缺氧對大鼠主動脈平滑肌細胞之平滑肌α肌動蛋白、平滑肌二十二α蛋白、平滑肌肌凝蛋白重鏈基因表現的影響

Effects of hypoxia on smooth muscle ɑ-actin, smooth muscle 22ɑ, smooth muscle myosin heavy chain genes expression in rat aorta smooth muscle cells

中文摘要

血管細胞的增生對於動脈粥狀硬化，血管再狹窄，肺動脈性高血壓與傷口癒合之生理學和病理學的進程上是一個重要的特徵。在健康狀態下的血管組織，徹底分化或成熟的血管平滑肌細胞增殖的速率是非常低的。目前已在動脈粥狀硬化損傷動物實驗模型中發現，缺氧對平滑肌細胞和血管內皮細胞增生是一個重要的刺激。平滑肌細胞藉由調控它們的 phenotype 往來於分化和增生間，來反應生理學和病理學上的刺激。細胞骨架蛋白目前已被接受爲確實的分化標誌，提供作爲區分收縮（分化）態與增生態間的特徵，這些包括 SM α-actin、SM22α、smooth muscle myosin heavy chain（SM-MHC）。Myocardin 是一個轉錄協同因子，屬於 SAP 家族，與血清反應因子(serum response factors)協同作用，血清反應因子是普遍存在的轉錄因子，兩兩形成二聚物後結合在被稱爲 CArG box 的 DNA 序列 [CC(A/T)6GG]上，利用 dominant-negative 突變阻斷血清反應因子活性同時可以阻礙平滑肌收縮基因的表現。

根據我們所了解到的，在試管實驗中缺氧對動脈平滑肌細胞 SM α-actin、SM22α、SM-MHC 基因表現之影響還尚未被研究，依據先前對於 SM α-actin、SM22α、SM-MHC 在平滑肌細胞中扮演角色之研究，我們假設體外培養的平滑肌細胞 SM α-actin、SM22α、SM-MHC 基因表現也許會受到缺氧的影響，且經歷缺氧的這些細胞增生速率較高。本篇研究我們觀察到缺氧可誘導平滑肌細胞 phenotype 改變，從分化態轉變爲增生態，暴露在缺氧下增加平滑肌細胞的增生和抑制 SM α-actin、SM22α、SM-MHC mRNA 的表現，然而轉錄協同因子 myocardin 蛋白質與 mRNA 表現並未受到缺氧抑制，利用電泳膠移動實驗（gel mobility shift assay），我們發現受到缺氧的影響，myocardin-血清反應因子複合體與 CArG 序列的結合減少。我們推論，缺氧向下調控平滑肌細胞的 SM α-actin、SM22α、SM-MHC mRNA 表現，是由於減少 myocardin-血清反應因子複合體與 CArG box 的結合，干擾了三元複合體的形成。.

英文摘要

Angiogenesis and vascular cell proliferation are critical processes for tissue repair after ischemia and vascular injury. In healthy vascular tissue, the fully differentiated

or mature SMCs proliferate at an extremely low rate. Hypoxia is an important stimulus of smooth muscle cells (SMCs) proliferation and is found in vivo models of atherosclerosis. SMCs modulate their phenotype between differentiated and proliferative states in response to physiological and pathological stimuli.
Cytoskeletal proteins as reliable differentiation markers have allowed characterization of the contractile versus the synthetic phenotype. These include SM α-actin, SM22α, smooth muscle myosin heavy chain (SM-MHC). Myocardin is the SAP family transcription factor functionally cooperated with serum response factor (SRF). SRF is a ubiquitous transcription factor that binds as a homodimer to the DNA sequence CC(A/T)6GG, known as a CArG box. Blocked SRF activity with a dominant-negative SRF mutant has also shown to prevent expression of smooth muscle (SM) contractile genes.
Given the previously defined role of SM α-actin, SM22α, SM-MHC in SMCs, we postulated that SM α-actin, SM22α, SM-MHC genes expression of SMCs in culture may be affected by hypoxia, and that under hypoxic conditions, these cells proliferate at a higher rate. In this report, we observed that hypoxia induced SMCs phenotype switch, from contractile to synthetic phenotype. Exposure to hypoxia enhanced SMCs proliferation and suppressed expression of SM α-actin, SM22α, SM-MHC mRNA. The myocardin mRNA and protein expression was not changed by hypoxia. Using gel mobility shift assays, we demonstrated that association of myocardin/SRF complex with CArG box was diminished by hypoxia. We conclude that down-regulation of SMCs differentiation marker genes by hypoxia prevents ternary complex formation via reduced the myocardin/SRF complex interaction with CArG box.