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论著

ET-1介导损伤气道上皮诱导成纤维细胞活化的信号机制

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摘要 目的： 探讨p38丝裂原活化蛋白激酶(MAPK)、磷脂酰肌醇3-激酶(PI3K/Akt)在内皮素(ET)-1介导损伤气道上皮诱导上皮下成纤维细胞活化过程中的作用及对白细胞介素(IL)-6的影响。方法：将正常或经多聚左旋精氨酸(PLA)刺激的人气道上皮细胞与原代气道成纤维细胞共培养并分别加入p38 MAPK、PI3K特异性抑制剂SB203580、LY294002或ET受体A阻断剂BQ123，应用免疫组化、免疫印迹技术或ELISA检测成纤维细胞α-平滑肌肌动蛋白（α-SMA）表达、p38 MAPK、Akt的活化及成纤维细胞上清IL-6水平；制备成纤维细胞胶原凝胶并与不同方法处理的上皮细胞共培养，测量各组凝胶面积变化以了解上皮细胞对成纤维细胞收缩反应的诱导及其上述处理因素的影响。结果：与损伤上皮细胞共培养的成纤维细胞上清中ET-1、IL-6水平［（13.69±1.36）ng/L、(56.7±10.7) ng/L］明显高于与正常上皮细胞共培养的成纤维细胞上清［（3.79±0.64） ng/L、（15.5±3.2）ng/L］，BQ123、SB203580或LY294002皆不同程度减弱损伤上皮细胞诱导的IL-6释放［分别为(27.2±3.1) ng/L、(31.5±3.6) ng/L、(41.3±3.2) ng/L］；成纤维细胞与损伤气道上皮细胞共培养后p38 MAPK、Akt先后激活，BQ123减弱磷酸化p38 MAPK、Akt水平，SB203580浓度依赖性减弱Akt磷酸化水平，而LY294002对磷酸化p38 MAPK水平影响很小。与损伤气道上皮细胞共培养后成纤维细胞α-SMA表达增加，并且胶原收缩百分比明显大于与正常气道上皮共培养的成纤维细胞［(61.2±2.7)% vs (15.4±7.3)%］；BQ123、SB203580及LY294002皆不同程度减弱成纤维细胞α-SMA表达与凝胶收缩且BQ123、SB203580抑制凝胶收缩作用较LY294002更明显。结论：ET-1通过激活p38 MAPK、PI3K/Akt信号通路并促进IL-6分泌在损伤气道上皮诱导成纤维细胞活化过程中发挥关键作用。

关键词 内皮缩血管肽1； 气道上皮细胞； 成纤维细胞； p38 MAPK； PI3K/Akt信号通路

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Signal mechanism of endothelin-1-mediated activation of airway fibroblasts induced by injured airway epithelial cells

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Abstract

<FONT face=Verdana>AIM: To explore the effects of p38 mitogen-activated protein kinases (MAPK) and phosphoinositide 3-kinases (PI3K)/Akt on interleukin (IL)-6, the endothelin (ET)-1-mediated process of airway fibroblast activation induced by injured human bronchial epithelial cells (HBE). METHODS: Human primary cultured airway fibroblasts were co-cultured with HBE pre-treated with or without poly-L-arginine (PLA). The procedure was also performed in the presence or absence of p38 MAPK selective inhibitor SB203580, PI3K selective inhibitor LY294002 or ETA receptor blocker BQ123, respectively. Immunostaining, Western blotting or ELISA were used for detecting α-smooth muscle actin (α-SMA) expression, the activities of p38 MAPK and Akt in fibroblasts or IL-6 levels in supernatants of fibroblasts. In addition, fibroblasts were mixed with soluble collagen and cultured with HBE treated as the same mentioned above, the gel contraction was measured by serial area measurements. RESULTS: ET-1 and IL-6 levels ［(13.69±1.36) ng/L, (56.7±10.7) ng/L］ in the supernatants of fibroblasts cultured with injured HBE were significantly higher than those in the supernatants of fibroblasts cultured with HBE ［(3.79±0.64) ng/L, (15.5±3.2) ng/L］. BQ123, SB203580 or LY294002 decreased IL-6 levels ［(27.2±3.1) ng/L, (31.5±3.6) ng/L, (41.3±3.2) ng/L］ differently in the supernatants of fibroblasts induced by injured HBE. Activation of p38 MAPK preceded Akt in fibroblasts cultured with injured HBE. BQ123 reduced the

phosphorylation levels of p38 MAPK and Akt. SB203580 concentration-dependently attenuated Akt phosphorylation, while LY294002 had little effect on p38 MAPK phosphorylation. Fibroblasts expressed more α-SMA after cultured with injured HBE and showed significant increase in the gel contraction compared to fibroblasts cultured with HBE［percentage of gel contraction: (61.2±2.7)% vs (15.4±7.3)%］, all these effects were diminished or inhibited by BQ123, SB203580 or LY294002. Furthermore, the effects of BQ123 and SB203580 on decreased gel contraction were stronger than the effect of LY294002. CONCLUSION: ET-1 exerts a key role in the airway fibroblasts activation induced by injured HBE through activating p38 MAPK, PI3K/Akt signaling and promoting IL-6 expression.</FONT>

Key words Endothelin-1 Airway epithelial cells； Fibroblasts； p38 MAPK； PI3K/Akt signal pathway

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