Ruskinn 低氧工作站应用案例——肿瘤免疫治疗

文章题目： An HIF-1a/VEGF-A Axis in Cytotoxic T Cells Regulates Tumor Progression
细胞毒性 T 细胞 HIF-1a/VEGF-A 轴调节肿瘤进展

文章出处： Cancer Cell 2017 11 13;325(5) ； 10.1016/j.ccell.2017.10.003；剑桥大学生理发育与神经科学
工作站使用情况： Ruskinn Sci-Tive

Abstract

Cytotoxic T cells infiltrating tumors are thought to utilize HIF transcription factors during adaptation to the hypoxic tumor microenvironment. Deletion analyses of the two key HIF isoforms found that HIF-1 α , but not HIF-2 α , was essential for the effector state in CD8 T cells. Furthermore, loss of HIF-1 α in CD8 T cells reduced tumor infiltration and tumor cell killing, and altered tumor vascularization. Deletion of VEGF-A, an HIF target gene, in CD8 T cells accelerated tumorigenesis while also altering vascularization. Analyses of human breast cancer showed inverse correlations between VEGF-A expression and CD8 T cell infiltration, and a link between T cell infiltration and vascularization. These data demonstrate that the HIF-1 α /VEGF-A axis is an essential aspect of tumor immunity.

主要内容： 细胞毒性 T 淋细胞（CTL）又称杀伤性 T 淋巴细胞，是机体抗肿瘤机制的重要环节，也是肿瘤免疫过继疗法主要效应细胞之一。CD8+T 细胞中低氧诱导因子 HIF-1 α 的缺失减少了肿瘤浸润和肿瘤细胞杀伤，改变了肿瘤血管生成。CD8+T 细胞中 HIF 靶基因 VEGF-A 的缺失加速了肿瘤的发生，同时也改变了血管生成。人乳腺癌 VEGF-A 表达与 CD8+T 细胞浸润呈负相关，T 细胞浸润与血管生成呈正相关。这些数据表明，HIF-1 α /VEGF-A 轴是肿瘤免疫的一个重要方面。

