低氧/厌氧产品案例——3D 细胞培养

文章题目：Differential anti-tumour effects of MTH1 inhibitors in patient-derived 3D colorectal cancer cultures

MTH1 抑制剂对患者来源的结肠癌细胞 3D 培养的不同抗肿瘤效果

文章出处 Scientific reports, 9(1), 819.

荷兰乌得勒支实验室转化肿瘤学院；乌得勒支癌症中心

工作站使用情况及使用气体浓度：RUSKINN Invio2;低氧（0.1 % O_2, 5 % O_2）

主要内容：活性氧(ROS)作为信号转导的第二信使，但高水平的活性氧可导致细胞死亡。MTH 1 使氧化的核苷酸去磷酸化，保护肿瘤细胞免受氧化 DNA 损伤。MTH1 抑制剂 TH588 和(S)-crizotinib（S-克唑替尼）可抑制癌细胞增殖。然而，最近这些 MTH1 依赖性的药物抗癌作用遭受到质疑。本研究采用低氧/复氧诱发细胞氧化损伤，进而评估 TH588 和 S 克唑替尼在患者来源的结直肠癌 3D 培养中的抗肿瘤作用。研究发现缺氧/复氧可增加细胞内 ROS 水平，增加对 S-克唑替尼的敏感性，但对 TH588 没有影响。此外我们发现这两种化合物诱导的 DNA 损伤是添加 ROS 抑制剂 N-乙酰-L-半胱氨酸所不能阻止的。因此得出结论，TH588 和 S-克唑替尼在 3D 结直肠癌培养中具有非常明确和独特的抗肿瘤作用，这些作用很可能是通过非 ROS 依赖机制发生的。

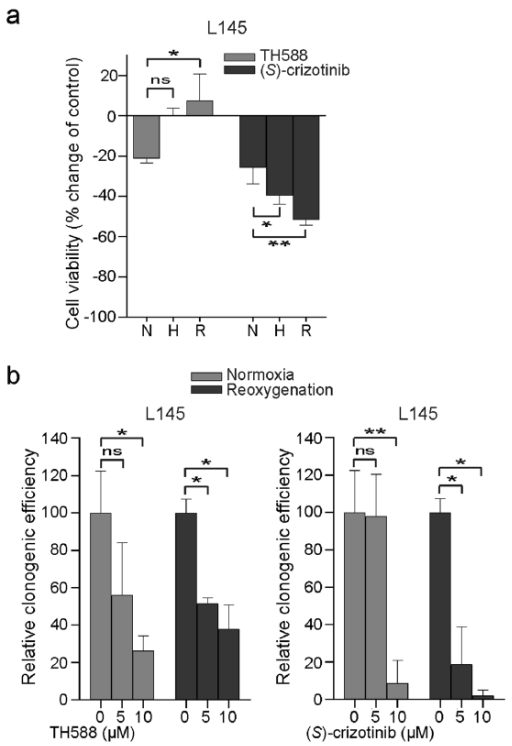
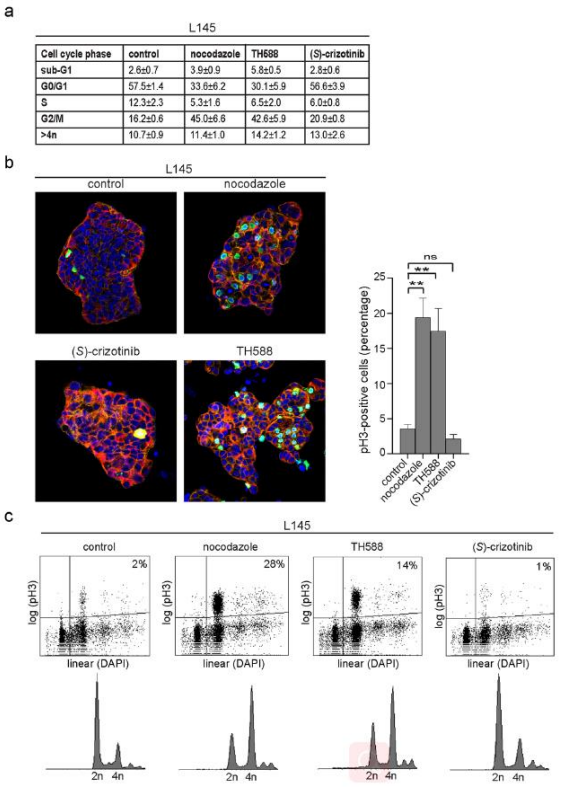


