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Co-delivery of drug nanoparticles and siRNA mediated by a modified cell penetrating peptide for inhibiting cancer cell proliferation

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Co-delivery of anti-cancer agent, Ellipticine (EPT) and Bcl-2 siRNA were mediated by a modified cell penetrating peptide, stearylated H16R8 (STR-H16R8). The stability and efficacy of the EPT nanoparticles was improved significantly by the amphiphilic peptide. Efficacy of STR-H16R8 stabilized EPT nanoparticles was further enhanced with Bcl-2 siRNA.

Combinations of therapeutic agents with different mechanisms have been proved effective in the treatment of many classes of cancer due to their ability to affect multiple intracellular pathways[1]. Over the past decade, combinations of conventional chemotherapeutics and RNA interference (RNAi) with small interfering RNA (siRNA)[2] have also shown promise with synergistic effects on the treatment of cancer. However, co-delivery of anticancer drugs and siRNA is particularly challenging due to the differences in the physicochemical properties of the two types of agents. Therefore, it is still in the early stage and to date, only several types of carriers for drug-siRNA combination have been developed, such as polyplex and lipoplex[3].

Ellipticine (EPT), whose mode of action is based mainly on DNA intercalation and inhibition of topoisomerase II, has exhibited significant antitumor activities[4]. In terms of drug-siRNA combinations for cancer treatment, it has been proved that siRNA induced knockdown of the gene expressing Bcl-2 protein, a key regulator of cell apoptosis[5], can sensitize cancer cells to anticancer drugs[6].

Moreover, stability of nanoparticles (NPs) in aqueous medium still an issue due to the agglomerations[7]. Here, by taking all this knowledge into account, we therefore designed a novel delivery system based on a stearylated and oligohistidylated cell penetrating peptide (CPP), stearyl-(histidine)16-(arginine)8 (STR-H16R8), which was recently developed by us for highly efficient siRNA delivery[8]. The stabilized EPT nanoparticles were prepared by a solvent evaporation method. Afterwards, Bcl-2 siRNA was introduced into the drug nanosuspension to form complexes with STR-H16R8. Finally, the anti-proliferation effect of this co-delivery system was investigated on cancer cells.

In order to develop a NP delivery system intended for intravenous administration, stable NP dispersion without agglomeration in the vehicles are a prerequisite. In this study, though TEM imaging showed the presence of distinct spherical NPs (Fig. 1A), NPs prepared in water agglomerated significantly (Fig. 1B, right bottle), However, EPT NPs prepared in STR-H16R8 showed a stable nanosuspension (Fig. 1B, left bottle). DLS measurements also showed larger particles of the NPs without STR-H16R8 (Fig. 2A).

Figure 1

Figure 1. A) TEM imaging of EPT NPs in STR-H16R8 (STR-H16R8/EPT, 0.375: 1, molar ratio); B) Photographs of EPT NPs in STR-H16R8 (STR-H16R8/EPT, 0.375: 1, molar ratio) (left) and water (right) after preparation.

With the molar ratio of STR-H16R8/EPT of 0.0025: 1, particle size was reduced significantly to 420 nm (Fig. 2A). When the ratios increased from 0.015: 1 to 0.375: 1, the particle sizes decreased further from 215 nm to 92 nm. With the ratios of 0.75: 1 and 1.5: 1, the particle sizes were 90 and 91 nm, which were almost the same as that at the ratio of 0.375: 1, suggesting that the nanoparticles have been stabilized completely. The results indicated that amphiphilic STR-H16R8 stabilized EPT NPs effectively, leading to the nanosuspension with the particle size of ca. 90 nm. As shown in Fig. 2B, the ζ-potential of bare hybrid NPs was -16.4 mV, but increased to 12.1 mV at the ratio of 0.0025: 1. The ζ-potentials were further increased from 19.5 to 38.5 mV when the ratios altered from 0.15: 1 to 1.5: 1. Cationic STR-H16R8 did not aggregate, but was freely dissolved in water showing no ζ-potential[8] and therefore, the ζ-potential shifting from negative to positive charges confirmed that cationic STR-H16R8 anchored on the surface of the NPs.

Figure 2

Figure 2. (A) Particle size and (B) ζ-potential of EPT NPs in various STR-H16R8 solutions (n=5); (C) ζ-potential and particle size (D) of EPT NPs/STR-H16R8/siRNA in water (n=5).

