Novel bacterial genotoxin-loaded nanoparticles for targeting therapy of radioresistant prostate cancer

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Abstract

Cytolethal distending toxin subunit B (CdtB) is a genotoxin acts as a type I deoxyribonuclease (DNase I), which is responsible for creating DNA double-strand breaks (DSBs). Nanoparticles loaded with antitumor drugs and specific ligands that recognize cancerous cell receptors are promising methods to overcome the therapeutic challenges. In this study we employed HA-decorated nanoparticles-encapsulated CdtB (HA-CdtB-NPs) and explored the targeted therapeutic activity in radioresistant PCa cells. Our results showed that HA-CdtB-NPs sensitized radioresistant PCa cells by enhancing DSB and causing G2/M cell-cycle arrest. The results demonstrate that HA-CdtB-NPs possess maximum target-specificity and delivery efficiency of CdtB into the nucleus, thereby enhancing the effect of radiation in radioresistant PCa cells. These findings indicate that HA-loaded CdtB nanoparticles can be developed as an effective agent for overcoming radioresistance in PCa.

Results

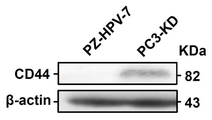

Figure 1. CD44 expression level in PC3-KD cells. CD44 expression levels in PZ-HPV-7 and PC3-KD cells were analyzed by western blot assay.

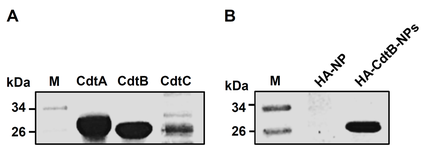

Figure 2. Preparation of HA-CdtB-NPs. SDS-PAGE analysis of (A) each recombinant CDT subunit and (B) HA-CdtB-NPs.

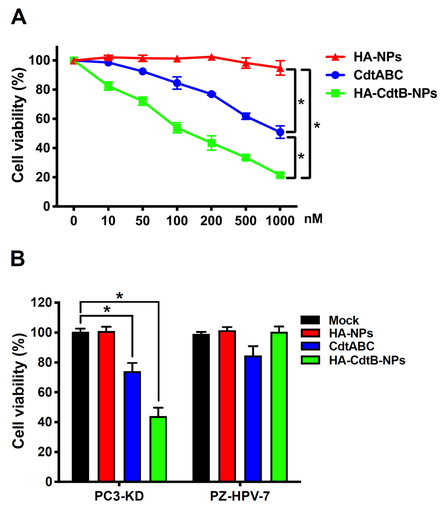

Figure 3. HA-CdtB-NPs inhibit PCa cell proliferation. (A) PC3-KD cells were treated with HA-NPs, CDT holotoxin or HA-CdtB-NPs at the indicated concentrations (0-1000 nM) for 48 h. (B) PZ-HPV-7 and PC3-KD cells were treated with HA-NPs, CDT holotoxin or HA-CdtB-NPs at the concentration of 100 nM for 48 h. Cell viability was assessed using MTT assay. *, $p < 0.05$..

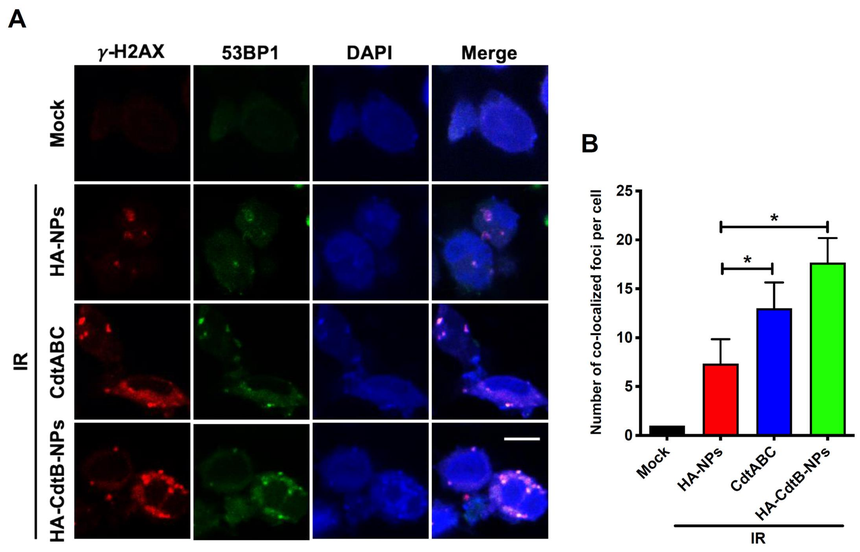

Figure 4. HA-CdtB-NPs promote DSB in radioresistant PCa cells. PC3-KD cells were mock-treated or exposed to IR (2 Gy) then incubated with 100 nM HA-NPs, CDT holotoxin, and HA-CdtB-NPs for 24 h. (A) Fluorescent immunostaining of γ-H2AX (red) and 53BP1 (green) was shown. DAPI (blue) was used as a tracer for cell nucleus. Scale, 10 μm. (B) The foci of γ-H2AX and 53BP1 colocalization in the nuclei were counted. *, $p < 0.05$.

Table 1. Loading efficiencies of HA-CdtB-NPs.

Hyaluronic acid (μg/mL)	Loading efficiency (%)
0	76.95
312.5	68.08
625	60.34
1250	58.15
2500	72.48
5000	65.23

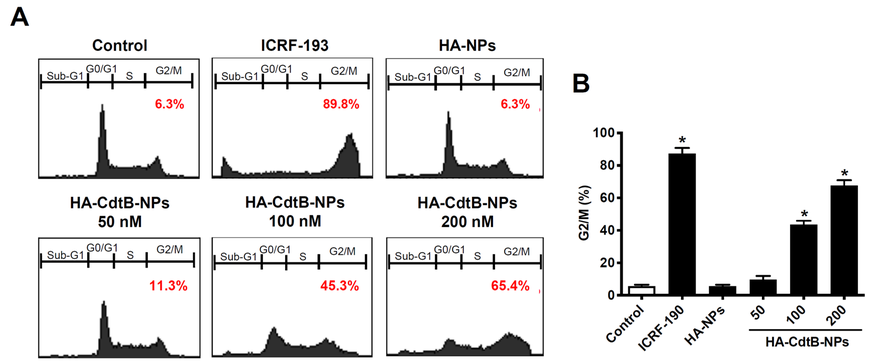

Figure 5. HA-CdtB-NPs arrest cell cycle. (A) PC3-KD cells were mock-treated, exposed to ICRF-193 or HA-CdtB-NPs (50, 100 and 200 nM) and incubated for 48 h. Cell cycle distribution based on DNA content was analyzed using flow cytometry. (B) The percentage of cells at G2/M phase were calculated. *, $p < 0.05$..

Conclusions

1. **Our results demonstrate that HA-CdtB-NPs possess activity similar to CDT holotoxin, but with superior advantages.**
2. **The potential effects include maximum target-specificity and delivery efficiency of CdtB into the nucleus, and enhancement of the effect of radiation in PCa cells.**

Acknowledgments: This study is supported by Taiwan Ministry of Science and Technology (108-2911-I-005-509, 109-2911-I-005-503), Chang Gung Memorial Hospital (CMRPD1I0061-3, CMRPD1J0021-3).

Author disclosure: Some results have been accepted by the journal *Biomedicines* and will be published in February 2021.