中国药理学与毒理学杂志 2013 27 (2): 156-162 ISSN: 1000-3002 CN: 11-1115/R

论著

白藜芦醇通过上调小鼠胚胎干细胞过氧化物酶体增殖激活受体γ共激活子1α表达促进其分化为心肌细胞

方海琴[1,2]，赵君[1]，崔亚雄[1]，袁海涛[1]，杨嵘[1]，荣靖[1]，赵增明[1]，何俊[1]，彭双清[1]
1. 军事医学科学院疾病预防控制研究所毒理学评价研究中心，北京 100071;
2. 国家食品安全风险评估中心卫生部食品安全风险评估重点实验室，北京 100021

收稿日期 2012-12-27 修回日期 2013-3-22 网络版发布日期 2013-4-23 接受日期

摘要 目的 观察白藜芦醇对小鼠胚胎干细胞(ESC)分化为心肌细胞的调节作用，并探讨其机制。方法 采用悬滴悬浮培养法培养ESC。白藜芦醇0.44，4.4和44 μmol·L^{-1}处理ESC 96 h。光学显微镜下记录每组自发心肌搏动数；透射电镜观察细胞内线粒体结构；实时PCR方法测定α-肌球蛋白重链(α-MHC)、过氧化物酶体增殖物激活受体γ(PPARγ)、PPARγ共激活子1α(PGC-1α)，核呼吸因子-1(NRF-1)、线粒体转录因子A(mtTFA)和线粒体呼吸链复合体Ⅳ(COX Ⅳ)的基因表达；Western蛋白印迹法检测PPARγ，α辅肌动蛋白和PGC-1α蛋白表达。结果 与正常对照组相比，白藜芦醇0.44和4.4 μmol·L^{-1}可增加ESC细胞分化为自发搏动的心肌细胞数，并明显上调分化的ESC心肌特异性基因α-MHC表达，约分别为正常对照组的5.6和3.7倍；上调心肌细胞特定标识蛋白α辅肌动蛋白的表达，约为正常对照组的1.7和2.1倍；提示白藜芦醇可以促进ESC分化为心肌细胞。白藜芦醇干预各组均可上调PPARγ基因和蛋白表达，同时白藜芦醇0.44和4.4 μmol·L^{-1}可以明显上调线粒体生物合成相关因子基因表达；白藜芦醇4.4 μmol·L^{-1}处理组线粒体数目增多，提示线粒体生物合成可能是ESC分化为心肌细胞的重要机制。结论 白藜芦醇可以通过激动PPARγ受体并上调由PGC-1α介导的线粒体生物合成，从而促进ESC分化为心肌细胞。

关键词 白藜芦醇 胚胎干细胞 氧化物酶体增殖物激活受体γ共激活子1α 心肌细胞 线粒体生物合成
分类号 R966

Resveratrol promotes expression of peroxisome proliferator activated receptorγ coactivator 1α during cardiomyocyte differentiation of murine embryonic stem cells *in vitro*

FANG Hai-qin[1,2], ZHAO Jun[1], CUI Ya-xiong[1], YUAN Hai-tao[1], YANG Rong[1], RONG Jing[1], ZHAO Zeng-ming[1], HE Jun[1], PENG Shuang-qing[1]

1. Evaluation and Research Centre for Toxicology, Institute of Disease Prevention and Control, Academy of Military Medical Sciences, Beijing 100071, China;
2. Key Laboratory of Food Safety Risk Assessment of Ministry of Health, China National Center for Food Safety Risk Assessment, Beijing 100021, China

Abstract

OBJECTIVE To observe the modulation of resveratrol on cardiomyocyte differentiation of murine embryonic stem cells (ESCs) and investigate the underlying mechanism. **METHODS** The murine ESCs were differentiated as embryonic bodies (EB) in hanging drops and treated by resveratrol 0, 0.44, 4.4 and 44 μmol·L^{-1} for 96 h. The number of EB containing spontaneously contracting cells was recorded under the light microscope. The mitochondrial ultrastructure was observed in transmission EM photos. The gene expression of α-myosin heavy chain (α-MHC), peroxisome proliferator activated receptorγ (PPARγ), PPARγ coactivator 1α (PGC-1α), nuclear respiratory factor-1 (NRF-1), mitochondrial transcription factorA (mtTFA) and mitochondrial respiratory chain complexⅣ (COXⅣ) was detected by real-time PCR while the expression of α-actinin, PPARγ and PGC-1α proteins was measured by Western blotting. **RESULTS** Resveratrol 0.44 and 4.4 μmol·L^{-1} increased the number of contracting myocardial cells in differentiated ESCs. Compared with normal control group, the expression of cardiac-specific α-MHC genes at the concentration of 0.44 and 4.4 μmol·L^{-1} resveratrol increased 6,6-fold and 4.7-fold, and cardiac-specific α-actinin protein increased 2.7-fold and 3.1-fold. Furthermore, the increase in the number of mitochondria was observed in transmission EM photos of differentiated ESCs treated by resveratrol 4.4 μmol·L^{-1}. PPARγ gene and protein expression were increased in all groups treated by resveratrol at different concentrations. Meanwhile, the expression of genes correlated with mitochondrial biogenesis increased significantly after treatment by resveratrol, especially at the concentration of 0.44 and 4.4 μmol·L^{-1}. **CONCLUSION** Resveratrol induces murine ESC differentiation to cardiomyocytes *in vitro* by agitating PPARγ and promoting mitochondrial biogenesis modulated by PGC-1α.

Key words resveratrol embryonic stem cells PPARγ coactivator 1α cardiomyocyte mitochondrial biogenesis

DOI: 10.3867/j.issn.1000-3002.2013.02.006

通讯作者 彭双清,E-mail:pengsq@hotmail.com,Tel:(010)66948462 pengsq@hotmail.com