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利用奈米修飾病毒CRISPR/Cas9技術克服非小細胞肺癌EGFR標靶治療之抗藥性 (3/3)

Using nano-modified virus CRISPR/Cas9 to overcome EGFR-TKI resistance in NSCLC (3/3)

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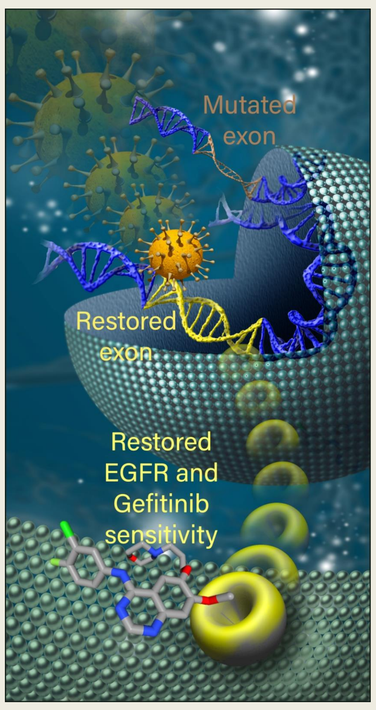
CRISPR as gene editing

The emergence of drug resistance to tyrosine kinase inhibitors (TKIs) has hindered the therapeutic outcomes for NSCLC patients. In this study, we have developed an approach using recombinant adeno-associated virus serotype 5 (rAAV5) to deliver both the clustered regularly interspaced short palindromic repeats (CRISPR)-associated 9 (Cas9)^{D10A} protein and adenine base editor (ABE) for the purpose of repairing TKI resistance-associated mutations. Following infection with rAAV5, NSCLC cells regain sensitivity to TKIs, offering a potential solution to combat drug resistance in NSCLC



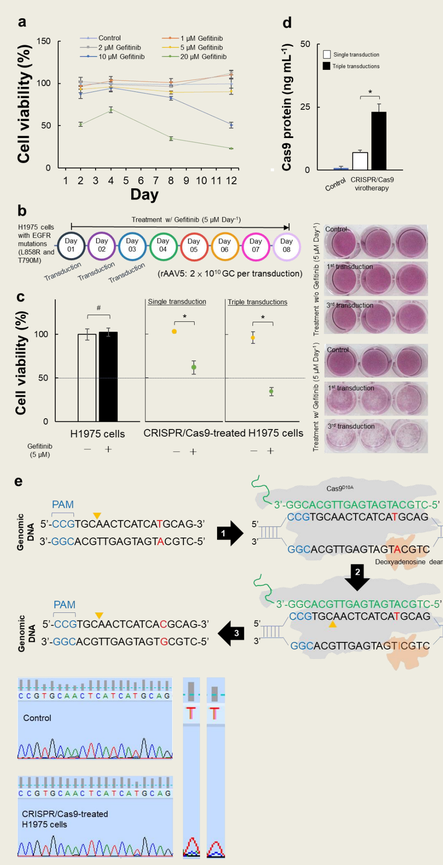


Figure 1. *In vitro* CRISPR/Cas9 virotherapy in NSCLC with EGFR-TKIs resistant. (a) Cell viability of H1975 cells after exposure to various concentration (μM) of Gefitinib over period. Cell viability is given as the percentage of viable cells remaining after treatment for various incubation periods measured by CCK-8 assay and compared against the unexposed. The bars represent the mean ± standard deviation (n = 6). (b) CRISPR/Cas9 treatment protocol for *in vitro* H1975 cells with various transduction conditions. (c) Cell viability of H1975 cells after exposure to various treatments. Cell viability is given as the percentage of viable cells remaining w/o or w/ the CRISPR/Cas9 virotherapy under exposed to Gefitinib measured by the MTS assay and compared against the unexposed ($^{\#}$, $P > 0.5$; *, $P < 0.05$; based on a two-tailed t test, assuming unequal variances). The bars represent the mean ± standard deviation (n = 6). Right panel, pararosaniline-stained photographs of H1975 cells after various treatments. (d) Qualitative determination of Cas9 protein in H1975 cells treated with single or triple transductions (*, $P < 0.05$; based on a two-tailed t test, assuming unequal variances). The bars represent the mean ± standard deviation (n = 6). (e) Schematic diagram of on-target (790, exon 20) adenine conversion mediated by the catalysis activity of an ABE. Step one: genomic DNA binding with Ca9^{D10A} and opening; step two: deamination of target A and nicking of top strand; step three: genomic DNA replication or repair. Lower panel: the target sequences in exon 20 from H1975 cells after CRISPR/Cas9 treatment at Day 8.

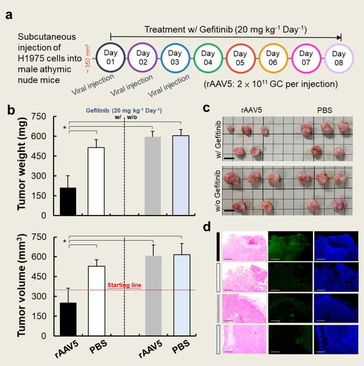
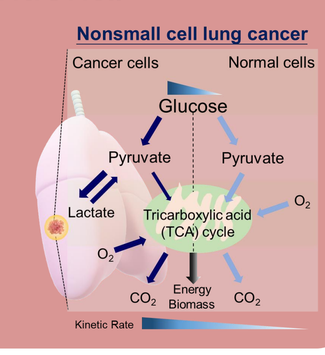


