Ruskinn 低氧工作站应用案例——卵巢癌干细胞

文章题目：Hypoxia-inducible factor-1α promotes cell survival during ammonia stress response in ovarian cancer stem-like cells **低氧诱导因子 HIF-1α促进卵巢类癌干细胞氨应激反应中的细胞存活**

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工作站使用情况：Invivo2

使用气体浓度: 5% CO_2, 1%O_2

主要内容：细胞对氨胁迫的耐受是肿瘤发生和肿瘤生长的重要特性，谷氨酰胺合成酶（GS）是导致细胞凋亡的关键驱动因子，在驱动癌症干细胞的增殖潜能方面具有重要功能。在卵巢类癌干细胞中，GS 是氨胁迫和谷氨酰胺依赖性代谢下增殖的关键驱动因子。本研究揭示了 HIF-1α在类癌症干细胞的两相氨胁迫管理中的功能，其中 GS 促进细胞增殖，HIF-1α导致增殖减弱，但却能促进细胞存活。

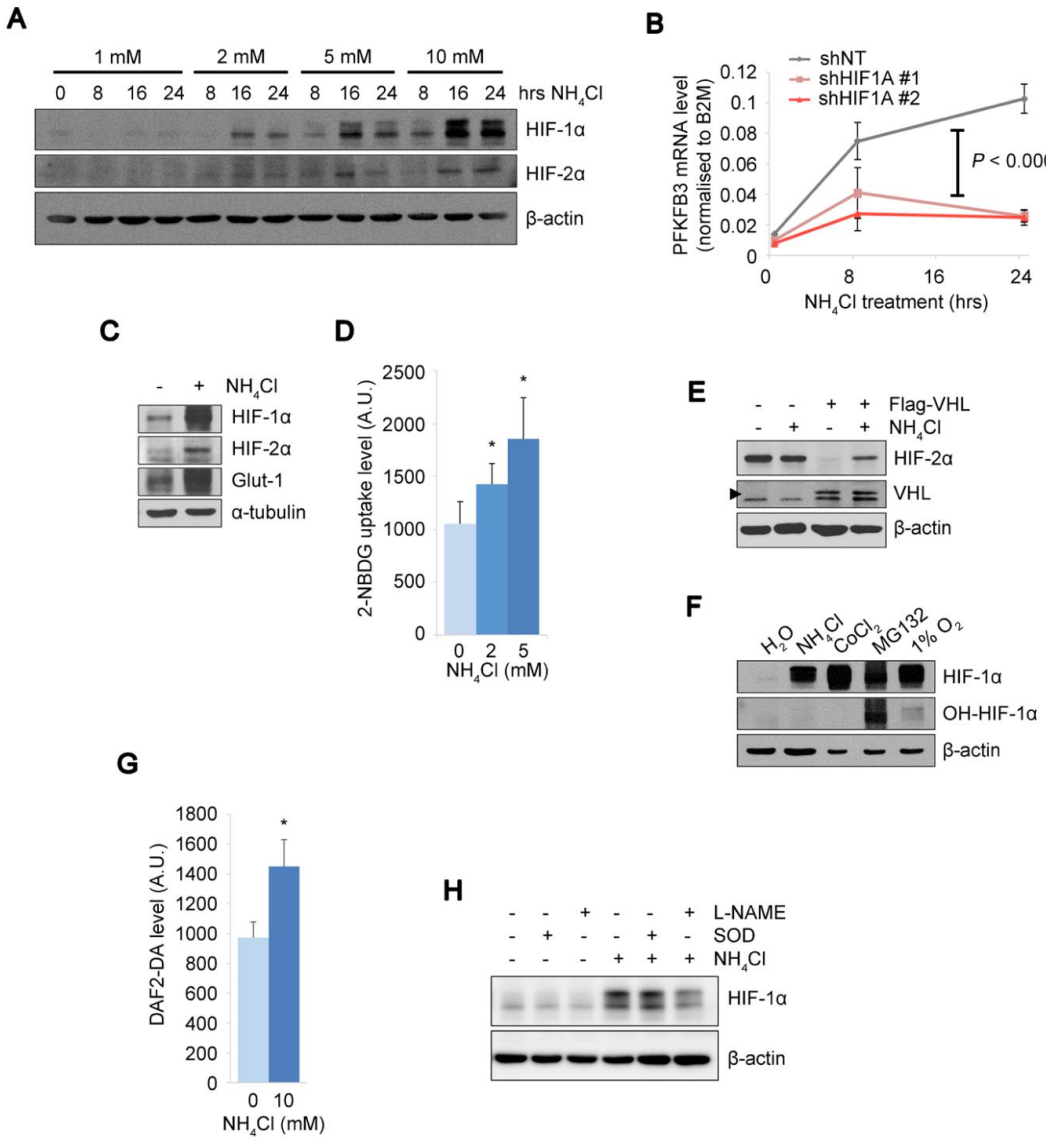

Figure 2: The PHD-HIF axis up-regulates glycolysis under ammonia stress.
(A) HIF-1α and HIF-2α levels were increased by NH_4Cl treatment in a dose- and time-dependent manner. SKOV3 cells treated with NH_4Cl at the indicated concentrations and durations. β-actin expression was shown as the loading control. (B) Kinetic PFKFB3 mRNA expressions in PEO1 CD90+ cells upon NH4Cl treatment. The PFKFB3 up-regulation by NH4Cl observed in control cells (shNT) was almost completely abolished by two independent shRNA knockdown of HIF1A (HIF-1α). Each duplicated experiment was repeated twice. A two-way ANOVA and post-hoc tukey comparisons revealed a group effect between NT and KDs ($P < 0.0001$). (C) Protein expressions of HIFs, a HIF target GLUT1 and α-tubulin as a loading control were determined in SKOV3 cells by immunoblot upon NH4Cl treatment (10 mM) for 16 hrs. (D) Glucose uptake assay with NH4Cl treatment at 0, 2 or 5 mM for 16 hours in CD90– and CD90+ PEO1 cells. Intracellular glucose levels were determined using 2-NBDG fluorescence signals. (E) Immunoblot of A498 cells and their derivative cells expressing WT-VHL upon treatment with 10 mM NH4Cl. Arrow head indicates the ectopic expression of WT-VHL. (F) Comparison of prolyl-hydroxylation levels of HIF-1α between the indicated treatments. SKOV3 cells were treated with NH4Cl (10 mM for 16 hrs), CoCl2 (150 μM for 1 hr), MG132 (10 μM for 2 hrs) or hypoxia (1% O2 for 2 hrs). The amount of protein loaded was adjusted to show approximately the same level of whole HIF-1α protein (refer to β-actin levels). (G) Cellular NO levels were determined by the fluorescent signal from DAF2-DA dye. (H) Effect of L-NAME or SOD on HIF-1α expression levels by NH4Cl. The data in D and G show the mean of at least n =3 independent experiments. Error bars indicate s.e.m. *$P < 0.05$; (Student's t-test).

HIF-1α和 2α蛋白随 NH4Cl 剂量和时间增加积累增多，即 HIF 被氨稳定和激活，且在氨胁迫条件下葡萄糖的摄取显著增加，即表明糖酵解在氨反应中通过 HIF 通路激活而上调，这与缺氧条件下 HIF 的生理作用是一致的。