論　文　内　容　要　旨

Effect of transforming growth factor - β1 on function and expression of monocarboxylate transporter 1 in alveolar epithelial cells

(肺胞上皮細胞におけるモノカルボン酸トランスポーターMCT1の発現・機能に及ぼす transforming growth factor-β1 の影響解析)

主指導教員：高野 幹久 教授
(医系科学研究科　医療薬剤学)
副指導教員：松浪 勝義 教授
(医系科学研究科　生薬学)
副指導教員：横大路　智治　准教授
(医系科学研究科　薬物治療開発学)

MOHI UDDIN

(医歯薬保健学研究科　薬科学専攻)

Introduction

Pulmonary fibrosis is a chronic respiratory disease in which the lung tissues become scarred, thickened, and stiff, resulting in serious breathing problems. In most cases, the definitive cause and underlying mechanism of the disease remain unclear. However, it is now recognized that epithelial mesenchymal transition (EMT) plays an essential role in the development of pulmonary fibrosis and that transforming growth factor (TGF)-β1 can induce EMT by activating important signaling pathways. On the other hand, recently, it has been reported that the EMT involves complex metabolic reprogramming. Monocarboxylate transporter (MCT1) plays an essential role in metabolic reprogramming via regulation of the lactate shuttle, suggesting the hypothesis that MCT1 may be closely related to pulmonary fibrosis via EMT process.

However, information concerning MCT1 activity in alveolar epithelium, which is mainly injured during pulmonary fibrosis, is limited and the contribution of MCT1 to EMT has not been well investigated. If the association of MCT1 with EMT is proven, MCT1 inhibition may be used to prevent EMT-related diseases, such as pulmonary fibrosis. Therefore, the aims of the present study were to characterize MCT1 function and to examine the changes in expression and function of MCT1 during TGF-β1-induced EMT using A549 cells, an in vitro model of human alveolar type II epithelial cells derived from human lung carcinoma.

Methods

To characterize MCT1 function in A549 cells, [^{3}H]γ-hydroxybutyrate (GHB) and [^{3}H]lactic acid were used as substrates for MCT1, and α-cyano-4-hydroxycinnamic acid (CHC), phloretin, lactic acid, and AR-C155858 (AR) were used as MCT1 inhibitors. To examine the effect of TGF-β1 on the function and expression of MCT1, the cells were treated with TGF-β1 (10 ng/mL) for 72 hr, and then transport activity and mRNA/protein expression of MCT1 were estimated by uptake study, real-time PCR analysis, and western blot analysis, respectively. In addition, SB431542 (SB), a TGF-β type I receptor kinase inhibitor, was used to confirm the contribution of TGF-β signaling pathway to TGF-β1-mediated regulation of MCT1.

Results

Characterization of MCT1 function in A549 cells

A linear time-dependent increase up to 1 min in [^{3}H]GHB and [^{3}H]lactic acid uptake was observed in A549 cells. Compared to the uptake of both substrates at 37 °C, the uptake was markedly decreased at 4 °C. As MCT1 is known to be a proton-coupled symporter, the effect of extracellular pH on GHB and lactic acid uptake was examined. As expected, [^{3}H]GHB and [^{3}H]lactic acid uptake at pH 6.0 was significantly higher than that at pH 7.4. In addition, the inhibitors including CHC, phloretin (data not shown), and AR significantly suppressed

[^{3}H]GHB and [^{3}H]lactic acid uptake in a concentration-dependent manner. These findings indicate that MCT1 is a functioning monocarboxylate transporter in A549 cells.

Role of TGF-β1 signaling pathway in functional expression of MCT1 in A549 cells

We confirmed that 10 ng/mL of TGF-β1 treatment for 72 h markedly induced EMT-like phenotypical changes in A549 cells[1]. Accordingly, the functional expression of MCT1 during TGF-β1 treatment was examined. TGF-β1 significantly enhanced the uptake of [^{3}H]GHB and [^{3}H]lactic acid in A549 cells, and these changes were ameliorated by co-treatment with SB. In addition, TGF-β1 treatment led to the enhancement of mRNA and protein expression levels of MCT1, and SB suppressed the upregulation of MCT1 induced by TGF-β1. These results suggest that the TGF-β signaling pathway may be closely associated with the functional expression of MCT1 in A549 cells.

Contribution of MCT1 to TGF-β1-induced EMT in A549 cells

As CHC and AR inhibited the uptake of GHB and lactic acid, we investigated the effect of these inhibitors on TGF-β1-induced EMT in A549 cells. Both inhibitors had no effect on TGF-β1 induced alterations in mRNA expression levels of cytokeratin 19, an epithelial marker gene, and α-smooth muscle actin, a mesenchymal marker gene, in A549 cells. Considering that knockdown of MCT1, but not AZD3965, a promising MCT1 inhibitor, suppressed several growth factors-induced EMT-like cell scattering and wound healing[2], MCT1 transport activity itself may not be crucial to the EMT process. Further studies are needed to clarify the role of MCT1 in TGF-β1-induced EMT in alveolar epithelial cells.

Conclusions

MCT1 function in A549 cells was characterized, and the functional expression was upregulated by TGF-β signaling pathway, as evidenced by an inhibitory effect of SB. These findings may help in understanding the association of metabolic reprogramming with TGF-β1-induced EMT in alveolar epithelial cells.

References:

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