低氧/厌氧产品案例——糖尿病

文章题目：Human Adipose-Derived Mesenchymal Stem Cells Respond to Short-Term Hypoxia by Secreting Factors Beneficial for Human Islets In Vitro and Potentiate Antidiabetic Effect In Vivo

人脂肪间充质干细胞在体外通过分泌有益于人胰岛的因子对短期缺氧作出反应，并在体内增强抗糖尿病作用

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工作站使用情况：BAKER RUSKINN Invio2 200

使用气体 浓度：低氧（1 % O_2 , 5 % CO_2 ,94 N_2）

主要内容：脂肪源间充质干细胞(ASCs)在体外释放胰岛有益因子，并在糖尿病啮齿动物模型中预防高血糖。氧张力已被证明能引起代谢变化并改变可溶性因子的释放。但缺氧对 ASCs 抗糖尿病特性的影响尚未被探索。研究发现缺氧培养的 ASC 耐受性较好，与常氧培养相比，缺氧培养的 ASC 培养基中 VEGF-A、FGF-2 和 bNGF 水平显著升高，而 HGF、IL-8 和 CXCL1 水平显著降低；缺氧培养的 ASCs 培养基能明显改善人胰岛功能，减少培养后细胞凋亡，减少细胞因子诱导的细胞凋亡；在糖尿病小鼠模型中，与对照组相比，接受 ASCs 的两组小鼠胰腺胰岛素含量均较高，但接受低氧预处理的 ASCs 小鼠非空腹和空腹血糖较低，口服糖耐量也有所改善。总之，在缺氧条件下，ASCs 的胰岛保护潜能得到了提高，且低氧预处理增强了 ASCs 的体内抗糖尿病作用。

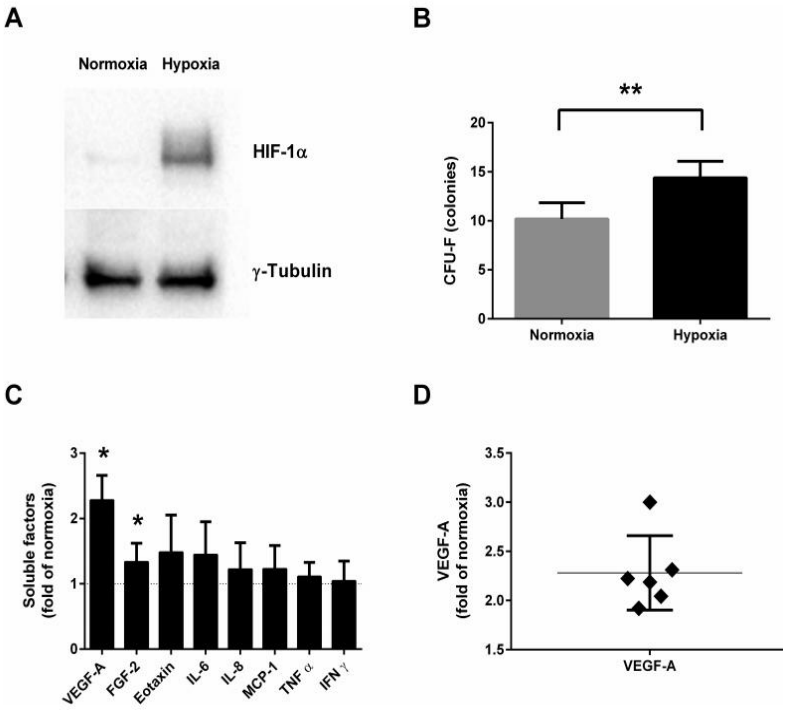

Figure 2. ASC response to hypoxia and altered levels of soluble factors in conditioned media (CM) after incubation in hypoxia (1% O2) compared to normoxia (21% O2). Representative Western blot of hypoxia-inducible factor 1a (HIF-1a) in ASC lysate after 48 h of incubation in normoxia or hypoxia (A). Colony number comparing cells incubated for 48 h in normoxia or hypoxia prior to colony-forming unit fibroblast assay (CFU-F) (n=5) (B). Levels of soluble factors in 1% O2 CM compared to 21% O2 CM in ASCs from six different donors (n=6) (C). Distribution of vascular endothelial growth factor A (VEGF-A) increase in 1% O2 CM compared to 21% O2 CM (D).

