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COMMENTARY

# From single-thread delivery to cascading therapy: The promise of nanoCRISPR in solid tumors

**KEY WORDS**

CRISPR/Cas9;
Gene editing;
Cancer therapy;
Delivery system;
Deep penetration

The CRISPR/Cas9 system is revolutionizing genetic medicine by enabling programmable genome editing with unparalleled precision and versatility[1]. Its therapeutic potential in oncology is particularly compelling, as targeted disruption of oncogenes or driver mutations could directly eliminate malignant cells[2]. However, translating CRISPR/Cas9-based therapeutics into effective treatments for solid tumors has been hindered by formidable biological barriers, notably the dense and heterogeneous tumor microenvironment (TME) that restricts vehicle diffusion and gene-editing efficiency[3]. Conventional nanocarriers primarily rely on paracellular transport routes, which remain ineffective in overcoming the structural and compositional heterogeneity of tumor stroma[4,5]. As a result, editing tends to be confined to superficial tumor regions, leaving deeper cancer cells largely unmodified and compromising overall therapeutic outcomes[6].

In a recent study published in *Acta Pharmaceutica Sinica B*, Liu and colleagues[7] reported the development of nanoCRISPR, a self-cascade nanoplatform designed to achieve robust gene editing and sustained tumor eradication through a unique transcellular penetration mechanism. This system simultaneously addresses the dual challenge of limited tumor penetration and uneven transfection efficiency by integrating programmable self-cascading behavior within a biocompatible nanostructure. The authors rationally designed nanoCRISPR to activate its self-cascade only upon encountering the matrix metallopeptidase 2 (MMP2)-enriched TME, thus ensuring spatial precision and minimizing off-target effects. Once triggered, nanoCRISPR initiates a synergistic cycle between CRISPR/Cas9-mediated apoptosis and transcellular transport, allowing apoptotic cells to facilitate further nanoparticle dissemination and gene transfer into neighboring tumor cells. This closed-loop "penetration−apoptosis−re-penetration" process represents a conceptual advance in CRISPR-based nanotherapeutics, transforming a traditionally single-pass system into a dynamic, self-propagating treatment modality.

Upon activation by MMP2, nanoCRISPR demonstrates dual internalization modalities: CD44 receptor-mediated endocytosis and TAT-assisted membrane perforation. Confocal microscopy and flow cytometry confirmed that this dual-entry strategy enables efficient cellular uptake even under serum-rich or endocytosis-inhibited conditions, underscoring the robustness of transcellular transport. Notably, nanoCRISPR achieved a transfection efficiency of up to 86% under physiological serum conditions, markedly surpassing that of the commercial reagent Lipofectamine 3000. These results suggest that the self-cascade platform achieves both high stability and superior gene delivery performance in biologically relevant conditions.

Functionally, nanoCRISPR targets the BCL-2/BAX apoptosis axis by co-delivering CRISPR/Cas9 plasmids for BCL-2 knockout (pCRISPR) and pro-apoptotic Bax plasmids (pBax). The system achieves approximately 17-fold higher BCL-2 editing efficiency and 130-fold Bax mRNA upregulation compared with conventional transfection approaches, indicating precise and effective pathway modulation. The coordinated regulation of these pathways induces potent apoptosis and proliferation inhibition in cancer cells under simulated TME conditions. Importantly, the induced apoptosis further enhances transcellular penetration, initiating a positive feedback loop that amplifies gene editing across neighboring tumor cells. Multi-round transfection and apoptosis assays demonstrated sustained activity, with second-round efficiencies of ∼48% and ∼27%, respectively— clear indicators of the system's self-cascading behavior.

