Ruskinn 低氧工作站应用案例——阿尔兹海默症

文章题目：**STAT1 drives M1 microglia activation and neuroinflammation under hypoxia**
低氧状态下 STAT1 驱动 M1 小胶质细胞活化和神经炎症

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使用气体浓度:低氧（1% O_2）,常氧（5%CO_2，95%空气）
工作站使用情况：Concept 400

主要内容：小胶质细胞是中枢神经系统中起第一个主动防御作用的免疫细胞。这些细胞不断监测组织微环境，并对缺氧、感染和损伤做出快速反应。本文通过证明低氧条件激活小胶质细胞向 M1 表型转化和促炎细胞因子的释放，同时验证了氧化应激诱导了 STAT1 磷酸化和 s-谷胱甘肽酰化，从而导致 STAT1 异常活化，揭示了 STAT1 信号在低氧条件下 M1 小胶质细胞活化中的中心作用。

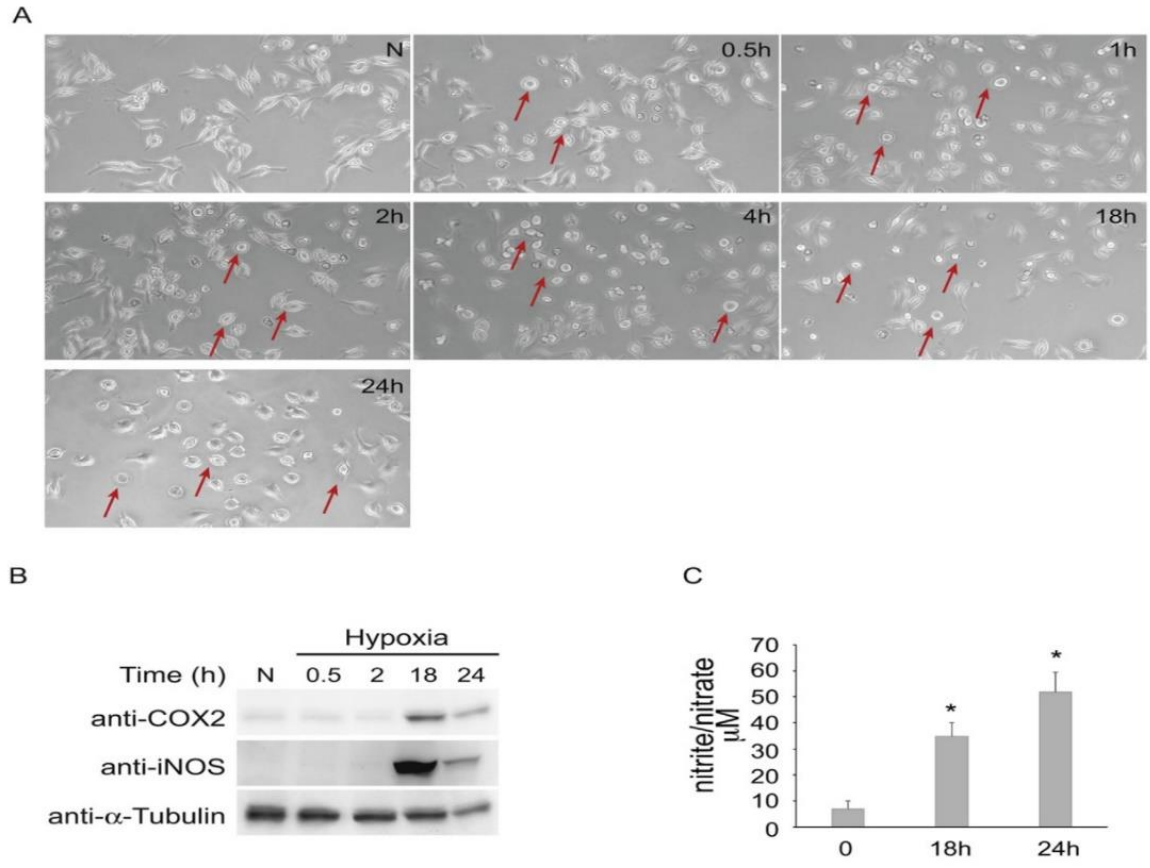

Fig. 1. Hypoxia induces M1 microglia activation. BV2 cells were exposed to hypoxia for the indicated time and different parameters of M1 microglia phenotype were analyzed. A) Phase-contrast microscopy images of BV2 cultured under 1%O_2 shown the morphological changes from ramified to a more amoeboid shape (arrow) (10x). Representative images from three separate experiments are shown. B) Total protein extracts were analyzed by western blot with anti-COX2 antibody and, after membrane stripping, with anti-iNOS antibody. The same blot was incubated with α-tubulin antibody to check the amount of loaded proteins. Data shown are representative of four independent experiments. C) The levels of NO^{2-}/NO^{3-} were evaluated in the culture medium of cells using Griess reaction. The results are presented as the means ± SD of at least six independent experiments. *P < 0.01 compared with the cells under normoxia.

