Experimental Cell Research 347 (2016) 312–321

Contents lists available at ScienceDirect

Experimental Cell Research

journal homepage: www.elsevier.com/locate/yexcr

Research Article

MiR-100-3p and miR-877-3p regulate overproduction of IL-8 and IL-1β in mesangial cells activated by secretory IgA from IgA nephropathy patients

Yan Liang [a,b], Guoqiang Zhao [c], Lin Tang [a,b], Junjun Zhang [a,b], Tianfang Li [b,d], Zhangsuo Liu [a,b,*]

[a] Department of Nephrology, The First Affiliated Hospital of Zhengzhou University, Zhengzhou 450052, China
[b] Research Institute of Nephrology, Zhengzhou University, Zhengzhou 450052, China
[c] College of Basic Medical Sciences, Zhengzhou University, Zhengzhou 450001, China
[d] Department of Rheumatism immunity, The First Affiliated Hospital of Zhengzhou University, Zhengzhou 450052, China

ARTICLE INFO

Article history:
Received 22 June 2016
Received in revised form
5 August 2016
Accepted 15 August 2016
Available online 16 August 2016

Keywords:
MiR-100-3p
MiR-877-3p
Mesangial cells
Secretory IgA
IgA nephropathy

ABSTRACT

IgA nephropathy (IgAN) is the most common type of primary glomerulonephritis, characterized by mesangial deposition of pathogenic IgA and the injury to mesangial cells. Our previous studies indicate that secretory IgA (SIgA) plays an important role in the pathogenesis of IgAN, and miR-16 is involved in destructive process in mesangial cells mediated by the SIgA from IgAN patients. Our current study aimed to study the role of miRNAs in the effect of SIgA from IgAN patients on mesangial cells. MicroRNA microarray and cytokines assay were performed to obtain the differential microRNAs expression profile in human renal mesangial cells stimulated by SIgA from IgAN patients (P-SIgA) with the cells treated by SIgA from healthy subjects (N-SgA) as control. The microRNAs with the most significant differences in microarray analysis were validated by quantitative RT-PCR. Among them, miR-100-3p and miR-877-3p were selected to predict target gene related to cytokines detecting in this study. Fifty-six differentially expressed microRNAs were chosen and 17 microRNAs with the most prominent changes were validated. Compared with N-SIgA, P-SIgA increased the production of interleukin (IL)-1β, IL-8, monocyte chemotactic protein-1 and transforming growth factor-β1. In addition, we for the first time demonstrated that over-production of IL-8 induced by the SIgA was regulated by down-expression of miR-100-3p in mesangial cells. Similarly, IL-1β over-production was regulated by down-expression of miR-877-3p. Our findings represent a pathogenic microRNAs expression profiling in human mesangial cells activated by P-SIgA. Furthermore, we provide a new explanation characterizing the molecular mechanism responsible for the regulation of IL-1β and IL-8 production in P-SIgA-triggered mesangial cells.

1. Introduction

IgA nephropathy (IgAN) is the most common type of primary glomerulonephritis and about 40% of IgAN patients develop end-stage renal failure [1]. The disease is characterized by mesangial deposition of pathogenic IgA, proliferation and cytokines production of mesangial cells, increased synthesis of the extracellular matrix, and inflammation [2,3]. However, the mechanism underlying the formation of IgAN is not completely understood. Previous studies reveal an association between the flare of IgAN and mucosal infection. Secretory IgA (SIgA) is the dominant immunoglobulin in mucosal immunity and plays an important role in the pathogenesis of IgAN [2–5]. Serum concentration of SIgA is associated with creatinine clearance, proteinuria, and renal pathological phenotypes [6]. It induces proliferation of human renal mesangial cells (HRMCs) and increases production of proinflammatory cytokines [7].

Recent studies have focused on aberrant regulation of gene expression in IgAN [8,9]. MicroRNAs (miRNAs) are short (approximately 22 bases) noncoding RNA molecules that post-transcriptionally

Abbreviations: IgAN, IgA nephropathy; sIgA, secretory IgA; HRMCs, human renal mesangial cells; miRNAs, microRNAs; UTR, untranslated region; IL, interleukin; SC, secretory component; FITC, fluorescein isothiocyanate; P-SIgA, sIgA from the saliva of patients; N-SIgA, sIgA from the saliva of healthy subjects; MCM, mesangial cell medium; qRT-PCR, quantitative real-time PCR; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; ELISA, enzyme-linked immunosorbent assay; MCP-1, monocyte chemotactic protein-1; TGF-β1, transforming growth factor-β1; SD, standard deviation; TNF, tumor necrosis factor; P-HRMCs, HRMCs activated by P-SIgA; N-HRMCs, HRMCs activated by N-SIgA