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The authors extended these findings to 3D tumor spheroid models and xenograft-bearing mice, confirming that nanoCRISPR achieves homogeneous intratumoral distribution and robust anti-tumor efficacy. *In vivo* imaging revealed preferential tumor accumulation within 12−24 h post-injection, consistent with active targeting and efficient penetration. Deep sequencing analysis of potential off-target loci in major organs showed negligible mutation frequencies (<1%), validating the genomic safety of the CRISPR/Cas9 component. Clinically, immunohistochemical evaluation of 48 human cervical squamous cell carcinoma samples revealed a compensatory imbalance in the BCL-2/BAX axis—characterized by high BCL-2 expression coinciding with low Bax levels—emphasizing the clinical relevance of dual-pathway regulation.

In murine tumor models, nanoCRISPR treatment achieved an impressive tumor growth inhibition rate of approximately 83%, far outperforming monotherapy controls. Histological analyses confirmed comprehensive regulation of the apoptotic pathway, featuring reduced BCL-2 expression, elevated Bax and cleaved caspase-3/9 levels, and extensive apoptotic regions. Equally important, hematological and biochemical parameters remained within normal ranges, suggesting minimal systemic toxicity and favorable translational potential.

The study by Liu et al.[7] marks a pivotal advancement in CRISPR-based nanomedicine by shifting the paradigm from passive delivery toward self-sustaining, active penetration. The introduction of a self-cascade mechanism—where gene editing-induced apoptosis actively enhances subsequent nanoparticle infiltration—overcomes one of the most persistent challenges in solid tumor therapy: the limited reach of therapeutic agents within heterogeneous tumor tissues. Unlike conventional strategies that focus solely on interstitial diffusion or extracellular matrix degradation, nanoCRISPR leverages intrinsic cellular processes to drive its own propagation, achieving uniform editing and apoptosis throughout the tumor mass.

Looking ahead, this self-cascade nanoCRISPR concept holds broad implications beyond oncology. The transcellular cascade mechanism could be adapted to other gene-editing platforms, such as base or prime editors, and applied to fibrotic or neurological diseases characterized by dense extracellular matrices. The study by Liu and colleagues establishes a compelling proof-of-concept for a self-cascade gene-editing nanoplatform that unites efficient transcellular penetration, precise apoptotic regulation, and sustained anti-tumor efficacy. By transforming CRISPR/Cas9 delivery into a self-propagating therapeutic cycle, nanoCRISPR sets a conceptual foundation for next-generation precision nanomedicines capable of overcoming the long-standing barriers of solid tumor treatment.

## References

1. Li T, Yang Y, Qi H, Cui W, Zhang L, Fu X, et al. CRISPR/Cas9 therapeutics: progress and prospects. *Signal Transduct Targeted Ther* 2023;**8**:36.
2. Wang N, Liu C, Lu Z, Yang W, Li L, Gong S, et al. Multistage sensitive NanoCRISPR enable efficient intracellular disruption of immune checkpoints for robust innate and adaptive immune coactivation. *Adv Func Mater* 2020;**30**:2004940.
3. Song X, Liu C, Wang N, Huang H, He S, Gong C, et al. Delivery of CRISPR/Cas systems for cancer gene therapy and immunotherapy. *Adv Drug Deliv Rev* 2021;**168**:158−80.
4. Zhou Q, Dong C, Fan W, Jiang H, Xiang J, Qiu N, et al. Tumor extravasation and infiltration as barriers of nanomedicine for high efficacy: the current status and transcytosis strategy. *Biomaterials* 2020; **240**:119902.
5. Munir MU. Nanomedicine penetration to tumor: challenges, and advanced strategies to tackle this issue. *Cancers (Basel)* 2022;**14**:2904.
6. Rosenblum D, Gutkin A, Kedmi R, Ramishetti S, Veiga N, Jacobi AM, et al. CRISPR-Cas9 genome editing using targeted lipid nanoparticles for cancer therapy. *Sci Adv* 2020;**6**:eabc9450.
7. Liu C, Xu Y, Wang N, Liu H, Yang X, Zhou S, et al. A self-cascade nanoCRISPR prompts transcellular penetration to potentiate gene editing and tumor killing. *Acta Pharm Sin B* 2025;**15**:5933−44.

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