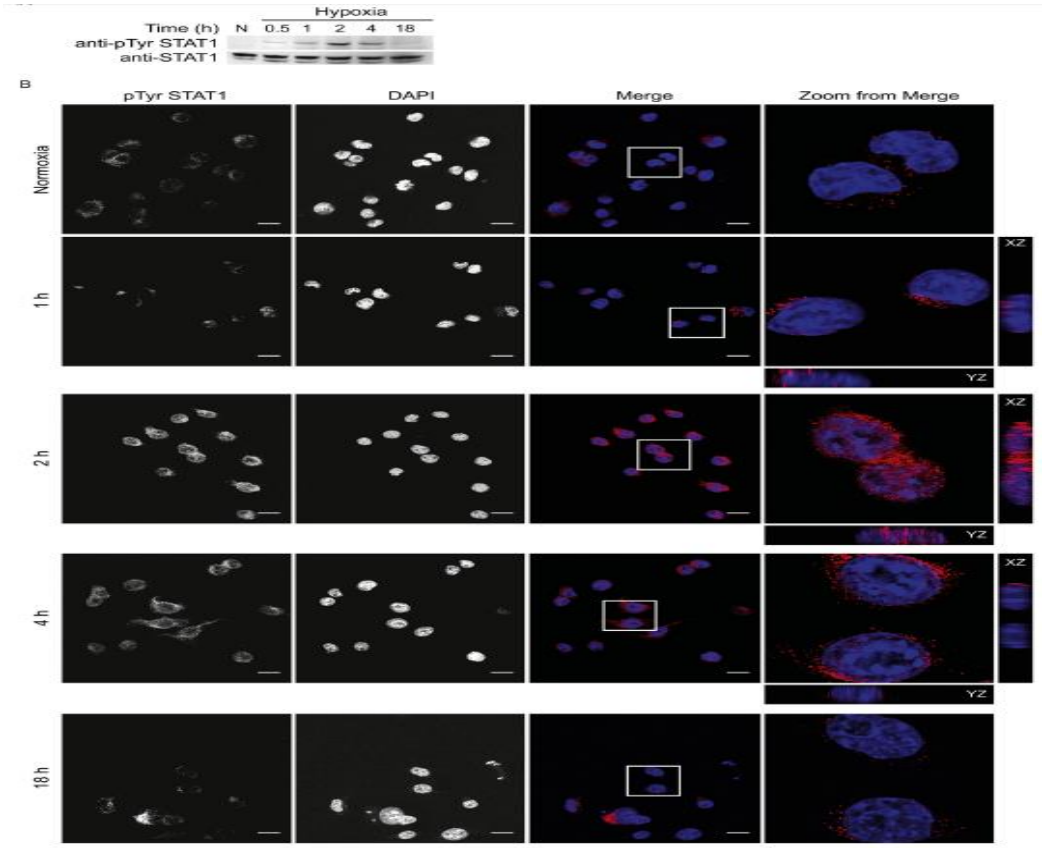

Fig. 3. Hypoxia induces STAT1 tyrosine phosphorylation and translocation into the nucleus. A) BV2 cells were exposed to hypoxia for the indicated time and total protein extracts were assessed by western blot with an anti-phosphoTyr701 STAT1 antibody and, after membrane stripping, with anti-STAT1 antibody. BV2 cells cultured under normoxia were used as control (N). Data shown are representative of five independent experiments. B) BV2 cells were kept under 1%O2 for the indicated time, immunostained with phosphoTyr701 STAT1 (red) and analyzed by confocal microscopy using lens 40x. Nuclei were stained with DAPI (blue). Scale bars indicate 50 μm. Representative images from three separate experiments are shown.

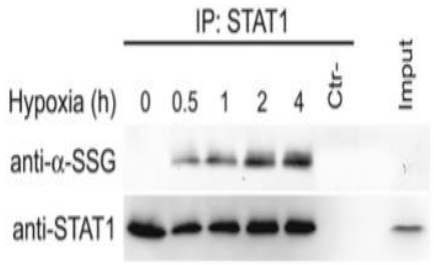

Fig. 5. Hypoxia induces S-glutathionylation of STAT1. BV2 cells were exposed to hypoxia for the indicated time and STAT1 was immunoprecipitated from total protein extracts using anti-STAT1 antibody. Immunoprecipitated STAT1 (IP: STAT1) was analyzed by western blot under non-reducing condition with anti-SSG and, after membrane stripping, with anti-STAT1 antibody. The same amount of total protein was immunoprecipitated with rabbit IgG and analyzed in western blot as described in Materials and Methods Section (Ctr-). Samples of cells lysates are reserved before pull-down (input). The images are representative of four experiments.

低氧（1% O_2）条件下，BV2 细胞中 M1 小胶质细胞的性状改变（分支状改变为弓形虫状），炎症蛋白 iNOS 和 COX2 的表达以及亚硝酸盐和硝酸盐(NO2-/NO3-)水平均显著升高；Cys324 和 Cys492 的 s -谷胱甘肽化激活了 STAT1 信号通路，且与常氧条件相比，磷酸化的 STAT1 在 2h 时明显升高。