Engineering Periodic Short Hairpin RNA Delivery Systems for Enhanced Therapeutic Efficacy

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ABSTRACT

RNA interference (RNAi) presents a highly promising approach for cancer therapeutics via specific silencing of disease-implicated genes, but its clinical translation remains severely limited by barriers in delivering short interfering RNA (siRNA). Numerous delivery vehicles have been developed to protect siRNA from degradation, promote target cell uptake, and facilitate endosomal escape into the cytoplasm, where RNAi occurs. However, *in vivo* instability, low silencing efficiency, undesired toxicity, and immunogenicity remain challenges for current siRNA delivery systems, particularly as the low valency and high rigidity of siRNA make it difficult to condense into stable nanoparticles. Here we engineer the siRNA cargo to make it more amenable to stable encapsulation by using a polymeric form of siRNA, or periodic short hairpin RNA (p-shRNA), as well as design a biodegradable polycationic carrier for efficient *in vivo* delivery of p-shRNA. Consisting of tens of linked siRNA repeats, p-shRNA is synthesized by the repeated action of T7 RNA polymerase around a circular DNA template. We first leverage molecular engineering design an open-ended p-shRNA structure that is efficiently processed inside cells into siRNAs, greatly enhancing its silencing potency. Furthermore, the much higher valency and flexibility of p-shRNA compared to siRNA enable more stable complexation with delivery materials. To exploit these advantages of p-shRNA, we optimize biodegradable polycations with hydrophobic regions that promote stable condensation and efficient intracellular release. Our approach unveils key design rules governing p-shRNA delivery, and we develop stabilized p-shRNA complexes that show *in vivo* therapeutic efficacy in a syngeneic melanoma mouse model. Finally, we extend our p-shRNA platform to act as a dual therapeutic agent, harnessing innate immune activation together with gene silencing. By modulating the surface of the p-shRNA complexes with an anionic polypeptide, we dramatically enhance innate immune recognition of p-shRNA by pattern recognition receptors while maintaining high silencing efficiency. These dually acting complexes can target ovarian tumors *in vivo* and prolong survival in a syngeneic ovarian cancer mouse model. Our findings establish a potent, multifunctional RNAi platform that can potentially move RNAi therapeutics closer to clinical translation by addressing the delivery and *in vivo* efficacy challenges faced by current siRNA systems.

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