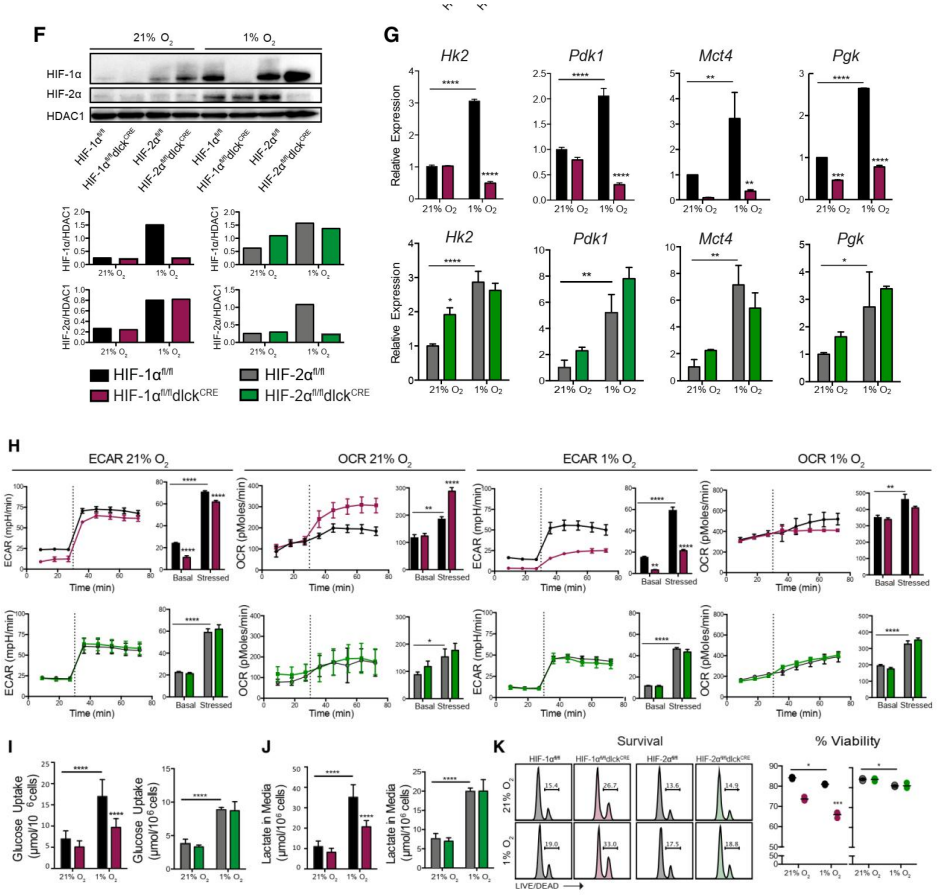

Figure 1. Hypoxia Promotes CD8+ T Cell Glycolytic Metabolism in an HIF-1a- but Not HIF-2a-Dependent Fashion(F) CD8+ T cells were isolated from spleens and activated with aCD3/CD28 for 48 hr, and then expanded for 3 days in IL-2 and subjected to 21% or 1% O2 for24 hr. Western blotting was performed on nuclear fractions; densitometric analyses are shown.(G) CD8+ T cells from HIF-1afl/fldlckCRE (maroon), HIF-2afl/fldlckCRE (green), and littermate controls (black for HIF-1afl/fl, gray for HIF-2afl/fl) were isolated, activated, expanded for 5 days in the presence of IL-2, and cultured for 24 hr under 21% versus 1% O2. qRT-PCR was performed for Hk2, Pdk1, Mct4, and Pgk (n = 3, error bars represent SD). (H) Extracellular acidification rate (ECAR) and oxygen consumption rate (OCR) of CD8+ T cells prepared as in (G) were measured by flux analysis, under basal conditions and after injection (dashed line) of oligomycin and FCCP (stressed) (n = 4 per genotype, error bars represent SEM).

在常氧(21%O_2)和低氧(1%O_2)条件下培养突变型和对照型 CTL 细胞(图 F)，当 CD8+T 细胞分化为效应细胞时，缺乏 HIF-1a 的 CD8+T 细胞，表现出糖酵解代谢相关基因的表达受损(图 G)。HIF-1a 在维持糖酵解(图 H)、葡萄糖摄取(图 I)和乳酸产生(图 J)方面起着至关重要的作用。如流式细胞仪分析所示，缺氧和 HIF-1a 的缺失，降低了 CD8+CTL 的存活率(图 K)。

Phenotypic switch of CD8+ T cells reactivated under hypoxia toward IL‐10 secreting, poorly proliferative effector cellsCD8+ T

低氧条件下 CD8+T 细胞向分泌 IL-10、增殖能力差的效应细胞的表型转换

文章出处：Eur. J. Immunol. 2015 Aug;458(8) ；10.1002/eji.201445284；日内瓦大学医学院
工作站使用情况：In Vivo2 300

Abstract

CD8+ T cells controlling pathogens or tumors must function at sites where oxygen tension is frequently low, and never as high as under atmospheric culture conditions. However, T‐cell function in vivo is generally analyzed indirectly, or is extrapolated from in vitro studies under nonphysiologic oxygen tensions. In this study, we delineate the role of physiologic and pathologic oxygen tension in vitro during reactivation and differentiation of tumor‐specific CD8+ T cells. Using CD8+ T cells from pmel‐1 mice, we observed that the generation of CTLs under 5% O_2, which corresponds to physioxia in lymph nodes, gave rise to a higher effector signature than those generated under atmospheric oxygen fractions (21% O_2). Hypoxia (1% O_2) did not modify cytotoxicity, but decreasing O_2 tensions during CTL and CD8+ tumor‐infiltrating lymphocyte reactivation dose‐dependently decreased proliferation, induced secretion of the immunosuppressive cytokine IL‐10, and upregulated the expression of CD137 (4‐1BB) and CD25. Overall, our data indicate that oxygen tension is a key regulator of CD8+ T‐cell function and fate and suggest that IL‐10 release may be an unanticipated component of CD8+ T cell‐mediated immune responses in most in vivo microenvironments.