EPT is practically insoluble in water with the solubility of 0.62 μM[9], but there would be still one or some electronegative groups in

this molecule, so the zeta potential of EPT NPs was negative. Normally, the nanoparticles with zeta potentials of less than -30 mV or more than 30 mV are stable in water due to strong electrostatic repulsion. As EPT nanoparticles were unstable in water, hydrophobic aggregation of nanoparticles overcoming the negatively electrostatic repulsion would play a predominant role in terms of agglomeration which results in the instability. Furthermore, we added octaarginine (R8), the positively charged hydrophilic segment of STR-H16R8, into the EPT nanosuspension. However, the maximum zeta potential was only 13.7 mV and the NPs still unstable in water. Therefore, we can conclude that the negatively charged surface of the NPs possessed the property of hydrophobicity and it was mainly based on the hydrophobic interaction between the surface of NPs and hydrophobic segment of STR-H16R8, rather than the coulombic interaction that STR-H16R8 anchored on the surface of EPT NPs.

After siRNA was added into the STR-H16R8 stabilized EPT NPs, the particle sizes were 95-105 nm, which did not change significantly (Fig. 2C), while the zeta potential decreased slightly (31-35 mV) (Fig. 2D). The reason would be that negatively charged siRNA interacted with the STR-H16R8 on the surface of NPs resulting in that part of the positive charges were neutralized. Also, some siRNA could formulate with the free peptide in the aqueous solution to form complexes, which showed lower zeta potentials in the range of 20~30 mV[8].

Cellular uptake of EPT after the cells were treated with bare EPT NPs and stabilized NPs was measured by fluorescence activated cell sorting (FACS). As cell viability was low after treated by the drug NPs with the ratio of STR-H16R8/EPT > 0.375, the reduced ratios of 0.015 and 0.15 were employed in this assay. As shown in Fig. 3A, in comparison to the EPT NPs only samples, which showed mean fluorescence intensities of 9.4 for EPT, the mean fluorescence intensity increased to 15.8 at the ratio of 0.015. The intensities were enhanced further with the ratio of STR-H16R8/EPT at 0.15 to 19.7, which implied that the efficacy of the EPT NPs would be improved with STR-H16R8.

Figure 3

Figure 3. (A) Cellular uptake of EPT after A549 cells were treated by bare EPT NPs and stabilized NPs with the molar ratios of STR-H16R8/EPT of 0.015 and 0.15 at 3 h, measured by FACS (green: untreated, blue: bare EPT NPs, red: STR-H16R8/EPT of 0.015, purple: STR-H16R8/EPT of 0.15). Data were collected up to 10,000 events; (B) Knockdown efficiency of STR-H16R8/Bcl-2 siRNA complexes in A549 cells (n=4). Lipofactamine 2000 (Lipo) was used as positive control (n=4); (C) Cytotoxicity of STR-H16R8 only and STR-H16R8/BCL-2 siRNA in A549 cells (n=5). The concentration of STR-H16R8 at the molar ratios of STR-H16R8/siRNA of 15, 30 and 60 were the same as those with the molar ratios of STR-H16R8/EPT at 0.375, 0.75 and 1.5, respectively; (D) Cytotoxicity of EPT NPs/STR-H16R8 and combination of EPT NPs/STR-H16R8/BCL-2 siRNA in A549 cells (n=5). The molar ratios of STR-H16R8/siRNA were 15, 30 and 60 with the molar ratios of STR-H16R8/EPT at 0.375, 0.75 and 1.5, respectively.

The knockdown efficiency of STR-H16R8/BCL-2 siRNA complexes was shown in Fig. 3B, which was comparable with Lipofactamine 2000. STR-H16R8 formulated with BCL-2 siRNA with the molar ratios of STR-H16R8/siRNA at 15, 30 and 60 did not induce any toxicity (Fig. 3C). Also, the viabilities of A549 cells treated with STR-H16R8 only were almost the same as untreated cells.

