The Research on the Mechanism of Isoproterenol Induced Fibrosis in Human Renal Mesangial Cells

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Objective: The research focused on the mechanism of isoproterenol induced fibrosis in human renal mesangial cells. Methods: Cells were divided into 3 groups: the control group, isoproterenol group and Stattc medication group. The expression of MMP2 and JAK/STAT signaling pathway proteins RTK, JAK1/2 as well as STAT in each group was detected by Western Blotting. Results: The results showed that the expression of MMP2 was increased combined the up-regulation of JAK/STAT pathway key proteins RTK, JAK1/2 and STAT in isoproterenol. The results also revealed that those abnormalities were alleviated by medication of Stattc. Conclusions: Over-activated JAK/STAT signaling pathway is involved in the pathogenesis of fibrosis in human renal mesangial cells.

1 Introduction

Glomerular mesangial cell is very active in glomeruli, which has the effect of secreting cytokines and removing large molecules [1-3]. Many kidney damages are associated with the dysfunction of renal mesangial cells. Fibrosis is a canal commune of a variety of pathological process, manifesting the increase of connective tissue and decrease of parenchyma cell in tracheal tissue [4-6]. The continuous fibrosis can cause organ structure damage, functional decline and seriously affect the patients’ health. JAK/STAT signaling pathway is associated with a variety of pathological process, especially the kidney tissue damage[7-9]. The present study aimed to explore the mechanism of isoproterenol induced fibrosis in human renal mesangial cells and provide a reference for clinical treatment.

2 Materials and methods

2.1 Cells and reagents

Renal mesangial cells were isolated and kept by our laboratory. Culture medium was purchased from HyClone (UT, USA). Tissue culture plate was purchased from Costar. The primary antibodies were purchased from Abcam, USA. The secondary antibodies and color reagent were purchased from Wuhan Boster Biological Technology, Ltd. Stattic was purchased from Sigma Aldrich. Other reagents were commercially available, analytically pure.

2.2 Instrument

Inverted microscope was purchased from Olympus, Japan. The CO2 incubator was purchased from Sanyo, Japan. The autoclave was the production of Xinhua Company. The centrifuge was purchased from Eppendorf, Germany. Real-time PCR System, electrophoresis apparatus and trans-blot were purchased from Bio-Rad.

2.3 Groups

Cells were divided into 3 groups: the control group, isoproterenol group and Stattc medication group. Isoproterenol group was given isoproterenol for 12 h of incubation. Stattc medication group was given Stattic for 6 h of incubation after the administration of isoproterenol for 6 h. Cells in the control group were given DMSO for equivalent time incubation.

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### 3.2 Up-regulated RTK expression of JAK/STAT signaling pathway by isoproterenol

It was found that in isoproterenol group, the expression of RTK of protein renal mesangial cell was significantly increased, and had significant difference compared with the control group, while in Stattci medication group, the up-regulated RTK expression was obviously inhibited by Stattci (Table 1).

![Figure 1](image1.png)

**Figure 1.** Up-regulated expression RTK protein of renal mesangial cell by isoproterenol and drug intervention. 

### 3.3 Up-regulated JAK1/2 expression of isoproterenol

The present study showed that in isoproterenol group, JAK1/2 expression of renal mesangial cell was significantly increased, and had significant difference compared with control group, while in Stattci medication group, the up-regulated JAK1/2 expression was obviously inhibited by Stattci (Figure 2).

![Figure 2](image2.png)

**Figure 2.** Up-regulated expression JAK1/2 protein of renal mesangial cell by isoproterenol and drug intervention. 

### 3.4 Up-regulated STAT protein expression of renal mesangial cell by isoproterenol

In the present study, we found that in isoproterenol group, the transcription factor STAT expression of renal mesangial cell was significantly increased, and had significant difference compared with the control group, while in Stattci medication group, the up-regulated STAT expression was obviously inhibited by Stattci (Figure 3).

![Figure 3](image3.png)

**Figure 3.** Up-regulated expression STAT protein of renal mesangial cell by isoproterenol and drug intervention.
4. Discussion

Tissue fibrosis is a major cause of many kinds of diseases for disability and death, seriously affecting the prognosis of patients. When the body tissue has less damage, the injured part will repair and restore the normal structure and function. While there are more damages or sustained damages, interstitial connective tissues of the body will increase largely to induce the occurrence of fibrosis [10]. In the present work, we studied the mechanism of fibrosis of renal mesangial cells induced by isoproterenol in which the results showed that the protein expressions of MMP2, RTK, JAK1/2 and STAT in isoproterenol group were significantly higher than that of the control group, and after the effect of Stattic, the above protein expressions obtained some recoveries. Therefore, isoproterenol induced the fibrosis of renal mesangial cells through activating JAK/STAT signaling pathway.

JAK/STAT signaling pathway involves in a variety of pathological models of kidney tissue. In the study of Tian et al.[11], they found that regulating and tonifying lung and kidney can obviously reduce lung and kidney tissue damage, of which the main way is associated with regulating the STAT signaling pathways. Ding et al.[12] believed that Yishen Capsule can delay the progress of diabetic nephropathy via inhibiting the activation of JAK/STAT pathway in diabetes nephropathy tissue. Lu et al.[13] also confirmed that Yishen Capsule reducing damage of diabetic nephropathy is also associated with the improvement of the STAT pathway. In the study of Astragalus and Salvia on the regulation of kidney JAK/STAT signaling pathway in UUO rats, it was found that Astragalus and Salvia play a protective role in unilateral ureteral obstruction of the glomerulus by improving the abnormal renal fibrosis and STAT signal pathway [14]. In the study of Shi et al.[15], it was found that JAK/STAT signaling pathway may involve in the process of early kidney changes in diabetes, and Benazepril may protect the kidney partly by affecting the activation of JAK/STAT signaling pathways. In the present study, we also found that isoproterenol can significantly up-regulate MMP2 expression. Meanwhile, it can also up-regulate the key protein expression of JAK/STAT signaling pathway. The up-regulated MMP2 expression is an important marker of tissue fibrosis, which involves in the fibrosis process of a variety of tissues [16]. Thus, the tissue fibrosis of renal mesangial cell caused by isoproterenol is accomplished by up-regulating JAK/STAT signaling pathway.

Therefore, the incubation of renal mesangial cell by isoproterenol can cause the occurrence of the fibrosis process, and the pathological process mainly needs to activate JAK/STAT signaling pathway. The inhibition of its signaling pathway can obviously inhibit the fibrosis degree.

Reference