MicroRNA expression profiling in peritoneal fibrosis

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Peritoneal fibrosis (PF) is an intractable complication leading to peritoneal membrane failure in peritoneal dialysis (PD). The aim of this study was to identify microRNAs (miRNAs) involved in PF. Peritoneal tissue from a PF rat model was screened for miRNA expression using microarray analysis. The expression levels of differentially expressed miRNAs were evaluated in serum and drained dialysate and associated with peritoneal membrane functions, as measured by the peritoneal equilibrium test in 33 PD patients. Furthermore, an miRNA inhibitor (anti–miRNA-21-5p locked nucleic acid (LNA): anti–miRNA-21-LNA) was intraperitoneally injected to PF model mice to investigate its effects on PF. The initial profiling study of PF rat peritoneal tissue identified 6 miRNAs (miRNA-142-3p, miRNA-21-5p, miRNA-221-3p, miRNA-223-3p, miRNA-34a-5p, and miRNA-327) whose expression was increased more than 2-fold and no miRNAs whose expression was decreased more than half. Among them, serum levels of miRNA-21-5p, miRNA-221-3p, and miRNA-327 and drained dialysate levels of miRNA-221-3p and miRNA-34a-5p were significantly correlated with peritoneal membrane functions in PD patients. Anti–miRNA-21-LNA significantly inhibited miRNA-21-5p expression in the PF mouse peritoneum, inhibited peritoneal fibrous thickening, and maintained peritoneal membrane functions. These results suggest that several miRNAs are involved in PF and that they may be useful as novel diagnostic biomarkers and therapeutic targets for PF. (Translational Research 2016;169:47–66)

Abbreviations: α-SMA = α-smooth muscle actin; DAPI = 4′,6-diamidino-2-phenylindole; FGF = fibroblast growth factor; FSP-1 = fibroblast-specific protein-1; MGO = methylglyoxal; miRNA = microRNA; PD = peritoneal dialysis; PDF = peritoneal dialysis fluid; PET = peritoneal equilibrium test; PF = peritoneal fibrosis; PPAR-α = peroxisome proliferator–activated receptor-α; qRT-PCR = real-time reverse-transcription polymerase chain reaction; siRNA = small interfering RNA; TGF-β_1 = transforming growth factor-β_1; VEGF = vascular endothelial growth factor

INTRODUCTION

Peritoneal fibrosis (PF) is a major complication in peritoneal dialysis (PD) patients. PF is associated with peritoneal membrane failure, which leads to withdrawal from PD.[1-3] PF is histologically characterized by loss of the mesothelial cell monolayer, excess accumulation of extracellular matrix components, such as collagen,

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AT A GLANCE COMMENTARY

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Background

Peritoneal fibrosis (PF) is a major complication in peritoneal dialysis (PD) patients, resulting in withdrawal from PD. No therapy and biomarker are available for PF. Few studies have been reported that have investigated microRNAs (miRNAs) in PF.

Translational Significance

The results of this study showed that miRNAs are involved in PF and that certain miRNAs may be useful as biomarkers to estimate PF in PD patients. Furthermore our results show that miRNAs are promising therapeutic targets for the treatment of PF.

and myofibroblast proliferation.[4,5] No therapy is currently available for the treatment of PF. Therefore, close monitoring of peritoneal membrane functions is important to prevent PF. To estimate peritoneal membrane functions, the peritoneal equilibrium test (PET) is usually performed; however, PET is an invasive and time-consuming method because it requires the taking of several sequential blood samples and takes half a day.[6,7] Therefore, the development of new biomarkers for the estimation of peritoneal membrane failure resulting from PF is required. In addition, new therapeutic approaches, including the study of target molecules that can inhibit PF, are warranted.

MicroRNAs (miRNAs) are small noncoding RNAs that inhibit the post-transcriptional processing of target messenger RNAs (mRNAs).[8] Several miRNAs have been reported to suppress the transcription of mRNAs that are critical for the regulation of fibrosis development in various organs, including liver, heart, lung, and kidney.[9-12] In addition, miRNAs are potential biomarkers for several pathologic conditions, including cancer, inflammation, metabolic disorders, and fibrosis.[13-18] Recently, several studies have reported associations between the expression levels of certain miRNAs and PF, indicating roles in the development of PF.[19-26] However, further studies are needed to determine the possibility of using miRNAs as biomarkers and treatment targets for PF. We, therefore, conducted a screen using an animal model to identify miRNAs involved in PF. We then investigated the miRNAs identified in the screen for use as biomarkers of peritoneal membrane failure in PD patients. Furthermore, we investigated the effects of a miRNA inhibitor on the inhibition of PF in an in vivo animal model.

MATERIALS AND METHODS

Ethical considerations. This study was conducted in accordance with the Declaration of Helsinki and was approved by the Ethics Committee of Jichi Medical University. Written informed consent was obtained from all patients. The study was registered with the University Hospital Medical Information Network Clinical Trial Registry and was given the identification number UMIN000014124. Animal experimental protocols were approved by the Animal Ethics Committee of Jichi Medical University and were performed in accordance with the Use and Care of Experimental Animals guidelines from the Jichi Medical University Guide for Laboratory Animals.

PD patients and healthy subjects. Serum and drained dialysate samples from 33 PD patients (20 males and 13 females, aged 53.1 ± 14.5 years) were investigated. The baseline characteristics of PD patients are shown in Table I. Sera from healthy subjects were purchased from Tennessee Blood Services (Memphis, Tenn).

PET for human subjects. Peritoneal solute transport was assessed with the PET.[7] PET details have been described previously.[7] Briefly, intra-abdominal fluid was drained, and PD fluid (PDF) containing 2.5% glucose was injected intraperitoneally. The creatinine (Cr) level in peritoneal effluents obtained 4 hours after the injection (D) was divided by that in plasma (P) to obtain the D/P-Cr ratio. The glucose level in peritoneal effluents obtained 4 hours after the injection (D) was divided by that obtained immediately after PDF injection (D0) to obtain the D/D0-glucose ratio.

Rat model of PF. Sprague Dawley male rats, aged 12 weeks and 230–250 g in body weight, were purchased from Japan SLC (Hamamatsu, Japan). Rats were housed in a room with controlled temperature and humidity under antiviral- and antibody-free microisolator conditions. To produce PF, rats were intraperitoneally injected with 100 mL/kg of PDF containing 20 mM of methylglyoxal (MGO; Sigma-Aldrich, St. Louis, Mo), 5 days a week for 3 weeks. This PDF contained 2.5% glucose, 100 mM of NaCl, 35 mM of sodium lactate, 2 mM of $CaCl_2$, and 0.7 mM of $MgCl_2$ (Midperic; Terumo, Tokyo, Japan).

Mouse model of PF. C57 black 6 male mice, aged 8 weeks and 20–25 g in body weight, were purchased from CLEA Japan (Tokyo, Japan). Mice were housed