低氧/厌氧产品案例——胆管癌干细胞

文章题目：Hypoxia induced Sonic Hedgehog signaling regulates cancer stemness, epithelial-to mesenchymal transition and invasion in cholangiocarcinoma

低氧通过 SHH 信号通路调节胆管癌细胞的干细胞特性、上皮-间质转化和侵袭

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工作站使用情况： Ruskinn Invio2 300

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

主要内容：肿瘤内缺氧可通过对各种信号通路的调节导致治疗抵抗。本文章研究了缺氧和 SHH 通路激活之间的关系以及这种相互作用对胆管癌发生过程中的癌干细胞和上皮-间质转化的影响。研究表明低氧通过上调 SHH 和 SMO 水平促进 SHH 通路激活，并增强胶质瘤相关癌基因同源物 GLI1 核易位；而 HIF-1α的沉默抑制 SHH 的上调；低氧还增强了胆管癌肿瘤干细胞(CSC)转录因子 NANOG，Oct4，SOX2，CD133 的表达及 EMT 标志物钙粘蛋白 N-cadherin，波形蛋白 Vimentin 的表达，促进 CSC 干细胞特性和侵袭。SHH 通路抑制剂环孢胺可抑制低氧诱导的 SHH 通路激活，从而通过下调 CSC 转录因子 CD133 和 EMT 标志物的表达来降低侵袭性。提示缺氧诱导的 SHH 通路激活对胆管癌的进展有调节作用。因此，SHH 信号可作为标准化疗胆管癌治疗的靶点。

