Extended Abstract Journal of Medical science 2020
Vol.4No.2

melatonin suppresses renal cortical fibrosis by inhibiting cytoskeleton reorganization and mitochondrial dysfunction through regulation of mir-4516

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Abstract

Renal fibrosis, a major risk factor for kidney failure, can lead to chronic kidney disease (CKD) and is caused by cytoskeleton reorganization and mitochondrial dysfunction. In this study, we investigated the potential of melatonin treatment to reduce renal fibrosis by recovering the cytoskeleton reorganization and mitochondrial dysfunction. We found that miR-4516 expression was downregulated in the renal cortex of CKD mice and *P*-cresol-treated TH1 cells. Decreased miR-4516 expression stimulated cytoskeleton reorganization and mitochondrial dysfunction, and induced renal fibrosis. Melatonin treatment suppressed fibrosis by inhibiting cytoskeleton reorganization and restoring mitochondrial function via increased miR-4516 expression. More specifically, melatonin treatment increased miR-4516 expression while decreasing ITGA9 expression, thereby inhibiting cytoskeleton reorganization. In addition, increased expression of miR-4516 by melatonin treatment reduced ROS formation and restored mitochondrial function. These findings suggest that melatonin may be a promising treatment for patients with CKD having renal fibrosis. Moreover, regulation of miR-4516 expression may be a novel strategy for the treatment of renal fibrosis.

Keywords: melatonin; mir-4516; renal cortical fibrosis; th1 cells; cytoskeleton reorganization

Introduction

Chronic kidney disease (CKD) presented a global all-age mortality rate of 45.5% between 1990 and 2017. CKD is defined as a gradual loss of kidney function and/or structure over a period of months to years . In patients with CKD, kidney dysfunction is mainly caused by progressive fibrosis. Although fibrosis is a damage repair response in connective tissues, an excess accumulation of the extracellular matrix results in loss of functional tissue when normal tissue is replaced [3,4]. Previous reports have shown that cellular damages, such as cytoskeleton reorganization, and mitochondrial dysfunction causes kidney fibrosis . Activation of cytoskeleton reorganization induces the expression of fibrosis-related proteins, such as fibronectin and collagen type 1. In addition, mitochondrial dysfunction induces renal fibrosis by inducing ROS generation, NLRP3 inflammasome activation, and pro-inflammatory cytokines: IL-1β, and IL-18 [7]. MicroRNAs are short noncoding RNAs which are 21–25 nucleotides in length, which play important roles in regulating various cellular and physiological processes by binding to the 3′-untranslated region (UTR) of the target mRNA to alter its expression functional studies have shown that miRNAs can act as critical modulators [9]. MiR-4516 is known to be associated with autophagy [10], and overexpression of miR-4516 is known to increase proliferation and invasion of glioblastomas [11]. Importantly, miR-4516 regulates the expression of fibronectin 1 and ITGA9, a regulator of cytoskeleton reorganization, and inhibits keratinocyte motility . In addition, miR-4516 suppress mitochondrial dysfunction by inhibiting mitochondrial.

Materials and Methods

4.1. Culture of Human Proximal Tubular Epithelial (TH1) Cells

TH1 cells were obtained from the American Type Culture Collection (Manassas, VA, USA). Cells were cultured in minimum essential medium (Gibco BRL, Gaithersburg, MD, USA) supplemented with 10% fetal bovine serum FBS Gibco and 100 U/mL penicillin/streptomycin (Gibco BRL). The cells were grown in a humidified 5% CO2 incubator at 37 °C.

4.2. Transfection of miRNA Inhibitor

For inhibition of miR-4516, cells were transfected with miR-4516 inhibitor (200 nM) using Lipofectamine 2000 reagent (Thermo Fisher Scientific, Waltham, MA, USA) following the manufacture's instruction. Reverse transcription-quantitative polymerase chain reaction analysis was performed to confirm successful transfection. Inhibitor of miR-4516 was purchased from abm Inc. (Richmond, BC, Canada).

Results

2.1. miR-4516 Is Downregulated in the Kidney Cortex of the CKD Mouse Model

To establish the CKD mouse model, BALB/c nude mice were fed 0.75% adenine for 1–2 weeks. The CKD mouse model was confirmed by the increased glomerulus size and expression of fibrosis-associated proteins in the kidneys (Figure 1A,B). Next, we used qPCR to show that miR-4516 expression is significantly downregulated in the kidney cortex of CKD mice (Figure 1C). We hypothesized that if miR-4516 expression was reduced, ITGA9 expression would increase leading to cytoskeleton reorganization. Indeed, CKD kidneys showed increased ITGA9 expression compared to a healthy kidney cortex (Figure 1D,E). To confirm activation of cytoskeleton reorganization, we measured the expression of GTPase signaling proteins, such as Rac1, RhoA, and CDC42, in the fibrotic area of the renal cortex.

Melatonin Injection Suppresses Renal Fibrosis in a CKD Mouse Model

To understand whether melatonin can suppress renal fibrosis, melatonin was intraperitoneally injected into CKD mice twice per week for two weeks. The mice were then sacrificed for detection of renal fibrosis using H&E staining.

The data show that melatonin injection markedly suppresses renal fibrosis in the CKD mouse model In addition, co-treatment with the miR-4516 inhibitor blocked the effect of melatonin on renal fibrosis Moreover, melatonin injections restored miR-4516 expression while suppressing mRNA and protein expression of ITGA9 in CKD kidneys. Treatment with miR-4516 inhibitor abolished these effects). We also measured Rho GTPase signaling proteins and fibrosis-related proteins in CKD kidneys. Interestingly, melatonin injections restored the changes in the GTPase signaling pathway and suppressed the expression of collagen type 1 and fibronectin These results show that melatonin recovers renal fibrosis by increasing miR-4516 expression.

Discussion

Renal disorder pathogenesis is led by progressive fibrosis, which is caused by an excessive repair response in the damaged tissues of patients with CKD. The renal cortex is composed of various types of cells that constitute the tubules, interstitium, and capillaries. Thus, dysfunction of the renal cortex with fibrosis attenuates the major kidney function of ultrafiltration. In this study, we demonstrated that miR-4516 expression is downregulated in CKD mice, and that renal fibrosis is suppressed when miR-4516 expression is restored by melatonin treatment. In this study, we demonstrated that miR-4516 expression is reduced in the renal cortex of CKD mice and *P*-cresol-treated TH1 cells. Decreased miR-4516 expression induced activation of cytoskeleton reorganization, mitochondrial dysfunction, and renal fibrosis. Melatonin treatment suppressed fibrosis by inhibiting cytoskeleton reorganization and restoring mitochondrial function by increasing miR-4516 expression. In particular, melatonin treatment increased miR-4516 expression and decreased ITGA9 expression, thereby inhibiting cytoskeleton reorganization. These results suggest that melatonin may be a therapeutic agent for patients with CKD with renal fibrosis, and that the regulation of miR-4516 expression may be a promising novel strategy for the treatment of renal fibrosis.

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This work is partly presented at Joint Event on 24th World Congress on Pharmacology & 7th World Heart Congress, August19- 20, 2019 at Vienna, Austria