低氧/厌氧产品案例——乳腺癌干细胞

文章题目：BRCA1 regulates the cancer stem cell fate of breast cancer cells in the context of hypoxia and histone deacetylase inhibitors

在低氧和组蛋白去乙酰化酶抑制剂条件下，BRCA1 调节乳腺癌细胞干细胞特性

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工作站使用情况： Ruskinn Invio2 400

使用气体 浓度： 低氧（1 % O_2 , 5% CO_2 ,95 N_2）

主要内容：肿瘤细胞的干细胞特性对于肿瘤恶性进展和克隆进化至关重要。肿瘤细胞由复杂的机制决定，包括细胞内在途径和来自肿瘤微环境的应激信号。本研究发现肿瘤抑制基因 BRCA1 是一种参与 DNA 修复和表观遗传调控的多功能蛋白，在肿瘤干细胞(CSC)样特征的调控中起着关键作用。 下调 BRCA1 促进乳腺癌细胞中肿瘤干细胞特性，BRCA1 重建的肿瘤细胞比 BRCA1 缺乏的细胞对组蛋白去乙酰化酶(HDAC)抑制剂诱导的干细胞丢失更敏感。此外，低氧优先阻断 HDAC 抑制剂诱导的乳腺癌细胞的分化，表明 BRCA1 和肿瘤乏氧应被视为可能影响 HDAC 抑制剂治疗效果的潜在重要临床参数。

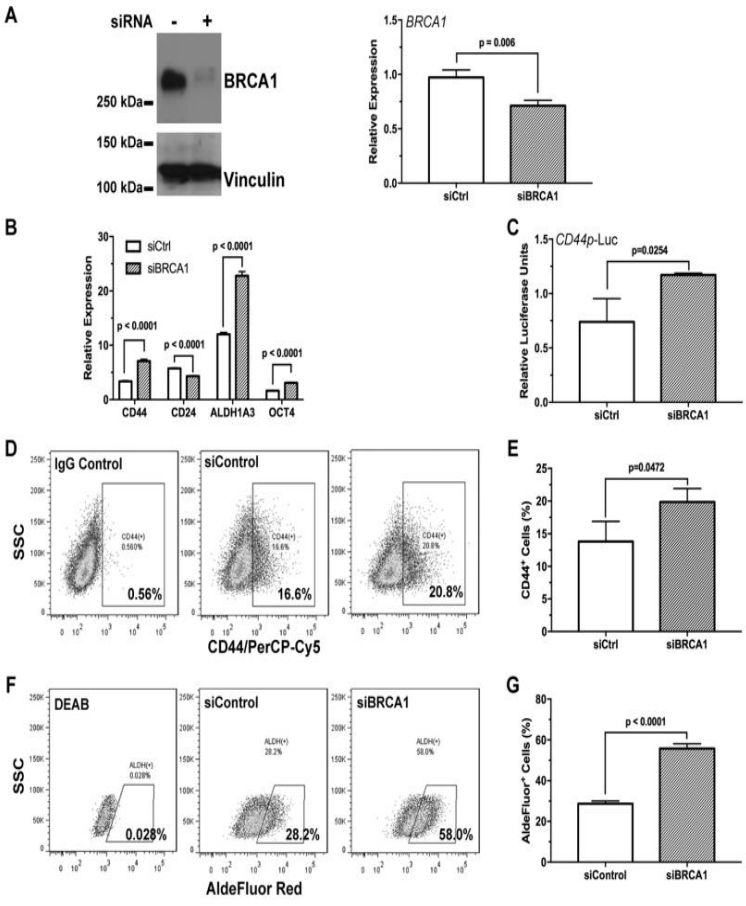
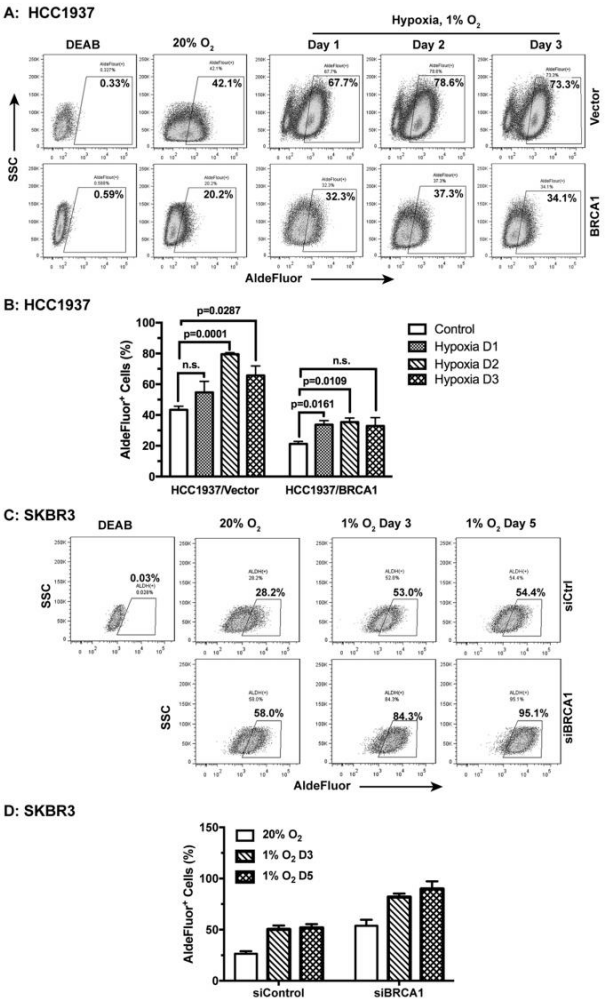


