Application of novel functional peptide to nanoparticle encapsulating siRNA for effective siRNA delivery

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Gene therapy using small interfering RNA (siRNA) is expected to be a novel strategy for the treatment of various diseases such as cancer, although there are some problems in clinical use of siRNA such as rapid degradation by nucleases after systemic administration and poor cellular uptake. Therefore, the delivery system that these problems have been improved is indispensable to establish the siRNA gene therapy. Previous study in my laboratory showed that lipid nanoparticle encapsulating siRNA (LNS) avoided the degradation of siRNA in the presence of serum. Preparation of LNS was indicated as follows: negatively-charged siRNA was mixed with the cationic peptide and nano-sized complex was formed; this cationic complex was encapsulated into the negatively-charged lipid particle based on dimyristoylphosphatidylglycerol (DMPG) and dioleoylphosphatidylcholine (DOPE). Owing to this lipid layer, siRNA was protected from the degradation by nucleases. However, LNS could not improve poor cellular uptake of siRNA because of its anionic surface charge. In the present study, the cell-penetrating peptide (CPP) was applied to LNS (PD-LNS) to solve this problem.

Firstly, RNA interfering (RNAi) effect of PD-LNS on murine B16F10 melanoma cells over-expressing luciferase (B16F10-Luc2) was examined. As a result, PD-LNS carrying siRNA targeting luciferase (siLuc2) significantly suppressed the expression of luciferase, whereas both naked siLuc2 and LNS carrying siLuc2 did not show. When optimal modification ratio of CPP to LNS was determined, it found that 6 mol% modification in total lipid of LNS was most appropriate to show RNAi effect. Furthermore, to elucidate the mechanism of highly RNAi effect of PD-LNS, surface charge alteration of PD-LNS with pH condition was examined. The result indicated that ζ-potential of PD-LNS was altered in pH-dependent manner: PD-LNS possessed cationic surface charge in acidic condition; the surface charge of particle was neutral in physiological condition (pH 7). It is considered that this feature of PD-LNS is suitable for not only escape from acidic endosome to cytoplasm but also improvement of biodistribution after systemic administration. The present study suggests that PD-LNS would be a potent siRNA delivery system for the treatment of cancer.