



Increased expression of miR-155 correlates with abnormal allograft status in solid organ transplant patients and rat kidney transplantation model

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ABSTRACT

Aims: Increasing evidence has shown the diagnostic value of miR-155 in organ transplantation. The dysregulation of miR-155 is reported to be associated with development of acute or chronic complications in solid organ transplant recipients. Here, we summarized related evidence to explore the correlation between the dysregulation of miR-155 and various allograft dysfunction in transplant recipients, and verified the dynamic change of miR-155 level in acute rejection (AR) using a rat renal transplantation model.

Main methods: Eligible studies were retrieved from PubMed, Embase, and Cochrane Library databases. A meta-analysis method was performed to evaluate the diagnostic value of miR-155 in transplant recipients. Furthermore, the F344-Lewis rat renal transplantation model was established to validate the dynamic change of miR-155 expression during AR.

Key findings: A total of 275 transplant patients, including renal, heart, and lung transplantation from 6 studies were analysed. The pooled SEN of miR-155 was 0.87 (95% CI, 0.78–0.93), the pooled SPE was 0.76 (95% CI, 0.63–0.85), the pooled PLR was 3.6 (95% CI, 2.2–5.8), the pooled NLR was 0.17 (95% CI, 0.09–0.31), the pooled DOR was 17.31 (95% CI, 7.20–41.65) and pooled AUC was 0.89 (95% CI, 0.86–0.92). The rat renal transplantation model (n = 24) and control model (n = 15) were successfully established. Expression of miR-155 in plasma was significantly increased in 7 d and 9 d post-transplantation compared to the control group ($P < 0.05$), and was consistent with the dynamic change of AR degree.

Significance: miR-155 is a potential biomarker for monitoring the abnormal allograft status in solid organ transplantation.

1. Introduction

Organ transplantation is the last resort for end-stage solid organ disease and can greatly improve the quality of life for patients. However, despite advanced surgical techniques and immunosuppressive medication, all kinds of allograft damages, especially allograft rejection, still represent the leading causes of impaired graft failure [1–3]. Currently, limited laboratory measurements are used for detecting the graft damage, and the following invasive examination and histological analyses are the standard method for diagnosis of different types of pathological processes after transplantation. Due to the difficulty in early diagnosis of allograft dysfunction, a minimally invasive,

sensitive, and specific bio-marker which can reflect the real-time allograft status as well as discriminate the early-stage dysfunction is urgently needed.

MicroRNAs (miRNAs) are small single-stranded RNA molecules (~22 nucleotides) that play a crucial role in extensive physiological and pathological processes. Due to their stable and detectable characteristics in serum, plasma, tissues, and urine, miRNAs are analysed as potential biomarkers in various types of solid organ transplantation [4–6]. According to Hamdorf et al., a total of 48 miRNAs, 40 miRNAs, 18 miRNAs and 6 miRNAs were found separately related to the dysfunction in lung, kidney, liver and heart transplantation, respectively. Among the allograft-related miRNAs, miR-155 was one of the most

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overlapped biomarkers [4]. Specifically, in this study, we aim to investigate the clinical significance of miR-155, which has been reported to be remarkably upregulated in all the cases of lung, kidney, liver and heart transplant rejection.

miR-155 is required for an immune response in humans. It is necessary for CD8⁺ T-cell proliferation, and its deficiency can lead to down regulation of T-cells proliferation [7,8]. Li et al. [9] reported that miR-155 suppressed the immune response in liver transplant mice through regulating Th1/Th2 differentiation, related cytokines, and the function of Kupffer cells. In a multi-centre clinical study, Duong et al. [10] found a remarkable dysregulation of miR-155 in the serum and cardiac grafts. Considering the limited number of included patients in each study, although the clinical significance of miR-155 was observed, the diagnostic efficiency of miR-155 in allograft dysfunction is still unclear.

In this research, we aim to reduce the heterogeneity, or inconsistency, among different reports. We summarized the published cohorts of solid organ transplant patients and analysed the overall diagnostic accuracy of miR-155. A total of 99 patients with various allograft damage and 176 patients with stable graft function from 6 studies were analysed. At the same time, the dynamic changes of the expression level of miR-155 in peripheral blood were also detected in the F344-Lewis rat renal transplant model. Based on this multi-study data analysis and animal model, our results suggest that miR-155 is a potential allograft dysfunctional biomarker for the screening and diagnosis of solid organ transplant patients.