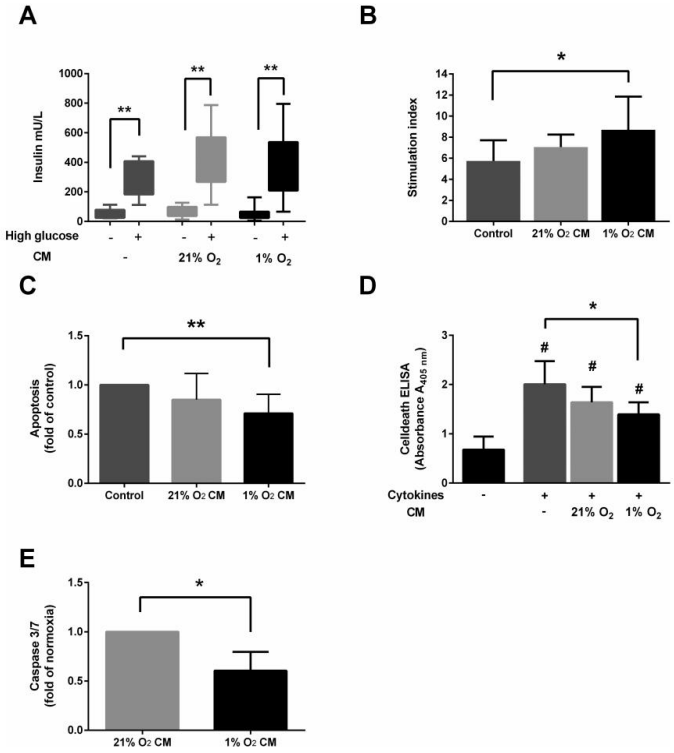

Figure 3. Exposing human islets to CM from ASCs incubated in hypoxia (1% O2 CM) significantly improves function and reduces apoptosis after culture and protects against cytokine-induced apoptosis. Human islets were cultured for 48 h in a 1:1 mixture of supple mented Connaught Medical Research Laboratories (CMRL) media and either unconditioned minimum essential media (MEM) a (Control), 21% O2 CM, or 1% O2 CM. Insulin release in low (-) and high (+) glucose (A) and calculated stimulation index (n=9) (B). Apoptosis measured by Cell Death ELISA after 48 h of culture (n=10) (C). Cell Death ELISA after 24 h of culture in control media, 21% O2 CM, or 1% O2 CM with or without a mixture of cytokines (TNF-a, IL-1b, and IFN-g) (n=6) (D). Caspase 3/7 activation in lysates of islets incubated for 24 h with a mixture of cytokines (n=6) (E).

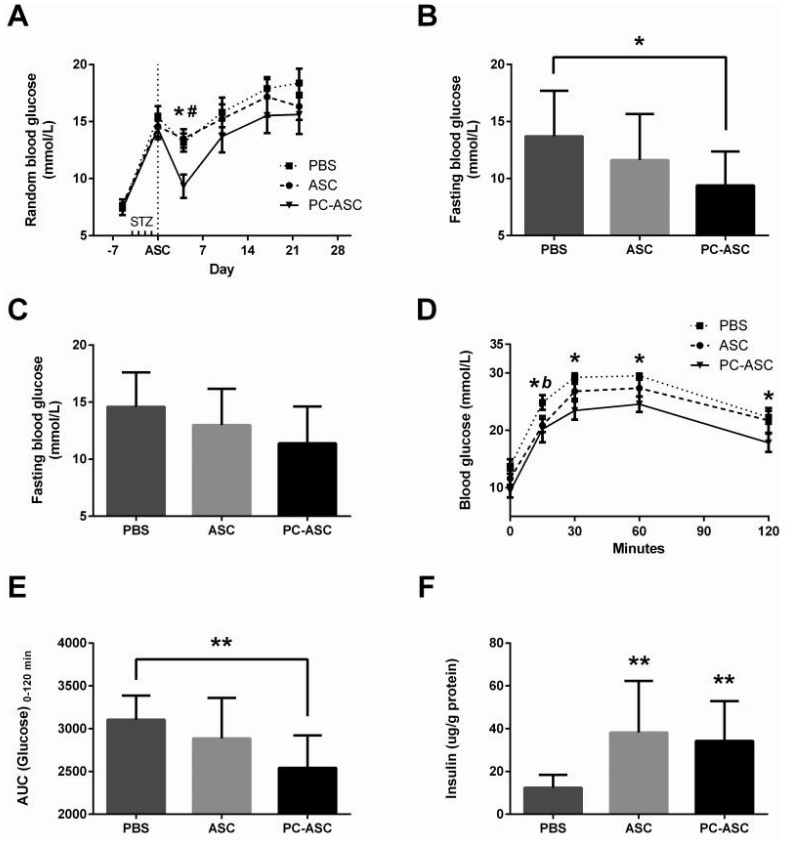

Figure 5. Hypoxic preconditioning potentiates antidiabetic effect of ASCs in mouse streptozotocin (STZ)-induced insulitis model. Balb/c recombination activating gene 1-deficient (Rag 1-/-)immunodeficient mice were intravenously injected with STZ (50 mg/kg) for 4 consecutive days. One day after the last STZ injection, mice received an intravenous injection with either 0.2 ml of PBS (PBS) (n=10 mice), 0.8´106 nonpreconditioned ASCs (ASC) (n=8 mice), or 0.8´106ASCs preconditioned for 48 h in 1% O2 (PC-ASC) (n=7 mice). Random glucose measured weekly (A). Fasting glucose measured on days 13–16 (B) and 21–23 after ASC injection (C). Oral glucose tolerance test performed on days 13–16 after ASC injection (D) with an area under the curve (AUC) analysis (E). Protein adjusted insulin content of pancreas after harvest on days 21–23 after ASC injection was analyzed by insulin ELISA (F).

短期缺氧（1% O_2）诱导脂肪源间充质干细胞(ASC)HIF-1a 增加，并促进 ASC 培养基中 VEGF-A 和 FGF-2 的分泌，而 eotaxin、IL-6、IL-8、MCP-1、TNF-a、IFN-γ的分泌无显著性变化（图 2）；1% O_2 ASC 培养基可改善人体胰岛功能，降低细胞凋亡，并抵抗炎症细胞因子（图 3）；与对照组相比，低氧预处理的 ASCs 的糖尿病小鼠非空腹血糖和空腹血糖显著降低（图 5A 和 B），口服糖耐量试验（OGTT）中血糖水平显著降低（图 5D），AUC 曲线下面积显著减少（图 5E），胰脏中胰岛素含量显著升高（图 5F），表明低氧预处理可增强 ASCs 对小鼠的抗糖尿病作用。