EPT NPs and EPT NPs/siRNA only exhibited low cell-killing abilities with the cell viability of ca. 90%, respectively (Fig. 3D). The cell viabilities decreased to 75% of STR-H16R8/EPT at 0.015 and 60% of STR-H16R8/EPT at 0.15, respectively which confirmed that the efficacy of the EPT NPs was improved with the promoted internalization mediated by STR-H16R8. However, at those low molar ratios of STR-H16R8/EPT, the addition of 100 nM siRNA did not change the cytotoxicity. The toxicity of EPT NPs were increased further with the viabilities of 35%, 24% and 15%, respectively when STR-H16R8/EPT was 0.375, 0.75 and 1.5. After Bcl-2 siRNA was involved, the viabilities of A549 cells were reduced to 32%, 15% and 4%, respectively (Fig. 3D). Taken together, the combination of EPT and STR-H16R8 induced an increased cytotoxicity, which was improved further by Bcl-2 siRNA.

In summary, EPT NPs with the particle size of ca. 90 nm were prepared successfully, which was further stabilized by water soluble STR-H16R8 in water. We demonstrated the stearylated peptide not only improved the stability of nanosuspension, but also significantly promoted the efficiency of the drugs due to the increased cellular uptake. Moreover, Bcl-2 siRNA further sensitized the cancer cells to the chemotherapeutics. In conclusion, a novel co-delivery system for drug and siRNA was developed, which showed the potential for the treatment of cancer.

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Notes and references

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† Electronic Supplementary Information (ESI) available: Materials and reagents, cell culture, transmission electron microscopy (TEM), particle size and zeta potential, fluorescence-activated cell sorting (FACS), gene silencing and cytotoxicity assay. See DOI: 10.1039/c000000x/

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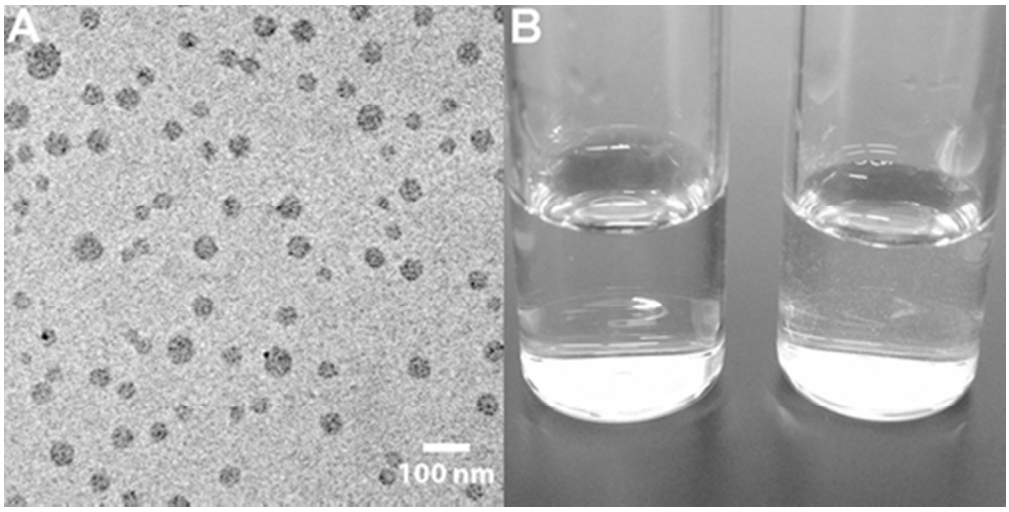

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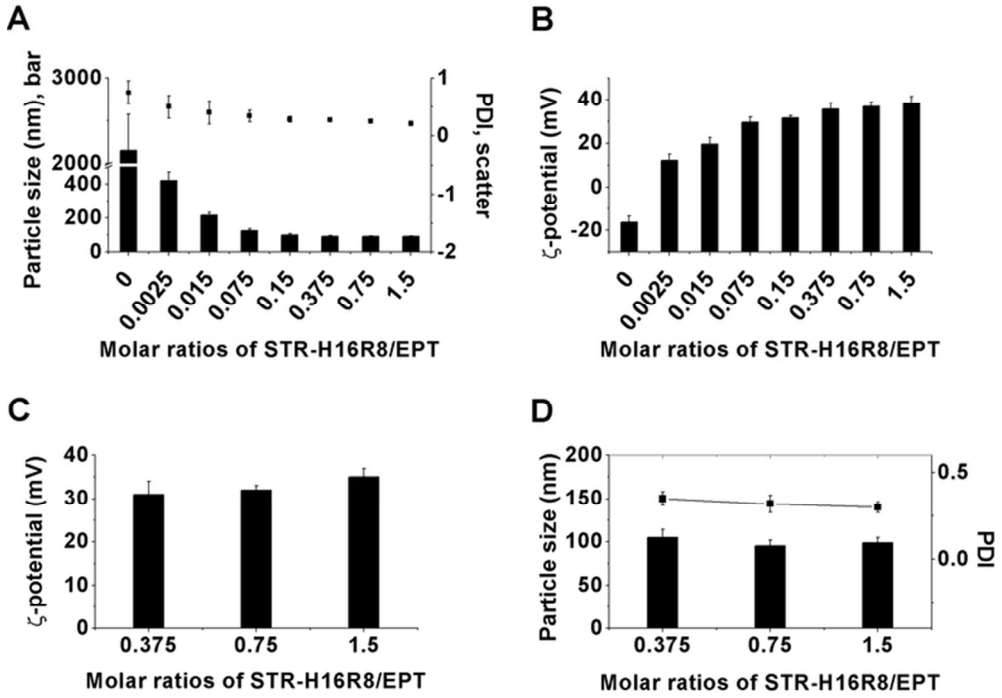