2. Materials and methods

2.1. Literature search strategies

The documents that met inclusion criteria were searched by two independent researchers (J. Liang and Z. Liu) using PubMed, Embase, and Cochrane Library databases up to December 15, 2018. The language was limited to English. The following keywords were used in different combinations: “miR-155” or “miRNA-155” or “microRNA-155” or “has-miR-155” and “transplantation” or “transplant” or “allograft” or “graft” or “rejection” or “reject” or “transplanted”. Additionally, potentially relevant studies were also screened from the reference lists of the full-text articles.

2.2. Inclusion and exclusion criteria

The eligible studies were included based on the following criteria: (1) the study subjects were patients with any kind of solid organ transplantation; (2) miR-155 expression levels were measured or verified by quantitative real-time reverse transcription polymerase chain reaction (qRT-PCR) or TaqMan miRNA assays; (3) patients were divided into stable graft function—and any kinds of graft dysfunction—subgroups based on the clear relevant diagnosis criteria; and (4) the diagnosis accuracy of miR-155 was reported such as sensitivity, specificity, area under the ROC curve (AUC) and 95% confidence interval (CI). Exclusion criteria were as follows: (1) abstracts, reviews, meta-analyses, letters, comments, and case report; (2) studies without patient samples; (3) studies without complete data to construct a four-fold contingency table; and (4) studies only focusing on the molecular mechanism of miR-155. For duplicate publications, only the most recent or most informative single article was included. For the study containing a tested dataset and a validated dataset, or different kinds of allograft dysfunction subgroup, all the data were included.

2.3. Data extraction and quality assessment

Data were extracted by two independent investigators (J. Liang and Y. Tang), and the inconsistencies in data extraction were listed and consulted by a third author (Y. Lu). Data includes authors, year of

publication, country, number of patients, dysfunctional types, sensitivity, specificity, defining methods of cut-off value, and area under the receiver operating characteristic (AUC) curve. Original data were requested from study authors if necessary. The quality of included studies was assessed by two other authors (Z. Zou and C. Zhou) according to the quality assessment of studies of diagnostic accuracy included in systematic reviews (QUADAS) checklist [11]. Twelve items of the QUADAS checklist and the quality of the studies are presented in Table 2.

2.4. Animal model

The animal study was performed at the Laboratory for Animal Research (West China Hospital). 32 male F344 rats (3 months old; weight 180–260 g) were used as donors and 32 male Lewis rats (3 months old; weight 220–300 g) as recipients. Rats were supplied by Beijing Vital River Laboratory (Beijing Vital River Laboratory Animal Technology Co., Ltd., Beijing, China). The rats were raised in a specific pathogen-free environment at 25 °C, 40–70% humidity with a 12-h light/dark cycle. All rats were allowed to move freely and had free access to food and water, and were fasted 12-h prior to operation. The experimental protocol of this study was approved by the West China Hospital of Sichuan University Biomedical Research Ethics Committee. Donor rats were anaesthetized with Pentobarbital® (Spofa, Prague) given intraperitoneally in a dose of 50 mg/kg body weight. The rats were placed on an operating table with a warming pad to maintain the body temperature at 37 °C constantly during surgery. The control group consisted of nephrectomised male Lewis rats (n = 15). All microsurgical techniques were performed by the authors (J. Liang, L. Tang and Z. Zou) according to the method summarized by Shrestha et al. [12]. Donor and recipient operations were performed sequentially.

2.5. Donor operation

The abdomen was opened through a midline incision from the xyphoid to the symphysis pubis. The bowel was covered with a moist gauze and retracted to the right to expose the left kidney. Separation and ligation of the branches of renal arteries and veins, aorta and inferior vena cava were performed. The left ureter was isolated distally to the bladder, exposing the ureter vesical junction. Fat and connective tissue adhering to the ureter were not completely removed. The inferior vena cava was clamped at the infrarenal end, and the aorta was clamped at the suprarenal end and 2 cm below the left renal artery. A 27-gauge needle was inserted into the abdominal aorta between the two vascular clamps for perfusion of the kidney with 3 mL of ice-cold normal saline containing heparin (25 U/mL). Blood and perfusate were drained through a vent in IVC. Perfusion was continued until the kidney became uniformly pale and the perfusate was clear. The left renal artery and vein were cut close to their junctions with the AO and the IVC. The ureter was cut close to the ureter-vesical junction. The left kidney, vessels, and ureter were placed, en bloc, into ice-cold normal saline.