主要内容：在体内 CD8+T 细胞在氧分压较低的地方发挥作用，故了解缺氧对 CD8+T 细胞影响是预测体内免疫功能的重要一步。分别在体外模拟生理氧和病理氧分压对 CD8+T 细胞重新激活和分化过程中的作用研究发现，与在大气氧含量(21% O_2)下产生的 CTL 相比，在 5% O_2(对应于淋巴结中的生理氧）下产生的效应信号更高；低氧(1% O_2)不会改变细胞毒性，但随着 O2 降低会依赖性地降低细胞增殖，诱导免疫抑制细胞因子 IL - 10 的分泌，并上调 CD137 和 CD25 的表达。

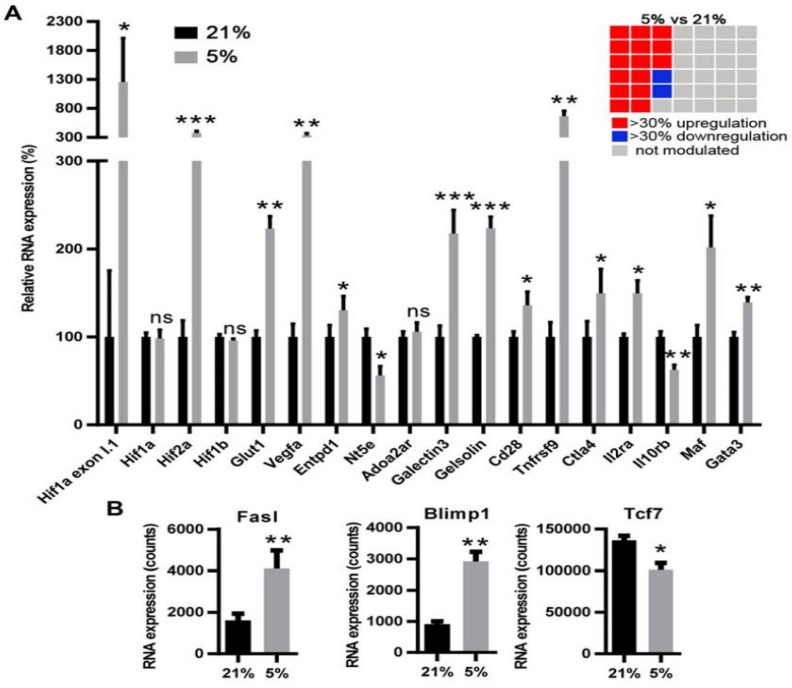

Figure 1. CTLs generated under physioxia have a higher effector profile than those generated under atmospheric oxygen fraction. (A and B) CTLs from Pmel-1 splenocytes were generated under 21% (black bars) or 5% O_2 (gray bars) and were analyzed for RNA expression. Results represent (A) the mean relative gene expression or (B) the RNA absolute count + SEM of five independent experiments (n = 5). The checkerboard represents the 42 genes analyzed (only genes modulated by more than 30% with a $p < 0.05$ are colored in red or blue).