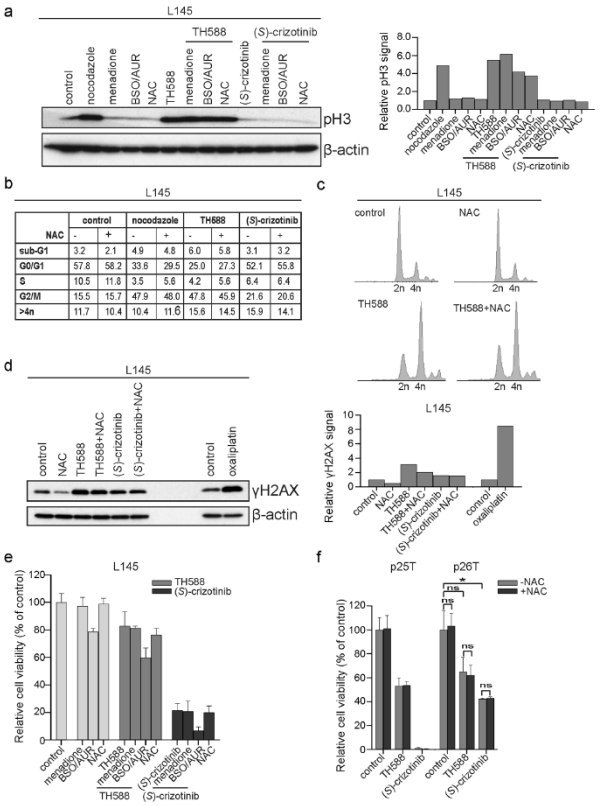
Figure 2. (S)-crizotinib sensitivity is enhanced during hypoxia and after reoxygenation whereas colorectal cancer spheroids are not sensitized to TH588 under these conditions. (a) Graph showing percentage change in cell viability after a 3 d treatment with 10 μM of TH588 or (S)-crizotinib compared to DMSO-treated controls.Human L145 CRC spheroids were cultured under normoxia (21% O_2) or transferred to a hypoxia chamber (0.1% O_2). After 72 h, CRC spheroids were either maintained under hypoxia (hypoxia) or returned to normoxia (reoxygenation). H, hypoxia; N, normoxia; R, reoxygenation.

将患者来源的结肠癌 3D 成球细胞系 L145 在低氧(0.1% O_2)条件下培养 24 h，然后复氧 4 h。研究发现低氧及低氧/复氧可增加 L145 细胞氧化损伤。继续将 L145 细胞用 MTH1 抑制剂 TH588 和(S)-crizotinib 处理暴露于低氧及复氧条件下，研究发现低氧和复氧增强了(S)-crizotinib 对 L145 细胞球的增殖及克隆形成的抑制作用，即低氧及复氧增加了 L145 细胞球对(S)-crizotinib 的敏感性，但不增加对 TH588 的敏感性（图 2）。



Cell cycle phase	control	nocodazole	TH588	(S)-crizotinib
sub-G1	2.6±0.7	3.9±0.9	5.8±0.5	2.8±0.6
G0/G1	57.5±1.4	33.6±6.2	30.1±5.9	56.6±3.9
S	12.3±2.3	5.3±1.6	6.5±2.0	6.0±0.8
G2/M	16.2±0.6	45.0±6.6	42.6±5.9	20.9±0.8
>4n	10.7±0.9	11.4±1.0	14.2±1.2	13.0±2.6

Figure 3. TH588 induces a mitotic arrest in human CRC spheroids. (a) Table showing the distribution of cells at the various phases of the cell cycle as determined by flow cytometry using DAPI staining. The values represent the mean percentage + s.d. and represent data from three independent experiments. (b) Immunofluorescent staining of the mitotic marker phospho-histone H3 (green), tubulin (red), F-actin (orange) and DAPI (blue) (left) and quantification of phospho-histone H3-positive cells (right). One representative Z-stack per condition is shown. A minimal of 1300 nuclei per condition were analysed. (c) Flow cytometryanalysis of DNA content (DAPI staining) and mitotic cell population (phospho-histone H3) of CRC spheroids after exposure to the indicated drugs or DMSO (control) for 1 d.



	control		nocodazole		TH588		(S)-crizotinib	
NAC	-	+	-	+	-	+	-	+
sub-G1	3.2	2.1	4.9	4.8	6.0	5.8	3.1	3.2
G0/G1	57.8	58.2	33.6	29.5	25.0	27.3	52.1	55.8
S	10.5	11.8	3.5	5.6	4.2	5.6	6.4	6.4
G2/M	15.5	15.7	47.9	48.0	47.8	45.9	21.6	20.6
>4n	11.7	10.4	10.4	11.6	15.6	14.5	15.9	14.1

Figure 5. ROS-modulating compounds do not alter sensitivity to TH588 and (S)-crizotinib. (a) Western blot analysis of phospho-histone H3 protein levels in L145 CRC spheroids following 1d treatment with TH588 or (S)-crizotinib combined with the ROS-scavenging compound NAC or ROS-inducing compounds BSO/AUR or menadione, (b) Table showing the distribution of L145 cells at the various phases of the cell cycle after 1 d treatment as determined by flow cytometry using DAPI staining. Some data (conditions without NAC) of Fig. 3a was reused in this table. (c) Flow cytometry plots of DAPI staining of L145 spheroids DMSO-treated or exposed to TH588 in the presence or absence of 0.5 mM NAC (logarithmic scale). (d) Western blot analysis of γH2AX levels following treatment with TH588 or (S)-crizotinib (1 d) in the presence or absence of NAC.L145 CRC spheroids exposed to two cycles of oxaliplatin were used as positive control (e) Analysis of L145 CRC spheroid viability following treatment with TH588 or (S)-crizotinib in the ± ROS-modulators. (f) Analysis of cell viabilityof organoid cultures after a 3 d treatment with 5 μM of TH588 or (S)-crizotinib in the presence or absence.

TH588 可诱导人结直肠癌细胞球 L145 有丝分裂停止,将细胞阻滞在 G2/M 期（图 3）；ROS 抑制剂 N-乙酰-L-半胱氨酸 NAC 可抑制细胞氧化损伤，但不能阻止 TH588 及(S)-crizotinib 诱发的人结直肠癌细胞球的氧化损伤（图 5），表明 TH588 和 S-克唑替尼在 3D 结直肠癌培养中的作用机制是不依赖 ROS 途径的。