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Differential expression of microRNAs in renal transplant patients with acute T-cell mediated rejection☆

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ABSTRACT

Background: MicroRNAs (miRNAs) regulate most of encoding genes and protein. In this study, we aimed to investigate the expression levels of miR-142-5p, miR-142-3p, miR-155 and miR-223 in paired biopsy and peripheral blood mononuclear cell (PBMC) samples of renal allograft recipients with acute T-cell mediated rejection (ATCMR), compared with normal allografts (NA).
Methods: In this study, the expression levels of individual miRNAs were determined in biopsy and PBMC samples of 17 recipients with ATCMR and 18 recipients with NA.
Results: Our results showed that the intragraft expression levels of all studied miRNAs were significantly higher in ATCMR than NA. However, regarding the PBMC samples, miR-142-3p and miR-223 were significantly increased in ATCMR than NA. Receiver operating characteristic (ROC) analysis showed that miR-142-5p, miR-142-3p, miR-155 and miR-223 in biopsy samples and miR-142-3p and miR-223 in PBMC samples could discriminate ATCMR from NA recipients.
Conclusion: It has been reported that high intragraft expressions of miRNAs have a profound role in the pathogenesis of ATCMR process. Our results showed that high expression of all the studied miRNAs in biopsies and miR-142-3p and miR-223 in PBMC samples could be used as suggestive diagnostic tools to discriminate ATCMR patients from NA.

1. Introduction

Solid organ transplantation is the most effective treatment for end-stage organ failure. However, the rejection of transplanted organs remains as a main factor which impacts on the organ transplant effectiveness [1]. Renal transplantation, compared with dialysis, improved health-related quality of life of end-stage renal disease (ESRD) patients and it is associated with a high cost-effectiveness ratio [2]. However, acute rejection (AR) of renal allografts is an immune response that may occur at any time after renal transplantation [3–7]. Furthermore, AR is the most important risk factor leading to chronic allograft nephropathy in renal allograft recipients [8], that remains the major cause of graft loss [9].

Currently, tissue biopsy is considered as a gold standard for diagnosis of allograft rejection [10]; however, it is highly invasive and requires risky procedures along with variation in reports of renal biopsies between pathologists [11,12]. The lack of non-invasive, accurate and specific diagnostic assays for graft rejection is a major difficulty in the management of renal transplant recipients [13,14]. Therefore, development of

Abbreviations: miRNA, MicroRNA; PBMC, Peripheral blood mononuclear cell; ATCMR, Acute T-cell mediated rejection; AR, Acute rejection; NA, Normal allograft; ROC, Receiver operating characteristic; ESRD, End-stage renal disease; CAF, Chronic allograft failure; FC, Fold change; IQR, Inter quartile range; AUC, Area under the curve; CI, Confidence interval.

☆ Authorship: Ehsan Soltaninejad participated in sample collection, performing the experiments and writing the paper draft. Mohammad Hossein Nicknam participated in designing the study and editing the manuscript. Mohammad Hossein Sharbafi participated in acquisition of clinical data and performing the experiments. Mohsen Nafar and Pedram Ahmadpoor were the responsible nephrologist of this study and participated in acquisition of clinical data and interpreting the data. Morteza Hosseinzadeh, Farshad Foroughi and Tayeb Bahrami participated in sample collection. Mir Saeed Yekaninejad participated in statistical analyzing the data. Ehsan Sharif-Paghaleh participated in editing the paper. Aliakbar Amirzargar is the main project manager, and participated in designing the study and editing the manuscript.

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sensitive and accurate biomarkers in the graft as well as non-invasive immunological biomarkers accessible in blood and other biological fluids corresponding to graft rejection is necessary.

MicroRNAs (miRNAs) are evolutionary conserved non-coding RNA molecules, small (≈19–25 nucleotides in length) that regulate gene expression by affecting the translational repression or by mRNA degradation [15]. miRNAs functions by binding through the 3′ untranslated region (3′UTR) of target mRNA. Also, it has been shown that miRNAs play important roles in many biological processes such as development [16], cell proliferation, differentiation, apoptosis, fat metabolism and oncogenesis [17]. Moreover, MiRNAs are stably expressed in biologically body fluids such as urine, saliva, serum, plasma and other body fluids. Furthermore, expression levels of miRNAs are correlated with human diseases such as various types of kidney diseases, cancers, stroke, heart diseases or in physiological states such as pregnancy [18–22]. This suggests that miRNAs could be used for non-invasive diagnosis and monitoring of a variety of diseases.

In this study, we focused on miR-142-5p, miR-142-3p, miR-155 and miR-223 because of their specificity to hematopoietic lineage [23,24]; meanwhile there are some evidences on over-expression of these miRNAs in biopsy/PBMC samples of AR patients [24,25]. To our knowledge, the expression analysis of miR-142-3p and miR-155 in PBMC samples of ATCMR recipients and NA has not been performed yet. Moreover, this study is the first study that evaluated expression of these miRNAs simultaneously in biopsy and PBMC samples of renal transplant patients.