2.6. Renal allograft preparation

Preparation of cuff for the renal vein was performed in the ice-cold heparinized saline. A polyethylene tubing (1–1.5 cm in length, AHMSIC, Co., Ltd., USA) was used as a cuff tube and the size was chosen according to the donor's vein size. There were two sizes used, the inner diameter of the tube (0.86 or 1.14 mm) and the outside diameter (1.32 or 1.63 mm). The donor renal vein was passed through the lumen of the cuff, and its end was then everted over the cuff. The donor renal vein was ligated to the cuff body using a circumferential 9-0 Nylon line.

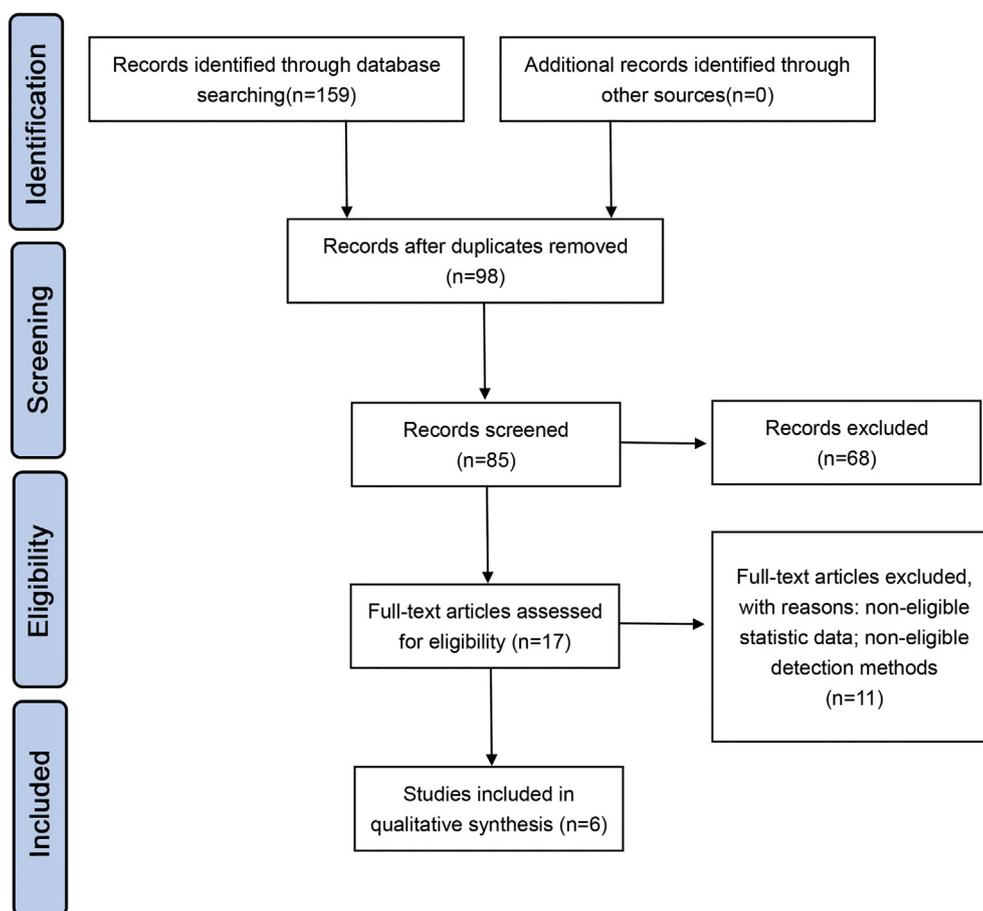


Fig. 1. Flow chart of the study search and selection.

2.7. Recipient operation

Following separation of the left renal vessels, the artery and the vein were clamped separately as close to the aorta and the caudal vena cava as possible. The ureter was isolated and transected distally. The renal vessels were transected close to the renal hilum and the kidney was removed. The ends of the renal vessels were carefully irrigated with saline. The donor kidney was placed orthotopically. The end-to-end anastomosis of the renal artery was performed first between the donor's and the recipient's renal artery using 10-0 Nylon lines and interrupted sutures. The venous end, which was everted over the cuff, was passed inside the recipient renal vein and secured with a circumferential 9-0 Nylon lines. We removed the vascular clamps after the anastomosis, making the kidney display rapid reperfusion and turn red. The bladder insertion technique was used to perform a small incision in the fundus of the recipient bladder and then pull the tip of the donor ureter into the bladder with two stitches for securing. Mild compression for 1 min was applied over the anastomosis with a small piece of gauge after recirculation. This was usually sufficient to prevent bleeding. Right nephrectomy was performed after renal transplantation in the same operation and the adrenal gland was preserved. The abdomen was closed in one layer. Injection of 2–4 mL physiologic saline subcutaneously at the end of the whole procedure was considered effective for restoring volume. No immunosuppression was used in the recipients. The establishment of rat renal transplantation model was considered a success if the transplanted animal survived for at least 3 days.

