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Title: Phytoestrogen attenuates hypoxia-induced expression of annexin A1 in alveolar epithelial cells through down-regulating NF- κ B and HIF-1 α

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关键词: 缺氧; 肺泡上皮细胞; 植物雌激素; 膜联蛋白A1

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摘要: 目的 从人肺泡上皮细胞甲酰肽受体激动剂——膜联蛋白A1 (Annexin A1, AnxA1) 的表达变化, 探讨植物雌激素影响缺氧肺部炎症浸润的机制。 方法 人肺泡II型上皮细胞 (A549) 接种于60 mm培养皿中常规培养, 待培养皿中细胞长满约70%, 根据是否用药分为: 对照组 (溶剂DMSO对照)、染料木黄酮 (genistein, Gen) 组、大豆苷元 (daidzein, DD) 组, 每组细胞再根据是否缺氧再分为常氧组(N)、缺氧组(H)。Gen组、DD组于缺氧处理前分别加入Gen及DD, 终浓度均为15 μ mol/L。缺氧组细胞置于缺氧培养箱 (1%O₂) 24 h。取细胞培养上清及细胞裂解离心上清, 结合甲酰肽受体特异性抑制剂tBoc2, 通过趋化实验, 检测其甲酰肽受体激动活性。用Western blot分析AnxA1、缺氧诱导因子-1 α (HIF-1 α)、核因子- κ B(NF- κ B)在蛋白水平的变化, 用RT²-PCR分析其mRNA水平的变化。 结果 缺氧后的细胞冻融上清及培养上清趋化活性显著高于常氧组 ($P < 0.05$), 而植物雌激素处理后, 两种上清的趋化活性均显著高

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于对照组 ($P<0.05$)。Western blot与RT²-PCR检测结果显示, 缺氧组AnxA1在蛋白水平和mRNA水平均高于常氧组 ($P<0.05$), 两种植物雌激素不同程度地削弱了缺氧对AnxA1的上调效果, 同时对HIF-1 α 、NF- κ B的蛋白水平也产生同样的影响。 结论 植物雌激素削弱了缺氧对AnxA1基因表达的上调效应, 其机制与HIF-1 α 、NF- κ B信号途径有关。

Abstract: **Objective** To determine the effects of phytoestrogen on the expression of formyl peptide receptor (FPR) and Annexin A1 (AnxA1) in human alveolar epithelial cells under hypoxia exposure. **Methods** Human alveolar type II epithelial cells (A549) were cultured and divided into normoxia (5%CO₂ and 95%O₂) and hypoxia (1%O₂ 5%CO₂, and 94%N₂ for 24 h) groups. Each group was further divided into the control, genistein treatment group (15 μ mol/L before hypoxia) and daidzein treatment group (15 μ mol/L before hypoxia). Chemotaxis assay was used to detect the activity of FPR agonists in the supernatants of culture media and lysates of the treated cells in presence or absence of FPR specific inhibitor tBoc2. The protein levels of AnxA1, hypoxia inducible factor 1 α (HIF-1 α) and nuclear factor κ B (NF- κ B) were detected by Western blotting. The mRNA level of AnxA1 was assayed by RT²-PCR. **Results** Hypoxia induced both cultural supernatants and lysate supernatants had significantly higher chemotactic activity than normoxia ($P<0.05$). Phytoestrogen treatment also induced higher chemotactic activity than the control groups ($P<0.05$). Western blotting and RT²-PCR indicated that hypoxia up-regulated the expression of AnxA1 significantly at the levels of protein and mRNA ($P<0.05$). The elevation was inhibited in the presence of phytoestrogen so as well NF- κ B and HIF-1 α . **Conclusion** Phytoestrogen attenuates hypoxia-induced up-regulated expression of AnxA1 through NF- κ B and HIF-1 α signal pathways.

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