Targeted delivery of CRISPR/Cas9 protein complex by functional nanoparticle for fusion gene editing in cancer cells

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Introduction

Development of nanomedicine

Key is “Nanoplatform”

1) Discovery of a new nano-structured materials
2) Development of systems via a novel approach
3) Application in the field of unmet need

Research area of Nanomedicine

Publication No.

Year

Development of CRISPR/Cas9 protein complex delivery system

Overall strategy

TEM image of Nanocarriers

Regulation of GFP expression

<table>
<thead>
<tr>
<th>particle</th>
<th>BET surface area (m²/g)</th>
<th>Pore volume (mL/g)</th>
<th>Mean pore size (nm)</th>
<th>Zeta potential (mV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>npSi</td>
<td>1130</td>
<td>0.67</td>
<td>3.07</td>
<td>25.2</td>
</tr>
<tr>
<td>pSi</td>
<td>359.75</td>
<td>1.18</td>
<td>20.25</td>
<td>24.5</td>
</tr>
</tbody>
</table>

(unpublished data)
Regulation of target gene *in vitro*

**Cell viability**

(a) Cell viability (%)

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Lipo only</th>
<th>pSi only</th>
<th>TACC3/Lipo</th>
<th>TACC3@pSi</th>
<th>FGFR3/Lipo</th>
<th>FGFR3@pSi</th>
</tr>
</thead>
<tbody>
<tr>
<td>cas9 delivery</td>
<td><img src="image1" alt="Graph" /></td>
<td><img src="image2" alt="Graph" /></td>
<td><img src="image3" alt="Graph" /></td>
<td><img src="image4" alt="Graph" /></td>
<td><img src="image5" alt="Graph" /></td>
<td><img src="image6" alt="Graph" /></td>
<td><img src="image7" alt="Graph" /></td>
</tr>
</tbody>
</table>

(b) Relative Caspase 3/7 activity

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<td><img src="image9" alt="Graph" /></td>
<td><img src="image10" alt="Graph" /></td>
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<td><img src="image13" alt="Graph" /></td>
<td><img src="image14" alt="Graph" /></td>
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</tbody>
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**RT-PCR for mRNA Lv.**

(a) GDH, TACC3, FGFR3

(b) Relative Expression Lv.

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<tbody>
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<td><img src="image16" alt="Graph" /></td>
<td><img src="image17" alt="Graph" /></td>
<td><img src="image18" alt="Graph" /></td>
<td><img src="image19" alt="Graph" /></td>
<td><img src="image20" alt="Graph" /></td>
<td><img src="image21" alt="Graph" /></td>
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**Western blot for Protein Lv.**

(a) TACC3, GAPDH

(b) FGFR3, GAPDH

(unpublished data)
Regulation of target gene *in vivo*

Anticancer effect in HeLa-tumor bearing mice

(a) Drug injection scheme

(b) Tumor samples

(c) Relative tumor volume over time

Histological study

- **Cas9@pSi**
- **Cas9/Lipo**
- **pSi only**
- **Control**

*(unpublished data)*
Biodistribution in vivo

Real-time imaging of Cas9 protein

- Control
  - pre
  - 1 hr
  - 3 hrs
  - 6 hrs
  - 9 hrs
  - 24 hrs
  - 48 hrs

- Cas9
  - pre
  - 1 hr
  - 3 hrs
  - 6 hrs
  - 9 hrs
  - 24 hrs
  - 48 hrs

- Cas9@pSi
  - pre
  - 1 hr
  - 3 hrs
  - 6 hrs
  - 9 hrs
  - 24 hrs
  - 48 hrs

Real-time imaging of Nanocarrier

- Control
  - pre
  - 3 hrs
  - 6 hrs
  - 9 hrs
  - 24 hrs
  - 36 hrs
  - 72 hrs

- pSi
  - pre
  - 3 hrs
  - 6 hrs
  - 9 hrs
  - 24 hrs
  - 36 hrs
  - 72 hrs

(unpublished data)
In this study, we reported a novel therapeutic platform based on CRISPR/Cas9 protein complex for targeted cancer treatment. We developed an efficient protein delivery system based on silica nanocarrier with expanded pores.

The present work is the first demonstration of a Cas9 protein-based gene editing approach both in vitro and in vivo, with high gene regulation efficacy, low cytotoxicity and immune response achieved by the systemic administration.

We believe that the studies can provide a strong foundation for basic research in the field of nanomedicine and the long-term technical progress of gene therapy into an effective clinical application.
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Dongkap Kim

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Rashid Tonmoy
Thank you for your attention