2.8. Histological examination

The recipient rats were sacrificed on days 3, 5, 7 and 9 after

operation for histological investigations. Normal donor kidneys were used as a control. 4% formalin fixed paraffin embedded kidney specimens were stained with hematoxylin-eosin (HE). Two blinded observers independently evaluated histological changes of the kidneys using coded slides. We made a histological evaluation of acute rejection for each tissue specimen. Acute rejection was evaluated according to the Banff classification [13] by the two blinded observers, and an average score was calculated. This rejection score was divided semi-quantitatively into seven grades from 0 to 6 according to the principle stated by Watanabe et al. [14] as follow: 0 = normal, 1 = borderline changes, 2 = IA, 3 = IB, 4 = IIA, 5 = IIB, and 6 = III by Banff classification.

2.9. miRNA isolation and expression

Total plasma RNA, including small RNAs, was collected from the caudal vein on days 3, 5, 7 and 9 after operation and isolated using Qiagen miRNeasy Serum/Plasma Kit (Qiagen, Valencia, CA) according to the manufacturer's recommendations. The yield and purity of RNA was analysed using a NanoVue Plus spectrophotometer (Healthcare Bio-Science AB, Uppsala, Sweden). cDNA synthesis was performed with a PrimeScript RT reagent kit (TaKaRa Biotechnology Co., Ltd., Dalian, China) according to the manufacturer's protocol. To quantify the miRNAs, qRT-PCR was performed with specific primers for miR-155 (forward: CCGCGCCTTAATGCTAATTGTGAT) and U6 (forward: CGCA AATTCGTGAAGCGTTC) small nuclear RNA (endogenous control) from the Bulge-Loop qRT-PCR primer set (Guangzhou RiboBio Co. Ltd., Guangzhou, China) according to the manufacturer's instruction. Relative expression was calculated using the $2^{-\Delta\Delta Ct}$ method.

Table 1
Characteristics of included studies.

N	Author	Year	Country	Pathological type	Unstable group number	Stable group number	Sensitivity %	Specificity %	AUC (95%CI)	Organ	Samples	Quantitative method
1	Anglicheau et al. [16]	2009	America	AR	12	21	100	90	0.98 (0.94–1.01)	Renal	PBMCs	TaqMan miRNA assays
2	Soltaninejad et al. [17]	2015	Iran	ATCMR	17	18	100	62	0.75 (0.52–0.98)	Renal	PBMCs	qPCR
3	Neumann et al. [18]	2017	Germany	CAV	19	21	88.89	61.11	0.713 (0.5402–0.8857)	Heart	Plasma	qPCR
4	Millán O [19]	2017	Spain	AR	8	72	85	86	0.875 (0.784–0.966)	Renal	Urinary	qPCR
5	Zumani Vahed [20]	2017	Iran	IFTA	26	27	81	47	0.57 (0.419–0.784)	Renal	Plasma	qPCR
6	Xu [21]	2017	America	AR	20	21	81.2	85.7	0.876(0.743–1.01)	Lung	Serum	TaqMan miRNA assays
6	Xu [21]	2017	America	BOS	14	21	78	82	0.808(0.734–0.957)	Lung	Serum	TaqMan miRNA assays

ATCMR: acute T-cell mediated rejection; PBMCs: human peripheral blood mononuclear cells; CAV: cardiac allograft vasculopathy; IFTA: interstitial fibrosis and tubular atrophy; BOS: bronchiolitis obliterans syndrome.

2.10. Statistical analysis

The pooled sensitivity, specificity, positive likelihood ratio (PLR), negative likelihood ratio (NLR) and diagnostic odd ratio (DOR) with the 95% confidence intervals (95% CIs) were estimated using bivariate model. Then, the summary receiver operator characteristic (SROC) curve and the area under the curve (AUC) were calculated to evaluate the diagnostic power. Data heterogeneity was examined by Cochran's Q test and Higgins I squared (I^2) statistic. If the studies contained no, or moderate, heterogeneity ($P > 0.1$ or $I^2 < 50\%$), we used fixed-effect model. Otherwise, the random-effects model was applied [15]. Publication bias analysis was conducted using Deeks' test. The meta-analysis was performed by utilizing RevMan 5.3 software and STATA package version 12.0 (Stata Corporation, College Station, Texas, USA).