对一组 42 个基因与低氧、免疫功能或参与细胞生存的基因进行研究发现，一些与 CD8+ T 细胞存活和扩张正相关的基因被上调，包括 gata3、il2ra 和 cd28；与细胞凋亡增加和免疫调节 5 相关的 maf 和 ctla4 也被上调。在空气中氧浓度状态下产生的 CLT 表现出更高的效应，blimp1 和 fasl 的上调和 tcf7 的下调可以证明这一点。

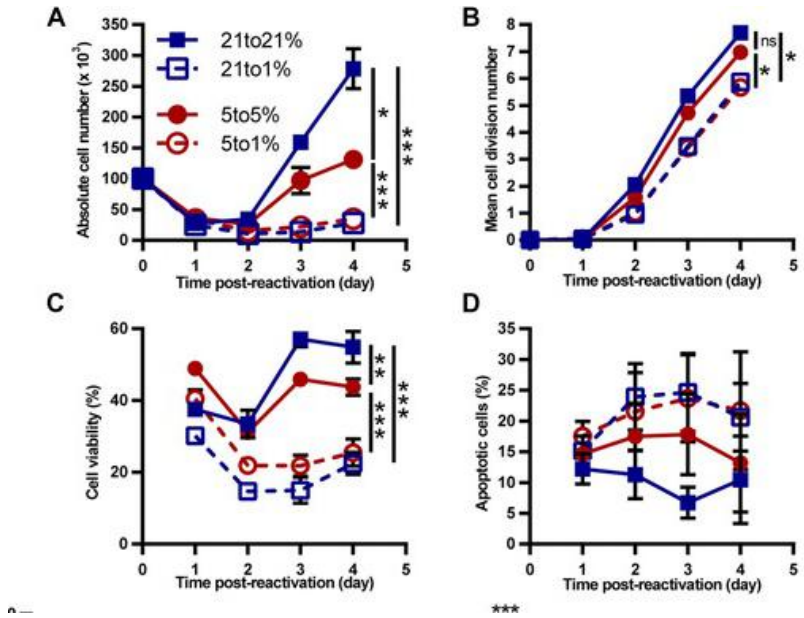

Figure 2. Hypoxia modulates expansion and RNA profile of reactivated CTLs. CTLs generated under 21% (squares) or 5% (circles) O_2 from Pmel-1 splenocytes were reactivated for indicated times under 21% O_2 (closed squares with solid line), 5% O_2 (closed circles with solid line) or 1% O_2 (open squares or open circles with dashed line). Results show (A) mean cell number, (B) mean cell division number, (C) mean cell viability, and (D) mean apoptotic cells ± SEM out of at least three independent experiments (n≥3). CTLs generated under (E) 21% or (F) 5% O_2 from Pmel-1 splenocytes were reactivated for two days under indicated oxygen fractions. (E) RNA profile from CTLs reactivated under 21% (black bars) or 1% O2(gray bars). (F) RNA profile from CTLs reactivated under 5% (black histograms) or 1% O_2 (gray histograms). Results represent the mean relative gene expression+ SEM out of four independent experiments (n = 4). The checkerboard represents the 42 genes analyzed (only genes modulated by more than 30% with ap < 0.05 are colored in red or blue). To display common genes modulated under each condition, genes composing the checkerboard are organized identically (in an arbitrary fashion). ns: not statistically significant, *p < 0.05, **p < 0.01, *** p <0.001 (A–D: Student's t-test; E, F: Three-way ANOVA)

通过平均细胞数、平均细胞分裂数、平均细胞存活率及平均凋亡细胞数可知，缺氧减少了重新激活的 CTL 的扩增。

Hypoxia Induced Impairment of NK Cell Cytotoxicity against Multiple Myeloma Can Be Overcome by IL-2 Activation of the NK Cells

NK 细胞 IL-2 活化可克服缺氧性 NK 细胞对多发性骨髓瘤的杀伤作用

文章出处：PLOS ONE. 2013；10.1371/journal.pone.0064835；荷兰马斯特里赫特大学医学中心

工作站使用情况：In Vivo$_2$ 1000

BACKGROUND:Multiple Myeloma (MM) is an incurable plasma cell malignancy residing within the bone marrow (BM). We aim to develop allogeneic Natural Killer (NK) cell immunotherapy for MM. As the BM contains hypoxic regions and the tumor environment can be immunosuppressive, we hypothesized that hypoxia inhibits NK cell anti-MM responses.