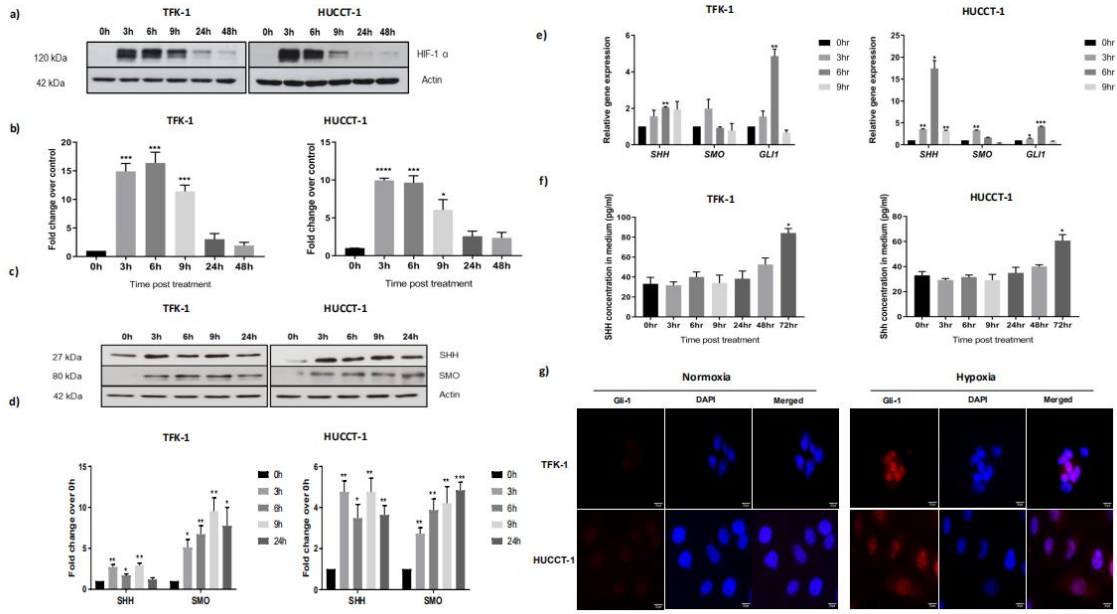

Figure1.Hypoxia induces HIF-1α expression and activates SHH pathway.(a) Western blot showing the effect of hypoxia on HIF-1α expression (b) Densitometric quantification of HIF-1α after normalizing with β-actin (c) Western blot analysis showing the effect of hypoxia on SHH and SMO expression (d) Densitometric quantification of SHH and SMO after normalizing with β-actin (e) RT-qPCR analysis showing the effect of hypoxia on SHH and SMO gene expression (f) SHH protein level in the culture medium of TFK-1 and HUCCT-1 cells after hypoxia treatment as measured by ELISA (g) GLI1 induction and nuclear translocation observed at 24hr post hypoxia treatment by immunofluorescence staining (63x magnification. Scale bar 20 μm). Data represents mean ± SEM of three independent experiments. * $p < 0.05$; * * $p < 0.01$; * * * $p < 0.001$; * * * * $p < 0.0001$.

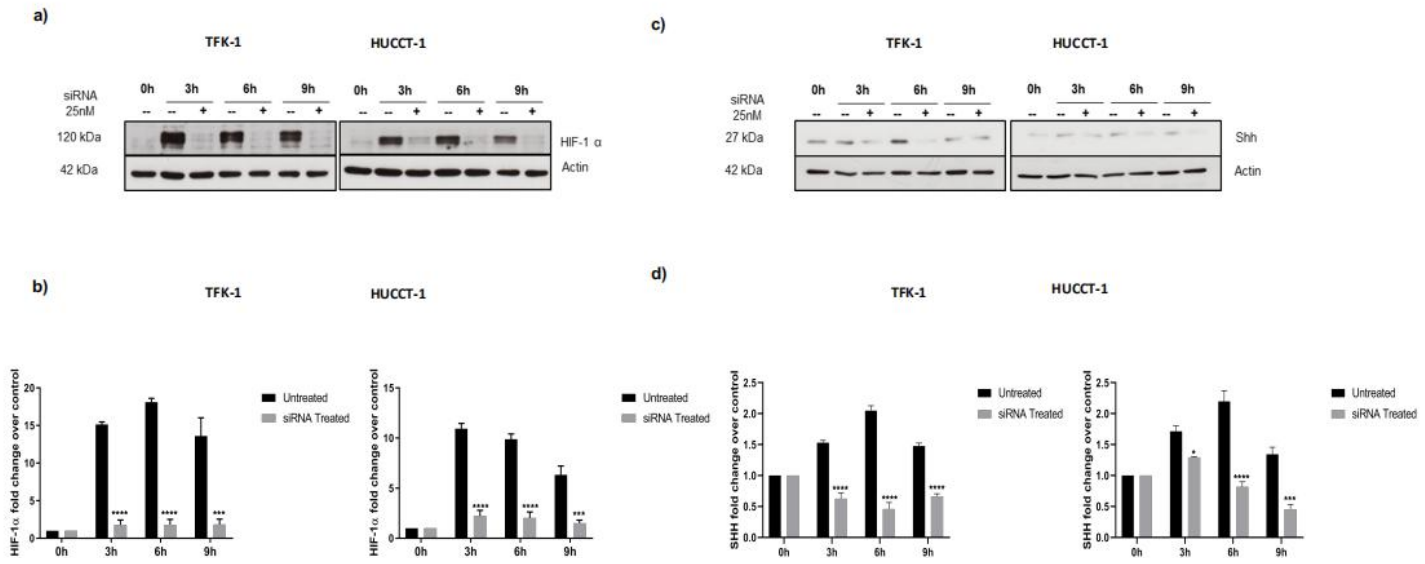

Figure2.Silencing of HIF-1α attenuates hypoxia-induced SHH pathway activation.(a) Western blot analysis of HIF-1α expression after using HIF-1α-siRNA under hypoxic conditions (b) Densitometric quantification of HIF-1 α after normalizing with β-actin (c) Western blot analysis of SHH expression after silencing HIF-1α under hypoxia (d) Densitometric quantification of SHH protein expression after normalizing with β-actin.