Experimental results were shown as the mean \pm standard deviation (SD). The data of two groups were compared with an unpaired standard Student's *t*-test using SPSS 16.0 (SPSS, Inc., Chicago, IL, USA). A *P*-value < 0.05 was considered to be statistically significant.

3. Results

3.1. Characteristic of the selected studies

According to the described search criteria, after excluding the duplicate articles and reading the title and abstract, 85 articles were collected for the initial consideration. A total of 68 articles were excluded as research articles about the mechanistic functions of miR-155 without clinical data of solid organ transplantation. Next, 17 full-text articles were downloaded and carefully read for eligibility. Among them, 11 studies were excluded because of lacking necessary data (Fig. 1). Ultimately, six studies involving a total of 317 patients were included [16–21], as shown in Table 1. Among the six studies, three different types of solid organ transplantation were investigated, including four cases of renal transplantation, one case of orthotopic heart transplantation, and one case of pediatric lung transplantation. The allograft dysfunctional pathological types included acute rejection (AR), acute T-cell mediated rejection (ATCMR), cardiac allograft vasculopathy (CAV), interstitial fibrosis and tubular atrophy (IFTA), and bronchiolitis obliterans syndrome (BOS). The expression of miR-155 in human peripheral blood mononuclear cells (PBMCs), urinary, plasma and serum were detected by quantitative real-time polymerase chain reaction (qRT-PCR) and TaqMan methods. The ROC curve was used to determine cut-off points of miR-155 in these studies.

3.2. Diagnostic accuracy of miR-155

In the heterogeneity analysis, I^2 of sensitivity and specificity was 33.47% ($P = 0.17$) and 74.11% ($P < 0.001$), respectively. Thus, the random effects model was applied. The sensitivity and specificity in the dysfunctional group were 0.87 (95%CI: 0.78–0.93) and 0.76 (95%CI: 0.63–0.85, Fig. 2A). The pooled DOR was 17.31 (95% CI, 7.20–41.65, Fig. 2B). In addition, the PLR was 3.6 (2.2, 5.8) and NLR was 0.17 (0.09, 0.31), respectively. The AUROC curve is presented in Fig. 3A with a value of 0.89 (95% CI: 0.86, 0.92). These results indicated that miR-155 was a valid diagnostic marker for allograft dysfunction with excellent sensitivity and specificity.

3.3. Quality of the studies

We assessed the results of the QUADAS and presented it in Table 2. All included studies were retrospective and not representative of the patient spectrum. All required information in the table from included studies were reported, except for two [16,21] studies that did not clearly describe the inclusion or exclusion criteria. Taken together, all six studies scored an “A”. According to the Deeks' funnel plot asymmetry test, publication bias was found in the pooled analysis ($P = 0.03$,

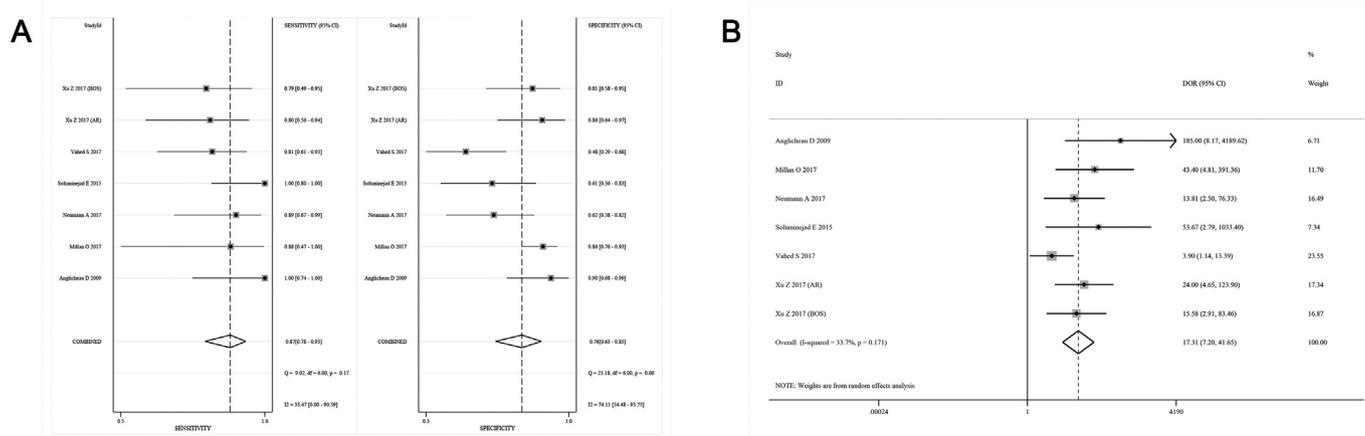


Fig. 2. A. Summary estimates of the sensitivity and specificity with forest plots analysis. B. Pooled DOR with forest plots analysis.

