低氧/厌氧产品应用案例——缺氧脑损伤研究

文章题目：Human 3D cellular model of hypoxic brain injury of prematurity
缺氧脑损伤的三维细胞模型

文章出处：斯坦福大学医学院, Nature Medicine, DOI: 10.1038/s41591-019-0436-0**使用气体浓度：**<1 %O_2; 21% O_2

主要内容：早产儿由于肺发育不成熟、低血压和缺乏脑血流调节，极有可能出现缺氧发作，并可发展成一种严重的早产儿脑病。早产儿大脑皮层的易感细胞类型和相关灰质缺陷的分子机制尚不清楚。本文使用人类三维大脑区域特异性的器官来研究缺氧对皮质形成的影响。作者发现了中间祖细胞的特定缺陷是一种与人类大脑皮层扩张相关的皮质细胞类型，且这些缺陷与未折叠蛋白的反应和变化有关。此外，本文在人原始皮层组织中验证了这些发现，并证明了一个未折叠蛋白反应通路的小分子调节剂可以防止中间祖细胞在缺氧后的减少。因此这个人类细胞模型将对研究人类大脑发育过程中潜在的环境和遗传因素具有价值。

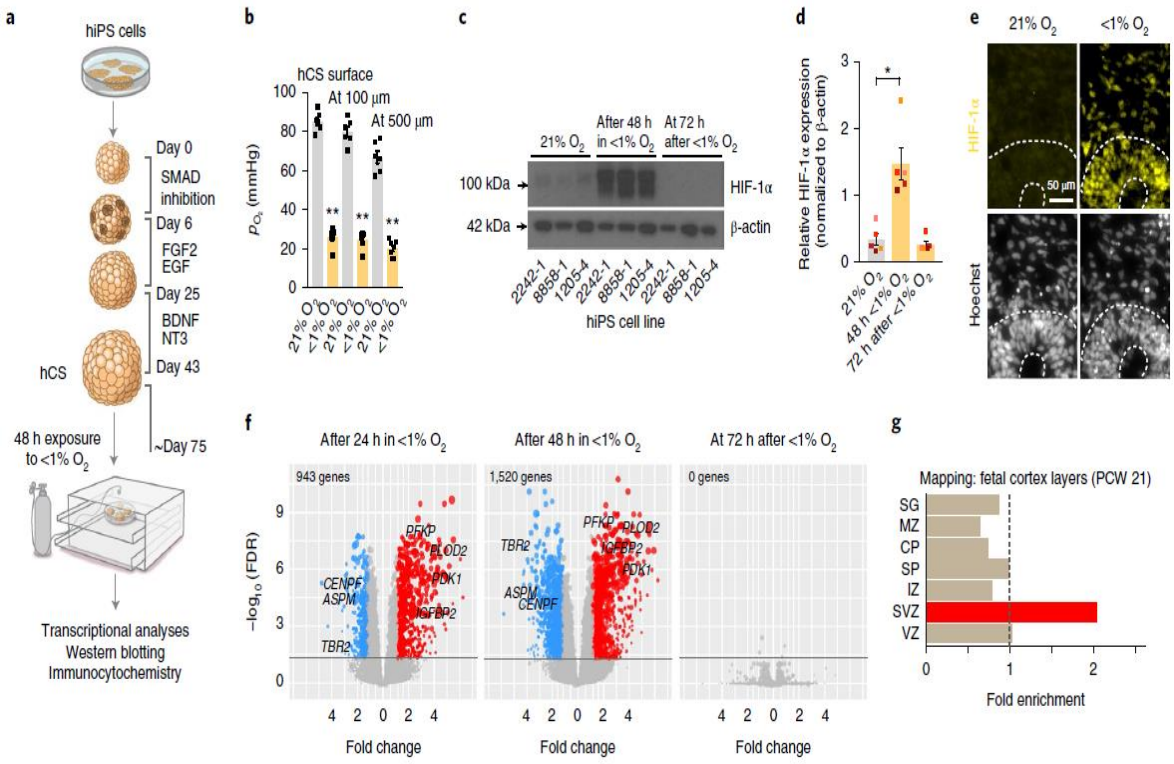

Fig. 1 Human cellular model for studying changes in oxygen tension in hCS. **a,** Schematic of the major stages in the generation of hCS from hiPS cells as escribed in ref. At days 74–78 of in vitro differentiation, hCS are exposed for 48h to <1 %O_2 in a gas-controlled culture chamber and then maintained for another 72h at 21% O_2. Control hCS are maintained at 21% O_2 throughout. **b,** Oxygen tension levels (Po2, mmHg) measured with an optical sensor (100and 500 µm) at 21% O_2

(n=6 hCS) and after 48h of exposure to <1% O_2 (n=7); hCS from three hiPS cell lines; Kruskal - Wallis test, P < 0.0001, Dunn's multiple-comparison test, **P=0.002 at hCS surface, **P = 0.008 at 100 µm, **P = 0.01 at 500 µm. **c,d**, Representative western blots and quantification of HIF-1α protein expression in hCS after 48 h of exposure to <1% O_2 and after 72h of reoxygenation versus the unexposed samples (21% O_2); normalized to β-actin (n=5 differentiated hiPS cell lines with at least two hCS per condition; Friedman's test, P=0.02, Dunn's multiple-comparison test versus 21% O_2, *P = 0.02 for 48h and P > 0.99 for 72h). Data are mean ± s.e.m. Individual values are indicated by dots. Western blots were cropped to show the relevant bands; molecular weight markers are indicated on the left (in kDa). See Supplementary Table 2 for quantifications, and uncropped blots are available as source data. **e,** Representative immunostaining of HIF-1α (yellow) in hCS exposed for 48 h to <1% O_2 versus 21% O_2 hCS. Experiment performed in two hiPS cell lines. Nuclei labeled by Hoechst staining. **f,** Volcano plots showing the results of RNA-seq experiments after exposure to <1% O_2 for 24h or 48h versus 21% O_2, as well as after 48h of exposure to <1% O_2 followed by 72h of reoxygenation (total time of 120h). Each dot represents a single gene, with genes that are significantly upregulated shown in red, genes that are significantly downregulated in blue and non-significant genes in gray (determined on the basis of FDR ≤ 0.05 and fold change ≥ 1.5). The size of the points corresponds to the difference in expression level between low-oxygen-exposed hCS and unexposed hCS (difference of medians); n=24 samples from hCS derived from three hiPS cell lines. **g,** Overlap between hypoxia-related transcriptome changes in hCS and layer-specific transcriptome signatures in the developing human cortex at PCW 21 as described in ref. 16. Strong enrichment is observed only in SVZ (P value corrected for multiple-comparisons). SG, subpial granular zone; MZ, marginal zone; CP, cortical plate; SP, subplate; IZ, intermediate zone; VZ, ventricular zone.

与常氧(21% O_2)相比，大脑皮层球状体缺氧(<1% O_2培养48h后)条件下表层、100和500µm处氧张力明显降低(b)，HIF-1α相对表达和定量表达明显升高(c,d,e)，基因的表达(包括与低氧反应有关的基因如*PLOD2*、*PFKP*、*PDK1*和*IGFBP2*)也表现出明显差异(f)，且在hCS的SVZ出现了明显的双倍富集。

hCS：人类大脑皮层球状体
hiPS：人诱导多能干细胞
PO_2：氧张力
SVZ：脑室下区
At 72 h after <1% O_2：缺氧条件下培养72h后恢复到常氧条件培养
SG、MZ、CP、SP、IZ、VZ：人类大脑皮层不同亚区