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ACTIVATION OF ERK 1/2 AND SP1 MAY CONTRIBUTE TO THE EXPRESSION OF TISSUE INHIBITOR OF METALLOPROTEINASE-1 INDUCED BY TRANSFORMING GROWTH FACTOR-β1 IN HUMAN PULMONARY ARTERIAL SMOOTH MUSCLE CELLS

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Objectives Tissue inhibitor of metalloproteinases-1 (TIMP-1) is considered to play a key role in the development of pulmonary arterial hypertension (PAH). However, the molecular regulatory mechanisms of TIMP-1 in the pulmonary arteries were not very clear, especially in the human pulmonary arterial smooth muscle cells (HPASMCs). This study try to investigate the signalling pathway involved in the regulation of TIMP-1 in HPASMCs stimulated with transforming growth factor (TGF)-β1.

Methods Cultured HPASMCs were incubated with different concentrations of TGF-β1 (0, 2.5, 5, 10, 20 or 40 ng/ml) for 24 h, or with 10 ng/ml TGF-β1 for different time (1, 4, 8, 12, 24 or 48 h). Western blot and real-time PCR were employed to detect the protein and mRNA expression of TIMP-1 in HPASMCs, and enzyme-linked immuno sorbent assay (ELISA) was used to detect the secretion of TIMP-1 in the culture medium. Then, the activities of three mitogen-activated protein kinases (MAPK), including extracellular signal-regulated kinase 1/2 (ERK1/2), p38 and c-Jun NH2-terminal kinase (JNK), were respectively inhibited with their specific inhibitors, U0126, SB202190 and SP600125. The protein and mRNA of TIMP-1 were also detected to help distinguishing that which kinase was involved in the regulation of TIMP-1 in HPASMCs induced by TGF-β1. Besides this, mithramycin, a specific inhibitor of Sp1 transcription factor, and curcumin, a specific inhibitor of activator protein-1 (AP-1), were used to block the DNA-binding activity of Sp1 or AP-1 respectively. And electrophoretic mobility shift assay (EMSA), Western blot and real-time PCR were carried out to help confirming that which transcription factor was involved in the regulation of TIMP-1 in HPASMCs.

Results Western blot, real-time PCR and ELISA analysis showed that TGF-β1 could time- and dose-dependently enhance the expression and secretion of TIMP-1. Furthermore, TGF-β1 could phosphorylate two kinases of MAPK, ERK1/2 and p38, but not JNK, and the phosphorylation of p38 was weaker compared with ERK1/2. Of these kinases, only the inhibition of ERK 1/2 by U0126 effectively blocked the TGF-β1-induced expression of TIMP-1. Mithramycin also significantly reduced the expression of TIMP-1 via blocking the DNA-binding activity of Sp1. However, the inhibition of AP-1 by curcumin couldn't achieve this result. Additionally, the results of EMSA showed that TGF-β1 could up-regulate the DNA-binding activity of Sp1, and that U0126 and mithramycin could effectively suppressed this activation in a dose-dependent manner.

Conclusions TGF-β1 could time- and dose-dependently stimulate the expression and secretion of TIMP-1 in HPASMCs, and ERK1/2 and Sp1 signalling pathways might be involved in these activities.