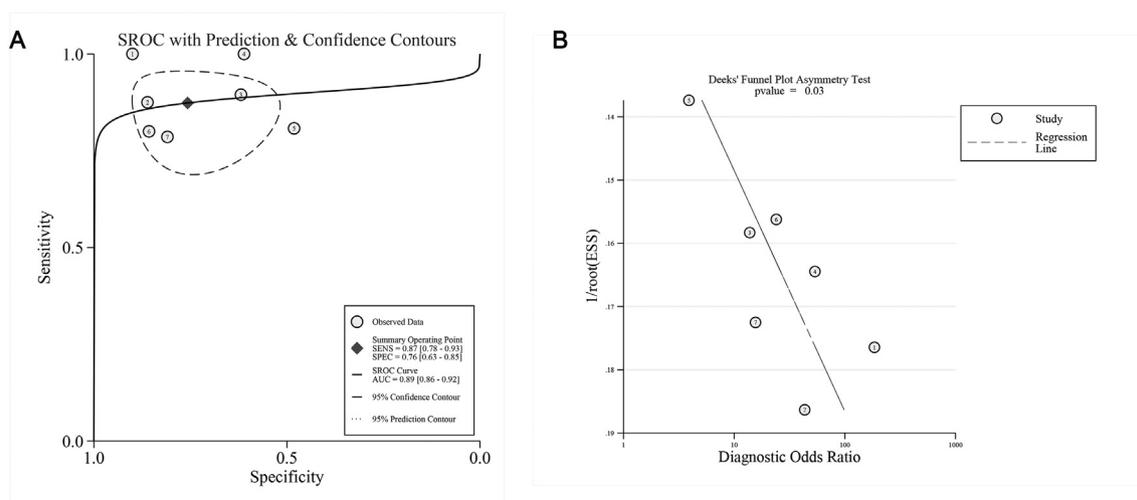


Fig. 3. A. SROC analysis of the diagnostic performance of miR-155. B. Deeks' funnel plot of publication bias.

Table 2

Summary of methodological quality of the included studies in the QUADAS checklist for each study.

Study number	1	2	3	4	5	6
Representative patient spectrum?	No	No	No	No	No	No
Selection criteria	Unclear	Yes	Yes	Yes	Yes	Unclear
Acceptable reference standard?	Yes	Yes	Yes	Yes	Yes	Yes
Acceptable delay between tests?	Yes	Yes	Yes	Yes	Yes	Yes
Partial verification avoided?	Yes	Yes	Yes	Yes	Yes	Yes
Differential verification avoided?	Yes	Yes	Yes	Yes	Yes	Yes
Incorporation avoided?	Yes	Yes	Yes	Yes	Yes	Yes
Index test execution	Yes	Yes	Yes	Yes	Yes	Yes
Reference standard execution	Yes	Yes	Yes	Yes	Yes	Yes
Reference standard results blinded?	Yes	Yes	Yes	Yes	Yes	Yes
Index test results blinded?	Yes	Yes	Yes	Yes	Yes	Yes
Relevant clinical information?	Yes	Yes	Yes	Yes	Yes	Yes
Quality of the studies	A	A	A	A	A	A

Fig. 3B).

3.4. Animal model establishment

In order to evaluate the dynamic expression level of miR-155 in a typical dysfunctional organ transplantation, we established the rat renal transplantation model. Considering acute rejection was one of the most representative adverse immune responses of transplant recipients, we chose the F344-Lewis pair as our rat transplantation model, which was

reported to develop a self-limited acute rejection and developed a similar progression of events following human renal transplantation [22]. A total of 24 ($n = 32$, success rate: 75%) procedures were successful. Cold ischemia time of donor kidney was 38.5 ± 2.5 min (range, 35–42 min). End-to-end arterial anastomosis time was 17.8 ± 2.2 min (range, 15–21 min), and venous anastomosis time was 4.8 ± 0.9 min (range, 3–6 min). Warm ischemia time of donor kidney was < 30 min in all procedures. Ureter vesical anastomosis time was 5.2 ± 0.7 (range, 4–6 min, Fig. 4A). There were no vascular events and hydronephrosis observed upon gross examination, with the renal arterial and venous anastomosis appearing patent without thrombus when the left 24 successful recipients were scarified.