METHODS:NK cells were isolated from healthy donors by negative selection and NK cell function and phenotype were examined at oxygen levels representative of hypoxic BM using flowcytometry. Additionally, NK cells were activated with IL-2 to enhance NK cell cytotoxicity under hypoxia.

RESULTS: Hypoxia reduced NK cell killing of MM cell lines in an oxygen dependent manner. Under hypoxia, NK cells maintained their ability to degranulate in response to target cells, though, the percentage of degranulating NK cells was slightly reduced. Adaptation of NK- or MM cells to hypoxia was not required, hence, the oxygen level during the killing process was critical. Hypoxia

did not alter surface expression of NK cell ligands (HLA-ABC, -E, MICA/B and ULBP1-2) and receptors (KIR, NKG2A/C, DNAM-1, NCRs and 2B4). It did, however, decrease expression of the activating NKG2D receptor and of intracellular perforin and granzyme B. Pre-activation of NK cells by IL-2 abrogated the detrimental effects of hypoxia and increased NKG2D expression. This emphasized that activated NK cells can mediate anti-MM effects, even under hypoxic conditions.

CONCLUSIONS: Hypoxia abolishes the killing potential of NK cells against multiple myeloma, which can be restored by IL-2 activation. Our study shows that for the design of NK cell-based immunotherapy it is necessary to study biological interactions between NK- and tumor cells also under hypoxic conditions.

主要内容：缺氧是大多数肿瘤组织的特征，可以改变不同免疫细胞的功能，研究发现缺氧可消除 NK 细胞对多发性骨髓瘤的杀伤作用，故为了设计基于 NK 细胞的免疫治疗，也有必要研究 NK 细胞与肿瘤细胞在低氧条件下的相互作用。

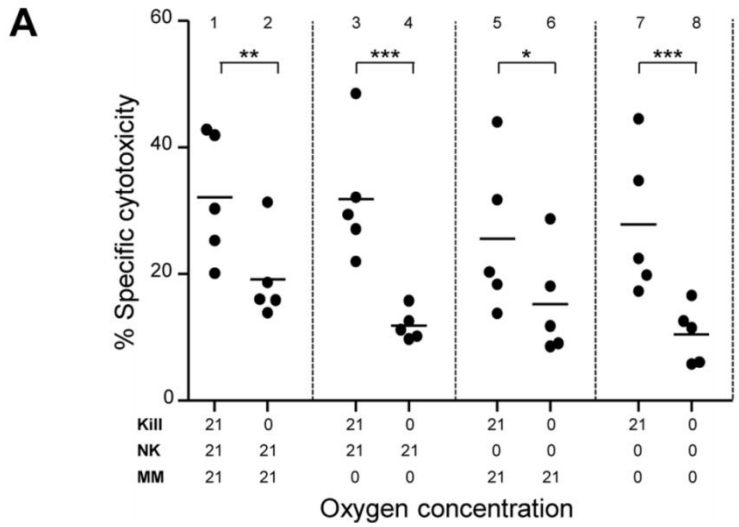

Figure 4. Oxygen concentration during NK cell killing is the key regulating parameter determining the cytotoxic potential. (A) Preincubation (16 hours) of MM- and NK cells followed by 4.5 hour assessment of cytotoxicity were performed at the O2 concentration depicted on the x-axis. Each dot represents mean of triplicate cultures of individual donors. Statistics were performed with one-way repeated measures ANOVA with Bonferroni correction * p,0.05, ** p,0.01, *** p,0.001.

将 NK 细胞、MM 细胞或两者都预先孵育在 21%或 0%的 O_2 中，并将它们组合在 21%或 0% O_2 中进行细胞毒性评估。在 0% O_2 中预孵育的 NK 细胞不影响其杀伤活性(图中第 3 组和第 5 组)。相反，在 0% O_2 条件下进行细胞毒试验(第 2、4、6 和 8 组)，与在 21% O_2(第 1、3、5 和 7 组)中进行细胞毒试验相比，均显示 NK 细胞的细胞毒性降低。