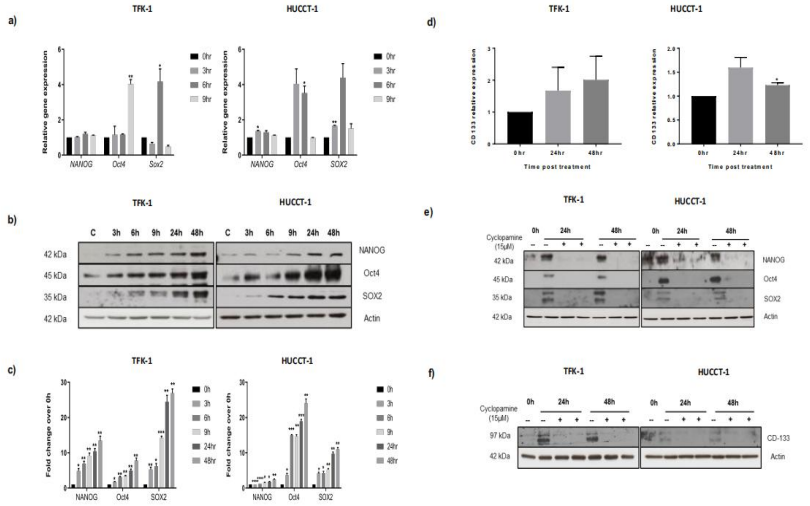

Figure6.Cyclopamine diminishes hypoxia induced expression of stem cell transcription factors and CD133 expression (a) RT-qPCR analysis of NANOG, Oct4, SOX2 in TFK-1 and HUCCT-1 cells under hypoxic conditions (b) Protein expression of NANOG, Oct4, SOX2 observed by western blot under hypoxic conditions (c) Densitometric quantification of NANOG, Oct4, SOX2 after normalizing with β-actin (d) RT-qPCR analysis of CD133 gene expression after 24hr and 48hr hypoxia treatment (e) NANOG, Oct4, SOX2 protein expression after co- treatment with cyclopamine and hypoxia (f) Protein expression of CD133 after cyclopamine and hypoxia co treatment.

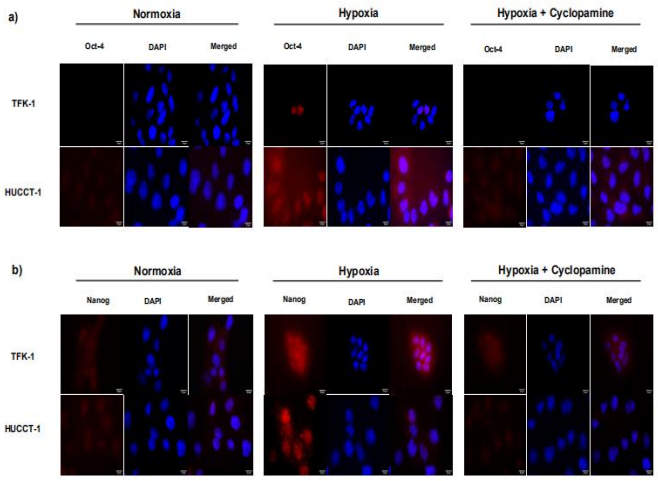

Figure7.Immunofluorescence staining showing the effect of cyclopamine on hypoxia induced upregulated stem cell transcription factors (a) Oct4 and (b) NANOG expression in TFK-1 and HUCCT-1 cells.

低氧（1 % O_2）上调 SHH 与 SMO 蛋白表达，促进 SHH 通路激活，诱导 HIF-1α表达，并增强 GLI1 核易位（图 1）；沉默 HIF-1α减轻低氧诱导的 SHH 通路的激活（图 2）；低氧（1 % O_2）可诱导胆管癌肿瘤干细胞（CSC）转录因子 NANOG、Oct4、SOX2 及 CD133 的表达，SHH 通路抑制剂环孢胺可抑制低氧诱导的 CSC 转录因子及 CD133 的表达（图 6 与图 7）。