3.5. The expression level of miR-155 was correlated with acute rejection

Acute rejection was evaluated by histological examination in days 3, 5, 7, and 9 (Fig. 4B). In the transplant group, all of 24 recipients detected the occurrence of AR and their average AR scores were increased from day 5 and peaked in day 9 (Fig. 4C). No AR was observed in the control group. The expression level of miR-155 in plasma was detected at the same time point after surgery. As a result, there was no significant difference on miR-155 expression level between AR group and control group in day 3 and day 5. Compared to the stable expression of miR-155 in the control group from day 3 to day 9 ($P > 0.05$), its expression level was found to be significantly increased in 7 d and 9 d post-transplantation in AR group ($P < 0.05$, Fig. 4D). Meanwhile, the raising

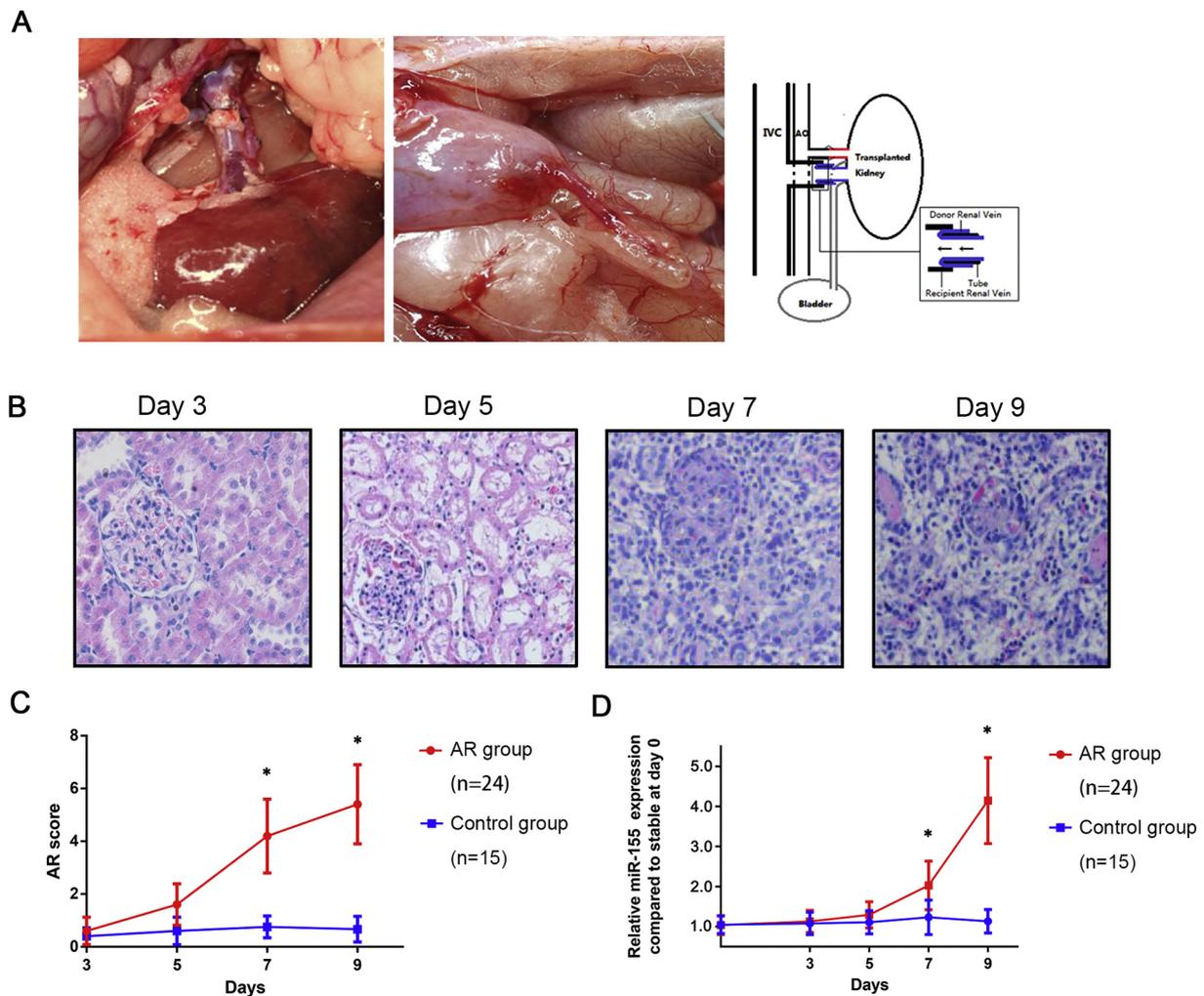


Fig. 4. Orthotopic allograft kidney transplantation model in rats. A. Left: Perfusion following transplantation; middle: ureteral reimplantation; Right: diagrams of transplant technique. B. H&E staining of renal tissue from the F344-Lewis rat renal transplant model. C. Dynamic tendency of AR score after transplantation. D. Dynamic tendency of relative expression of miR-155 after transplantation. Relative expression was calculated and compared to the stable level at day 0. * $P < 0.05$.

trend of miR-155 overexpression was also observed to be consistent with the increase in the degree of AR.

