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## EXPRESSION CHANGE OF IKCA CHANNELS IN ET-1 INDUCED PROLIFERATION OF PULMONARY ARTERIAL SMOOTH MUSCLE CELLS AND MODULATION MECHANISM

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Yajuan Guo, Aiqun Ma. *Department of Cardiology First Affiliated Hospital Xi'an Jiaotong University*

**Objectives** Pulmonary vascular remodelling is an important pathologic feature of Pulmonary hypertension (PH). Pulmonary arterial smooth muscle cells (PASMCs) increased proliferation is an important component of pulmonary vascular remodelling. Regulation of PASMCs proliferation may therefore be critical to pulmonary vascular remodelling in PH, however the underlie mechanisms are still controversial. However, the precise mechanism of PH still remains to be elucidated.

Changes to $Ca^{2+}$-activated potassium channels (KCa) in proliferating vascular smooth muscle cells (VSMCs) have been described, but no regulatory role in proliferation has been attributed to them. KCa channels as a bridge between $Ca^{2+}$ signal and electrical activation in excitable cells, including of PASMCs, became more and more popular in research of hypertension, PH and atherosclerosis. Intermediate conductance calcium-activated potassium channel (IKCa) and large conductance calcium-activated potassium channel (BKCa) are two subtypes of KCa family. In systemic circulation VSMCs, BKCa channel expression defines the contractile

phenotype of VSMCs, while expression of IKCa channels characterises proliferating cells. The role of IKCa channel in PASMCs proliferation is unclear.

Rho/Rho kinase signalling pathway plays an important role in various cellular functions which involved in the pathogenesis of PH. Rho/Rho kinase signalling pathway mediates vascular smooth muscle cell contraction and vasoconstriction, the signalling pathway mediates '$Ca^{2+}$ sensitisation' of spontaneous. The upstream signals responsible for activation of Rho/Rho kinase signalling in PH is not is unclear.

To investigate expression change of KCa channel and Rho/Rho kinase signalling pathway in ET-1 induced proliferation of pulmonary arterial smooth muscle cells and to investigate modulation mechanism.

**Methods** The primary human PASMCs in 5–10 generations were cultured in 37°C with 5% $CO_2$ and incubated with several concentration of ET-1 (0, 10, 100 nmol/l), or co-cultured with ET-1 (10 nmol/l) pretreated with Clotrimazole (10 μmol/l, IKCa inhibitor), Fasudil (100 nmol/l, Rho kinase inhibitor) or Bosentan (100 nmol/l, inhibitor) respectively for 24 h. After the PASMCs were harvested, we used real time RT-PCR and western blot analysis to evaluate the expression and characteristics of IKCa channels and RhoA. We also used the MTT analysis and flow cytometric analysis to investigate the effects of IKCa channels on cell proliferation and the mechanisms involved.

**Results** High concentration ET-1 induced cell proliferation in human PASMCs in a dose- dependent manner. The IKCa channels expression of mRNA and protein is up-regulated in ET-1 induced proliferation of human PASMCs in a dose-dependent manner. The RhoA expression of mRNA and protein is up-regulated in ET-1 induced proliferation of human PASMCs in a dose-dependent manner. Inhibition of IKCa prevents ET-1 induced proliferation of human PASMCs. Inhibition of Rho/Rho kinase signalling pathway prevents ET-1 induced proliferation of PASMCs. BKCa channel inhibition fails to prevent ET-1 induced PASMCs proliferation. Inhibition of Rho/Rho kinase signalling pathway prevents up-regulated change of IKCa channel express in ET-1 induced PASMCs proliferation. ET-1 induced cell proliferation model in human PASMCs is a simple and useful cell proliferation model for basic research of PH.

**Conclusions** There are an up-regulated change of IKCa channels expression and RhoA expression in ET-1 induced proliferation of human PASMCs. There are an ion channel remodelling of KCa channels, which including up-regulated change of IKCa channels expression and down-regulated change of BKCa channels expression in ET-1 induced proliferation of PASMC. IKCa channel inhibition prevents PASMCs proliferation, Rho/Rho kinase signalling pathway may be involved in partially. IKCa channels and Rho/Rho kinase signalling pathway are important in PASMCs cell proliferation, making the channel and the signalling pathway a potential therapeutic target in pulmonary vascular remodelling.