2. Objective

The aim of this study was to investigate, whether ATCMR is associated with changes in miRNA expression levels of miR-142-5p, miR-142-3p, miR-155 and miR-223 within biopsies and paired PBMC samples. If so, whether the expression levels of these miRNAs in biopsy and PBMC samples of ATCMR could be used as diagnostic and predictive biomarkers for ATCMR.

3. Materials and methods

3.1. Patients and sample collection

The study was approved by the Tehran University of Medical Sciences ethical committee; each patient signed written informed consent. In this cross sectional study, the patients were recruited from Shahid Labbafinejad Medical Center. Biopsy and PBMC samples were obtained from renal transplant patients with ATCMR (n = 17) and NA (n = 18) without histopathological evidence of rejection as controls. The exclusion criteria were BK virus infection, calcineurin inhibitor nephrotoxicity, urinary tract obstruction and patients with ≥2nd transplantation. The inclusion criteria were patients undergoing their first transplantation and signing the inform consent. Renal allograft biopsies were classified according to the 2009 update Banff classification criteria [26]. In our study, all 17 AR subjects developed a T-cell mediated rejection (12 IA and 5 IB) in category 4 based on the Banff diagnostic category [26]. The pathologists were blinded in their evaluation of the biopsies to determine the presence of rejection. Additional demographic and clinical characteristic information of the patients are shown in Table 1.

Renal allograft biopsy samples were immediately placed in RNA*later* solution (Ambion) according to the manufacturer's instructions. In summary, biopsy samples were stored at 4 °C overnight to allow penetration of the solution into tissues and subsequently stored at −80 °C until RNA extraction. Peripheral whole blood on EDTA were collected at the time of obtaining biopsy samples from the same patients with ATCMR or NA. PBMCs were isolated from whole blood of ATCMR and NA recipients using standard Ficoll density-gradient centrifugation. To protect the RNA from degradation, PBMCs were kept in RNA*later* solution and then transferred to 4 °C overnight then stored at −80 °C until RNA extraction.

Table 1
Demographic and clinical characteristics of renal allograft recipients.

	Normal allograft	Acute T-cell mediated rejection
Number of patients	18	17
Male (n; %)	11 (61.1%)	10 (58.8%)
Female (n; %)	7 (38.9%)	7 (41.2%)
Age (years; min–max)	55 (34-67)	47 (21-61)
Types of allografts		
Deceased	1	3
Living	17	14
Types of donors		
Related donor	1	0
Unrelated donor	17	17
Recipient CMV (pos)	1	2
CMV disease	0	0
Donor CMV (pos)	3	2
Donor HCV (pos)	0	0
Donor HBS Ag (pos)	0	0
Donor HBC Ab (pos)	1	1
Donor HIV (pos)	0	0
Blood creatinine level (mg/dl)	1.24 ± 0.21	5.18 ± 1.49
Date of biopsy (months post-transplant)	13.66 ± 4.21	11.65 ± 3.18

3.2. RNA isolation and cDNA synthesis

Total RNA samples were isolated from renal biopsies or PBMCs using the mirVana miRNA isolation kit according to the manufacturer's instructions (Ambion). The concentration and quantity of total RNA were measured at 260 nm and 280 nm (A260/280) using a NanoDrop 2000 spectrophotometer (NanoDrop Technologies). Total RNA (10 ng) from ATCMR and NA subjects were reverse transcribed into cDNA using specific primers and reagents provided by TaqMan® MicroRNA Assays (Applied Biosystems, Foster City, CA, USA) and Taqman® MicroRNA Reverse Transcription Kit (Applied Biosystems, Foster City, CA, USA) respectively.

3.3. Quantitative real-time PCR

Real-time quantitative PCR was performed to measure the expression levels of microRNAs with the TaqMan® MicroRNA Assays (Applied Biosystems, Foster City, CA, USA), using probes for miR-142-5p (assay ID: 002248), miR-142-3p (assay ID: 000464), miR-155 (assay ID: 002623), miR-223 (assay ID: 002295), RNU44 (Assay ID: 001006) on a StepOnePlus Real-Time PCR System (Applied Biosystems, Foster City, CA, USA). The data were normalized using RNU44 as endogenous control.

3.4. Data analysis

Threshold cycle number was used to calculate the relative expression between samples. We used the ΔΔCt (cycle threshold) method in which relative expression $= 2^{-\Delta\Delta Ct}$, where ΔΔCt = (ΔCt of ATCMR) − (ΔCt of NA). Non-parametric Mann–Whitney test was used for comparing miRNA expression between acute T-cell mediated rejection and normal allograft and between T-cell mediated grade IA and IB rejection groups. Non parametric Spearman correlation was used for calculating the correlation between miRNAs in biopsies and PBMCs for normal allograft and acute T-cell mediated rejection groups. Receiver operating characteristic (ROC) analysis was applied to find the best cut off values of miRNAs for diagnosing the acute T-cell mediated rejection from non-rejected patients. Also multivariate ROC curve analysis was used to find the discriminative value of all studied miRNAs for discriminating acute T-cell mediated rejection from non-rejected