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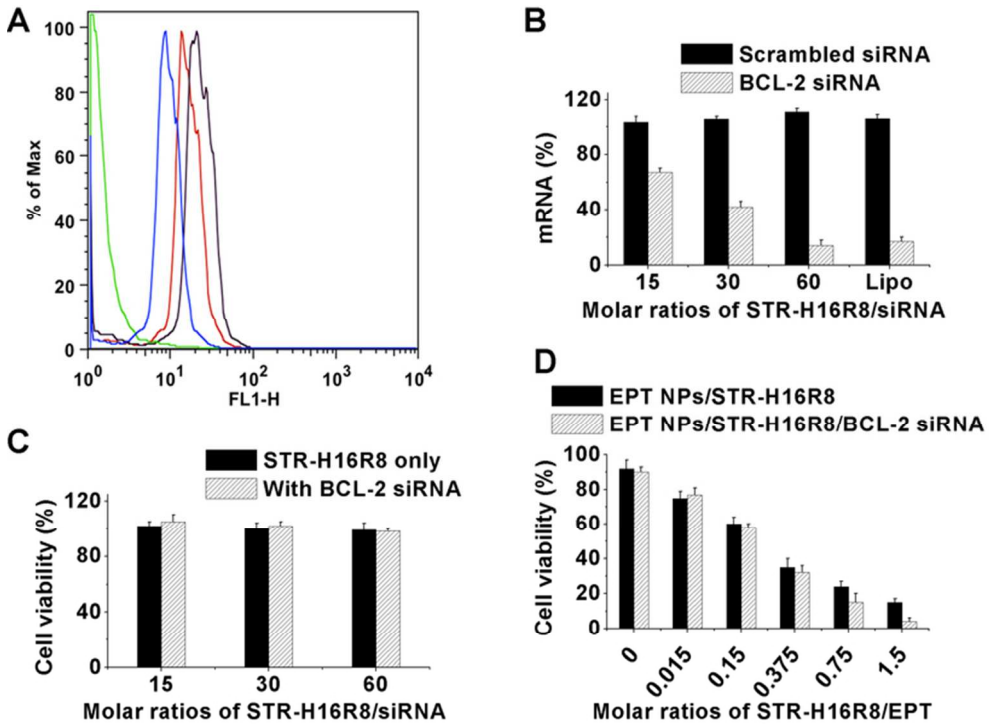

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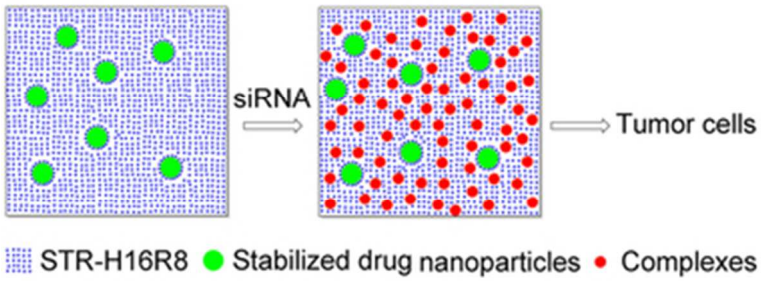
Graphic abstract

Modified cell penetrating peptide can stabilize drug nanoparticles with improved efficacy and co-deliver siRNA inducing synergy on the inhibition of cancer cell growth.



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