中国病理生理杂志 2008 24 (12): 2428-2432 ISSN: 1000-4718 CN: 44-1187/R

论著

低氧诱导肌成纤维细胞生成并通过ERK1/2途径促进Ⅰ型胶原的表达

郭丽萍[1，2],黄海长[1],李惊子[1△]

1北京大学第一医院肾内科，北京大学肾脏病研究所,北京100034;2航天中心医院,北京100049

收稿日期 2007-9-20 修回日期 2008-4-25 网络版发布日期 2009-2-22 接受日期 2008-4-25

摘要 目的：探讨低氧能否激活成纤维细胞转化为肌成纤维细胞,以及低氧环境对肾皮质肌成纤维细胞表达Ⅰ型胶原（Col-Ⅰ）的影响及其可能的信号通路。方法：（1）采用正常大鼠肾成纤维细胞系（NRK-49F），应用Western blotting方法检测低氧诱导因子-1α(HIF-1α)的表达;比较低氧和正常氧条件下α-平滑肌肌动蛋白(α-SMA)的蛋白水平。（2）原代培养正常大鼠肾皮质肌成纤维细胞，Western blotting方法检测低氧和正常氧条件下，HIF-1α和Ⅰ型胶原蛋白水平以及ERK1/2的活化及其特异阻断剂 PD98059的阻断效应；RT-PCR方法检测Ⅰ型胶原mRNA表达水平；细胞免疫化学法检测HIF-1α胞内表达部位的变化；明胶酶谱法检测细胞上清中基质金属蛋白酶-2（MMP-2）和基质金属蛋白酶-9（MMP -9）的活性。结果：（1）低氧刺激6 h，NRK-49F细胞和肌成纤维细胞胞内和核内均有HIF-1α蛋白表达，核内更明显。（2）低氧培养12 h,NRK-49F细胞表达α-SMA蛋白明显增高，是正常氧组的187%±32%（P<0.05），验证了低氧可引起成纤维细胞表型转化。（3）低氧刺激原代培养的正常大鼠肾皮质肌成纤维细胞6 h、12 h上清中Ⅰ型胶原蛋白水平增加，分别为正常氧组的171%±27%（P<0.05）和256%±61% （P<0.05）；低氧刺激4 h、6 h，Ⅰ型胶原mRNA表达增加，为正常氧组的189%±28%（P<0.05）和221%±44%（P<0.05）。（4）低氧刺激肌成纤维细胞6 h、12 h、24 h，培养上清液中MMP-9、MMP-2活性无明显变化。（5）低氧刺激肌成纤维细胞15 min 即可使ERK1/2活化，阻断实验显示，PD98059可以使低氧12 h引起Ⅰ型胶原增加（低氧刺激组为正常氧对照组的273%±51%，P<0.05)显著减少（PD98059 +低氧组为正常氧组的108%±19%，P>0.05)。结论： 低氧促肾纤维化可能与其诱导肾成纤维细胞转化为肌成纤维细胞并经ERK1/2途径增加Ⅰ型胶原蛋白的表达有关。

关键词 低氧 肌成纤维细胞 胶原Ⅰ型 ERK1/2通路

分类号 R692

Hypoxia induces myofibroblast formation and stimulates production of collagen I in myofibroblasts through ERK1/2 pathway

GUO Li-ping[1,2],HUANG Hai-chang[1],LI Jing-zi[1]

1Renal Division,Department of Medicine,Peking University First Hospital;Institute of Nephrology,Peking University,Beijing 100034,China;2Aerospace Center Hospital,Beijing 100049,China.E-mail: jingzi@bjmu.edu.cn

Abstract

<FONT face=Verdana>AIM: To investigate the effect of hypoxia on the myofibroblast transdifferentiation from fibroblasts,and associated signaling of hypoxia on the production of collagen I in cultured rat renal cortical myofibroblasts.
METHODS: The study is composed of two relevant parts.In the first part,a normal rat renal interstitial fibroblast cell line NRK-49F was treated with hypoxia (1% O2) or normoxia (21% O2) for 6 h,12 h and 24 h.The expression of hypoxia inducible
factor-1α (HIF-1α) was examined by Western blotting in order to make sure the hypoxic condition is reliable.The myofibroblast transformation from fibroblasts induced by hypoxia was assayed by detecting the protein levels of α-smooth muscle actin (α-SMA).In the second part,the object was done on the primary cultured rat renal cortical myofibroblasts.Myofibroblasts were subjected to hypoxic or normoxic conditions for variety of times.The levels of HIF-1α in cell lysates and collagen I protein in supernatant culture medium and the activation of extracellular signal-regulated kinase (ERK)1/2 MAPK pathway were analyzed by Western blotting.RT-PCR was carried out to measure the levels of collagen I mRNA at different time points (2 h,4 h and 6 h).The distribution of HIF-1α in myofibroblasts was demonstrated by immunocytochemistry.The changes of

collagen I production were detected after PD98059,a specific inhibitor of ERK1/2 activation pretreatment and during the hypoxia incubation.The activity of gelatinase matrix metalloproteinase-2(MMP-2) and MMP-9 in the supernatant medium from the cultured cells were assayed by gelatin zymography.
RESULTS: Significant increased levels of HIF-1α protein appeared in cell lysates under hypoxia for 6 h.Furthermore,HIF-1α was translocated into nuclei of myofibroblasts after 6 h exposure of myofibroblasts to hypoxia.The levels of α-SMA protein increased in NRK-49F under hypoxia for 12 h (187%±32%,P<0.05).The level of collagen I protein in culture medium was increased in hypoxia treated myofibroblasts at 6 h (171%±27%,P<0.05) and 12 h (256%±61%,P<0.05).Collagen I mRNA expression was increased in cells under hypoxia condition for 4 h (189%±28%,P<0.05) and 6 h (221%±44%,P<0.05).The activities of MMP-2 and MMP-9 in the supernatant medium were not significantly changed at different experimental time points between the normoxic and hypoxic conditions.Activation of ERK1 /2 occurred as early as 15 min,sustained the high level at 30 min and 60 min and was back to the baseline level at 2 h.Blockade of ERK activation with PD98059 abolished hypoxia-induced expressions of collagen I protein.
CONCLUSION: Hypoxia contributes to the renal interstitial fibrosis through inducing formation of myofibroblasts and stimulating the production of collagen I in myofibroblasts.</FONT>

Key words Hypoxia Myofibroblasts Collagen type I ERK1/2 pathway

DOI: 1000-4718

通讯作者 李惊子 jingzi@bjmu.edu.cn