Ruskinn 低氧工作站应用案例——人诱导多功能干细胞

文章题目：Modeling of LMNA-Related Dilated Cardiomyopathy Using Human Induced Pluripotent Stem Cells **人诱导多功能干细胞 LMNA 基因相关扩张型心肌病的模型研究**

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使用气体浓度:低氧（1% O_2, 5% CO_2, 94% N_2）,常氧（19% O_2, 5% CO_2,76% N_2）
工作站使用情况：Invivo2 400

主要内容：**扩张型心肌病(DCM)**是导致心力衰竭和心脏移植的主要原因之一，部分家族性 DCM 是由于 LMNA 基因突变所致，为了更好地对 DCM 进行病理学研究，文章使用**人诱导多能干细胞衍生心肌细胞**(hiPSC-CM)对与 **LMNA** 基因相关的 DCM 进行建模。对建模后的细胞研究得出，在常氧条件下，突变型 hiPSC-CMs 表现出几乎正常的肌节结构，而在缺氧条件下则观察到明显的**肌节损伤和细胞应激敏感性**的增加。**p.S143P hiPSC-CM 模型为**研究加速心脏退行性变的细胞机制提供了一个有用的工具。

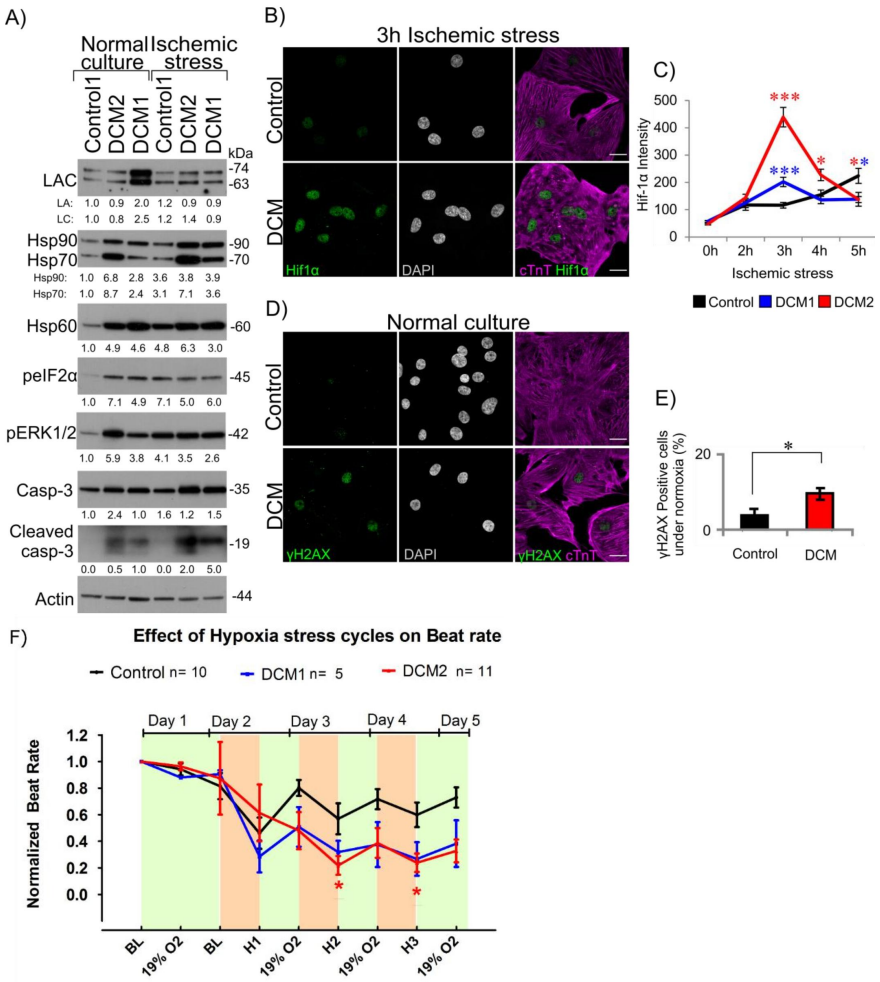

Figure 5. DCM hiPSC-CMs show increased elevated cellular stress.
(A) Western blot analysis of lamin A/C, phospho-eIF2α (peIF2α), Hsp90, Hsp70, Hsp60, phospho-ERK1/2 (pERK1/2) and cleaved caspase-3 under normal culture conditions and after exposure to ischemic stress for 3 h. Actin was used as a loading control. The average numerical values of signal intensities relative to the loading control (actin) are shown below each blot. n = 2 individual experiments. Control2 hiPSC-CMs were not qualified for analysis due to lower differentiation efficiency compared to other lines. (B) Confocal microscopy analysis of Hif-1α intensity. Control1, DCM1 and DCM2 hiPSC-CMs were cultured either in norma culture conditions or exposed to ischemic stress for 2 h, 3 h, 4 h and 5 h, fixed and stained for Hif-1α, cardiac marker cTnT and DNA (DAPI). A 3 h time point is shown. Scale bar 20 µm. (C) The fluorescence intensities of Hif-1α were determined from all the confocal sections of >15 randomly selected cells at different time points and the average normalized signals were plotted. (D) Control1 and DCM2 hiPSC-CMs were cultured under normal culture conditions, fixed and stained for γH2AX, cTnT and DNA (DAPI). (E) γH2AX positive cells from control1 and DCM2 were counted and plotted (n = 500). (F) Effect of three repeated 3 h cycles of hypoxia (1% O2) shown as H1, H2 and H3 and overnight re-oxygenation (19% O2) on beat rate of hiPSC-CMs recorded on MEA. Control data presented in F is combined from Control1 and 2. Data is expressed as mean ± s.e.m., (*) $p < 0.05$, (**) $p < 0.01$ and(***) $p < 0.001$.

氧的减少迫使心肌细胞从有氧呼吸途径切换到厌氧呼吸途径，导致糖酵解底物增加，ATP 生成紊乱了心脏收缩力。由于缺氧条件下，DNA 损伤增加以及 Hif-1α、HSP70 等因子持续上调等导致肌节受损日益严重，反复缺氧和常氧循环实验中所有实验组的搏动均降低，但对 DCM-CMs 的影响更严重，仅恢复了原始搏动率的 30-45%。本实验进一步验证了 hiPSC-CMs 用于心脏病和 I/R 损伤建模方面的可行性，以促进对心脏病理生理学的研究。