4. Discussion

In this research, we systemically explored the role of miR-155 in solid organ transplantation. Through summarizing and analyzing the data from five kinds of allograft dysfunctions in renal, heart, and lung transplantation, we found that the diagnostic performance of miR-155 in dysfunctional allograft was excellent. Furthermore, the correlation between the expression level of miR-155 and acute rejection degree was also observed in a F344-Lewis rat renal transplantation model. Based on these results, our study successfully validated the diagnostic efficiency of miR-155 from a unique perspective.

It has been > 10 years that miRNAs were reported to be the non-invasive biomarkers in the transplantation. Due to the variations in experiment protocols and platforms, limited numbers of transplant patients studied, and different pathological changes in each study, the diagnostic efficacy of miR-155 is controversial. Here, based on integrating analyses of all the miR-155 related diagnostic models in all kinds of organ transplantation, we were surprised to find that miR-155 could be an ideal biomarker in monitoring allograft status, regardless of different pathological types and organ sources. In fact, not only in the renal, heart and lung transplantation that were included in our analysis, but miR-155 was also reported to be the ideal bio-marker in liver

transplantation and allogeneic hematopoietic cell transplantation (HCT). Asaoka T. et al. reported that miR-155 could be an ancillary marker to discriminate AR predominant cases from recurrent hepatitis C in HCV positive patients after live transplantation [23]. Xiao et al. [24], Xie et al. [25] and Atarod et al. [26] found that miR-155 could serve as a noninvasive biomarker for acute graft-versus-host disease in HCT.

Based on the validation experiment in a rat model, the expression level of miR-155 was increased following the process of acute rejection. The dynamic changes verified a close association between miR-155 and immune status of allograft. In previous studies, Li et al. [9] had found that knockdown of miR-155 in Kupffer cells resulted in immunosuppressive effects and prolongs survival of mouse liver allografts. Meanwhile, miR-155 deficiency in Kupffer cells also ameliorated liver ischemia-reperfusion injury in mice model, according to Li et al. [27]. Taken together, these evidences in animal model support that miR-155 as a potential biomarker reflecting allograft status.

Some limitations in this research must be acknowledged. Firstly, due to the lack of prospective studies, all results included were retrospective. Secondly, although most of the studies used the same statistical method (ROC curve method) to confirm the best cutoff value, the cutoff points differed among the studies. Moreover, the publication bias was found here, indicating that there would be some negative or positive result deficiencies compared to a real-world study. As previously mentioned, some studies with positive results were excluded due to the

lack of detailed data or its author could not be contacted. On the other hand, some negative results were not reported. For example, Esmaeili-Bandboni et al. [28] recently found that compared to miR-326, miR-155 was not the best early diagnostic biomarkers for the detection of human acute heart allograft rejection. In light of the large number of miRNAs reported as biomarkers in the organ transplantation, we could speculate that in some cases, miR-155 with low diagnostic efficiency was not reported. We should also notice that even though miR-155 had high diagnostic efficacy in predicting dysfunction, it is lacking the ability of distinguishing specific pathological types in allograft dysfunction.

5. Conclusion

With its high diagnostic efficacy, objectivity, and minimal invasiveness, miR-155 is a useful biomarker for monitoring the allograft status and can aid with clinical decision-making. However, there is still a need for further studies to confirm the validity of employing miR-155 to distinguish different pathological types of dysfunctional allograft.

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Ethics approval and consent to participate: This study conformed strictly to the ethical guidelines of the Declaration of Helsinki and was approved by the West China Hospital of Sichuan University Biomedical Research Ethics Committee.

Competing financial interests: The authors declare no competing financial interests.

Author contributions

J. Liang participated in study design, performed the study, and prepared the manuscript. Z. Liu and Y. Tang participated in study design, data analysis, and manuscript editing. J. Liang, L. Tang and Z. Zou finished the animal model establishment. X. Wang, C. Zhou, K. Wu, F.X. Zhang and F. Zhang helped with data analysis. Y. Lu conceived study and participated in its design and coordination, and manuscript editing. All the authors read and approved this manuscript.

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