Figure 3. Down-regulation of BRCA1 promotes breast cancer stem cell characteristics. (A) BRCA1 was down-regulated by RNA inference in the BRCA1-competent SKBR-3 breast cancer cells, which was confrmed by Western blot and qRT-PCR (n=3). (B) Expression of breast cancer stem cell-associated genes in SKBR3±siBRCA1 cells were measured by qRT-PCR (n=3). (C) CD44 promoter activity in SKBR3±siBRCA1 cells was measured using luciferase assay (n=3). Te cell surface CD44 levels in SKBR3±siBRCA1 cells were analyzed by fow cytometry with representative fow cytometry data shown in (D) and quantitation in (E, n=3). Te ALDH activity in SKBR3±siBRCA1 cells were analyzed by fow cytometry with representative fow cytometry data shown in (F) and quantitation in (G, n=3). Te DEAB treated cells were used as negative control.



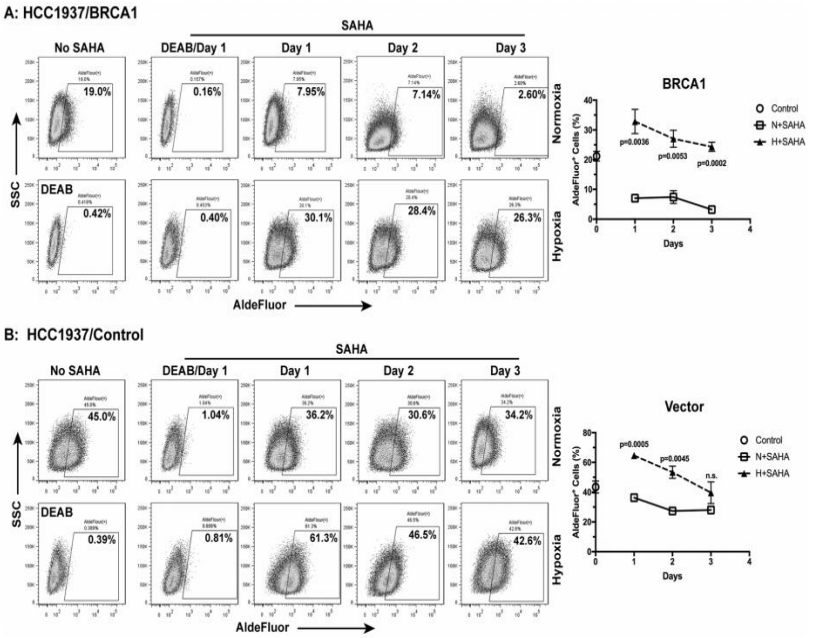


Figure 6. BRCA1 status and hypoxia determine breast cancer cell response to the histone deacetylase inhibitor SAHA. HCC1937±BRCA1 cells were treated with 1μM SAHA under either normoxia or hypoxia. ALDH activities (ALDEFLUOR) were measured at the indicated time following incubation (n=3). Te DEAB treated cells were used as negative control.

Figure 5. BRCA1 affects hypoxia-induced breast cancer cell stemness. HCC1937±BRCA1 cells were either maintained under ambient tissue culture conditions (20% O_2) or under hypoxia (1% O_2). Their ALDH activities were measured at the indicated time. Te representative fow data are shown in (A) and quantitation in (B, n=3). SKBR3±siBRCA1 cells were maintained under normoxia or hypoxia and their ALDH activities (ALDEFLUOR) were measured at the indicated time with the fow data shown in (C) and quantitation in (D, n=2). Te DEAB treated cells were used as negative control.

沉默 BCRA1 可上调肿瘤干细胞相关标志物 CD44、ALDH1A3 和 OCT4 的表达，下调 CD24 的表达，表明下调 BCRA1 表达可促进乳腺癌肿瘤干细胞特性（图 3）；低氧（1%O_2）促进乳腺癌肿瘤干细胞特性，BCRA1 抑制低氧诱导的乳腺癌肿瘤干细胞特性（图 5）；在常氧条件下，BCRA1 活性强的乳腺癌细胞对组蛋白去乙酰化酶抑制剂 SAHA 诱导的干性丧失更敏感，而 BCRA1 缺陷的乳腺癌细胞则不那么敏感，而低氧对 BCRA1 活性强的乳腺癌干细胞干性有更强的保护作用（图 6）。