roles in the fundamental biological processes, which warrant further comprehensive biological investigations.

Some limitations should be acknowledged in interpreting the results. First, a total of 5635 eligible lncRNAs were initially identified via mining Affymetrix HG-U133 Plus 2.0 microarray data and included in the current study, which accounted for about 35.7% (5635/15767, data from GENCODE) and the number of total identified lncRNAs in human beings is still growing [38]. Hence, the diagnostic lncRNAs identified here may not represent all the lncRNA candidates of diagnostic values for the prediction of AR. Second, the lack of traditional diagnostic parameters such as serum creatinine and blood urine nitrogen, which hindered the comparison of the diagnostic performance between the newly generated ARRS and traditional biomarkers. Third, even though two independent well-matched cohorts were included and robust statistical analyses were applied, the false-positive results do potentially exist, which need an external validation cohort to validate the results with different methods such as qRT-PCR.

Conclusion

In summary, by analyzing the genome-wide lncRNA expression profiles in the PB samples of renal transplant recipients from two independent well-matched cohorts, our study identified the specific circulating lncRNAs differentially expressed between AR and STA patients, and a molecular signature of two lncRNAs (AF264622 and AB209021) in PB, which could serve as a novel non-invasive biomarker for the early diagnosis of AR in both pediatric and adult renal transplant recipients. Further validation studies are warranted to verify our findings within prospectively designed clinical trials and comprehensive biological experiments.

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Disclosure Statement

The authors declare that they have no competing interests.

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