* Corresponding author at: Department of Nephrology, The First Affiliated Hospital of Zhengzhou University, Zhengzhou 450052, China.
E-mail address: zhangsuoliu@sina.com (Z. Liu).

http://dx.doi.org/10.1016/j.yexcr.2016.08.011
0014-4827/

Table 1
Quantitative RT-PCR primer sequences for miRNAs.

miRNAs	Primer sequences
miR-3613-3p	
RT-primer	5′GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACTGTTTTT3′
Forward primer	5′TCCGACTTCCCAACCCGAAAAA 3′
miR-223-3p	
RT-primer	5′GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACACAGTCA3′
Forward	5′TCCGAACCCCATAAACTGTT 3′
miR-1237-3p	
RT-primer	5′GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACAGGAAGA3′
Forward	5′TCCGAGACCCCCTGCCTCG 3′
miR-563	
RT-primer	5′GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACTCCAACT3′
Forward	5′TCCGACCCTTTGCATAC 3′
miR-100-3p	
RT-primer	5′GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACGTTCGAA3′
Forward	5′TCCGAGTATGGATATCTATG 3′
miR-19b-1-5p	
RT-primer	5′GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACTCAAAAC3′
Forward	5′TCCGACGACCTACGTTTGGAC 3′
miR-615-3p	
RT-primer	5′GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACAGGCTCG3′
Forward	5′TCCGATTCTCCCTCTGGGTC 3′
miR-16-2-3p	
RT-primer	5′GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACGGTTATA3′
Forward	5′TCCGAATTTGCTCGTGTCAT 3′
miR-508-5p	
RT-primer	5′GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACATGAGGT3′
Forward	5′TCCGAGTACTCACTGCGGGAG 3′
miR-877-3p	
RT-primer	5′GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACAGGAGAA3′
miR-301b-3p	
RT-primer	5′GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACGTCACGT3′
Forward	5′TCCGACGAAACTGTTATAGTA 3′
miR-490-5p	
RT-primer	5′GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACGGTACCT3′
Forward	5′TCCGATGGGTGGACCTCT 3′
miR-4499	
RT-primer	5′GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACTTCTGAC3′
Forward	5′TCCGAAGGGAGGAGA 3′
miR-6076	
RT-primer	5′GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACTCGTACT3′
Forward	5′TCCGAGGTGGAGAGGAGAC 3′
miR-1246	
RT-primer	5′GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACTTACCTA3′
Forward	5′TCCGAGGACGAGGTTTT 3′
miR-1224-5p	
RT-primer	5′GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACCACTCCT3′
Forward	5′TCCGAGGTGGAGGGCTC 3′
miR-146a-5p	
RT-primer	5′GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACACTCTTG3′
Forward	5′TCCGATTGGGTACCTTAAGT 3′
U6	
RT-primer	5′GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACAAAATATGGAACTGC3′
Forward	5′CTCGCTTCGGCAGCACA 3′
Conmon Reverse	5′GTGCAGGGTCCGAGGT 3′

regulate gene expression through incomplete base pairing with the 3′-untranslated region (UTR) of target mRNAs [9]. They regulate many biological processes including cell proliferation, inflammatory responses, and apoptosis [10,11]. Some miRNAs also play a crucial role in renal disease including IgAN [9]. However, very few studies have been performed to investigate the role of miRNAs in mesangial cells during IgAN formation and progression. The observations made in our own group suggest that SIgA mediated interleukin (IL)-6 production in human mesangial cells is regulated by miR-16 [7]. To further examine the role of miRNAs in the injury of mesangial cells activated by SIgA from IgAN patients, we performed miRNA microarray analysis to obtain the miRNA expression profile in HRMCs stimulated by SIgA from IgAN patients. The cells treated with SIgA from healthy subjects were used as control. Then, miR-100-3p and miR-877-3p was selected to investigate the proinflammatory mechanism of SIgA from IgAN patients in mesangial cells.

2. Materials and methods

2.1. Patients

Seventy-six patients were enrolled in the current study after pathological identification of primary IgAN with renal biopsy. Routine direct immunofluorescence showed a significant deposition of IgA with an absence of IgM in the glomerular mesangium. The demographic and clinical parameters of all patients were obtained before renal biopsy.