Figure 2. *In vivo* CRISPR virotherapy. (a) Treatment protocol for mice with H1975 xenografts treated with various conditions. (b) Tumor weight (mg) or tumor volume (mm^3) of various conditions-treated H1975 (EGFR$^{L858R/T790M}$) xenograft tumors *via* intratumoral injection. Tumor weights of mice were measured at Day 8 (*, $P < 0.05$; based on a two-tailed t test, assuming unequal variances). Tumor sizes were measured by a caliper on the described days (*, $P < 0.05$; based on a two-tailed t test, assuming unequal variances). Bars represent the mean ± standard deviation (n = 5). (c) Representative xenograft NSCLC tumors after various treatments. Bar = 1 cm. (d) Representative images of tumor sections from mice in each group (n = 5) after various treatments at Day 8 were stained with hematoxylin and eosin (H&E), terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) assay (green fluorescence), or DAPI (blue fluorescence). Bars = 500 μm.

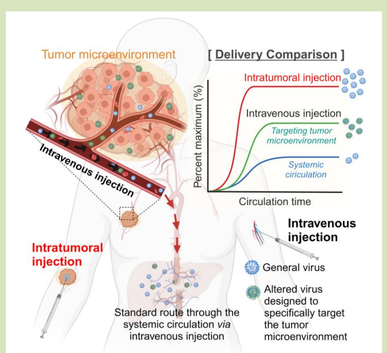
Potential therapeutics using tumor-secreted lactate in NSCLC

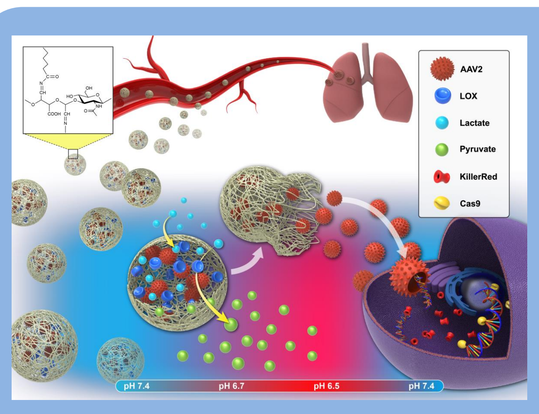
Non-small cell lung cancer (NSCLC) frequently presents targeted-drug resistance and lactate accumulation. Can lactate oxidation be exploited for enhancing targeted delivery and promoting an immune response for better clinical outcomes?

Targeted-therapy failure in treating NSCLC frequently occurs because of the emergence of drug resistance and genetic mutations. The same mutations also result in aerobic glycolysis, which further antagonizes outcomes by localized increases in lactate, an immune suppressor. Recent evidence indicates that enzymatic lowering of lactate can promote an oncolytic immune microenvironment within the tumour. Here, we review factors relating to lactate expression in NSCLC and the utility of lactate oxidase (LOX) for governing therapeutic delivery, its role in lactate oxidation and turnover, and relationships between lactate depletion and immune cell populations. The lactate-rich characteristic of NSCLC provides an exploitable property to potentially improve NSCLC outcomes and design new therapeutic strategies to integrate with conventional therapies.



In 2015, oncolytic virotherapy gained clinical approval, followed by recombinant AAV delivery approval in 2017. However, systemic administration faces challenges, with limited viruses reaching target sites. While the FDA permits higher AAV doses for improved transduction rates, most accumulates in the liver, raising toxicity concerns. Targeting the tumor microenvironment offers promise by enhancing specificity and efficacy. Recent findings support approaches such as modifying viral surfaces, modulating the immune system, and optimizing tumor microenvironment characteristics. Targeting tumor acidity shows potential for improved virotherapy outcomes and integration with standard treatments.





Approved targeted therapies for NSCLC though initially effective, often eventually fail due to emergence of drug resistance. This evolution is frequently associated with specific driver mutations and greater reliance on aerobic glycolysis, which can increase lactate production in the tumor microenvironment (TME). Meanwhile, oncolytic virotherapy has been approved since 2015, however systemic administration remains particularly challenging and viral delivery for the genome-editing tool of CRISPR system has continued to elicit major concerns due to off targeting. Nevertheless, sophisticated yet robust formulation of viral therapeutics stands to revolutionize their specificity. Notably, physico-chemical properties such as acidosis can regulate viral release and additionally promotes viral transduction. Herein, we report exploitation of NSCLC tumor-secreted lactate in designing an acid-degradable nanoparticle containing the acyclic acetal component of oxidized hyaluronic acid (HA) for release of virus. The virus, LOX, and hexanoamide are conjugated with aldehyde-HA through reductive amination. LOX catalyzes the oxidation of lactate to pyruvate, modulating a localized lowering of pH and triggering destabilization of the acyclic component-based nanoparticles. Site-specific delivery is proven by viral transduction in the NSCLC tumor-secreted lactate microenvironment, offering an avenue for improving drug-resistant NSCLC outcomes.

●Potential targeting of the tumor microenvironment to improve cancer virotherapy. ***Pharmacology & Therapeutics***. **2023**, 250, 10851.
●An acid degradable, lactate oxidizing nanoparticle formulation for non-small cell lung cancer virotherapy. ***Nano Today***. **2022**, 46, 101582.
●Enhanced targeting and immune activation of tumor microenvironment by nanomodified anti-PD1 in liver cancer. ***Advanced Therapeutics***. **2021**, 4, 2100048.
●Potential therapeutics using tumor-secreted lactate in nonsmall cell lung cancer. ***Drug Discovery Today***. **2021**, 26, 2508-2514.