Targeting Peptide-Mediated Delivery of siRNAs into Ovarian Cancer Cells

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Statement of Purpose: Ovarian cancer is the leading cause of gynecological cancer death in the world.[1] It is the 7th most diagnosed cancer in women worldwide with 230,000 yearly diagnoses and has a five-year survival rate of 46% due to late detection.[1,2] Treatment methods include surgery to remove cancerous tissue followed by chemotherapy.[2] Even with treatment, most women experience recurrence within 1.5 years, and 75% of those cases in advanced stages develop extreme drug resistance.[1,2] With such an unfavorable prognosis, the development of a better treatment strategy, which includes more precise therapeutics and delivery systems, is necessary.

In the past few decades, targeting moieties have increased in popularity for tissue-specific delivery of therapeutics.[3] There are several advantages of using targeting moieties, including increased drug concentration in targeted areas and decreased off-target effects.[3] A particularly advantageous targeting moiety is a targeting peptide. Peptides have high stability, relatively cheap manufacturing costs, high tunability, and low immunogenic response.[4,5] The conjugation of targeting peptides to therapeutics such as short-interfering RNAs (siRNAs) is a potential mechanism for increased therapeutic efficacy. siRNAs use RNA interference to inhibit gene expression through sequence-specific cleavage of messenger RNA.[6] Even with an array of siRNAs available to target and silence oncogenic genes, there are drawbacks that must be addressed.[6] Limitations of siRNA include their high negative charge, susceptibility to degradation by RNases, and lack of cell targeting capability.[6] These drawbacks can be addressed through complexing siRNAs with targeting peptides.

This project evaluates the potential of a luteinizing hormone-releasing hormone (LHRH) targeting peptide for increasing delivery and uptake of siRNAs into ovarian cancer cells. The LHRH receptor has been found to be overexpressed on ovarian cancer cells; thus we have selected a targeting peptide that has been demonstrated to have specificity for this receptor.[7] In this research, we characterize the ability of the peptide to bind siRNAs and protect them from degradation.

Methods: siRNAs were complexed with an LHRH-targeting peptide to form nanocomplexes. Agarose gel shift, protection assays, dynamic light scattering, and MTS studies were conducted to characterize size and zeta potential of the complexes, siRNA protection capabilities, minimum N/P ratios for complexation, and biocompatibility. Western blotting was also performed to examine the basal expression of LHRH in human ovarian cancer cell lines OVCAR3 and CAOV3 and in heathy ovarian cell line HOSEpic.

Results: Western blots confirmed that LHRH was overexpressed on OVCAR3 and CAOV3 cells, and little to no expression was observed in the healthy ovarian cell line. The LHRH peptide forms uniform nanocomplexes with siRNA. The average particle size was 147 ± 9 nm and particles were positively charged (Fig 1A). The minimum N/P ratio required to completely bind free siRNAs was 40:1 (Fig 2B). Protection assays revealed that the LHRH peptide could better protect siRNAs from degradation in 50% FBS compared to incubation with RNases. Cell viability studies demonstrated that the targeting peptide was not cytotoxic to ovarian cancer cells.

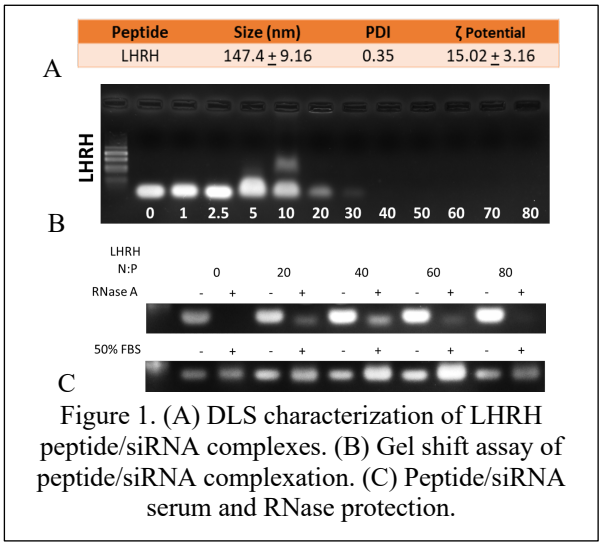

Figure 1. (A) DLS characterization of LHRH peptide/siRNA complexes. (B) Gel shift assay of peptide/siRNA complexation. (C) Peptide/siRNA serum and RNase protection.

Conclusions: The LHRH receptor is a promising target due to its overexpression on ovarian cancer cells and not on healthy ovarian cells. The LHRH peptide successfully complexes with siRNAs. The nanocomplexes have a positive zeta potential, which can enable interactions with cell membranes and complexing with negatively charged siRNAs. With exposure to serum, the peptide was able to protect the siRNAs from degradation. In future studies, the ability of the LHRH peptide to mediate targeting and enhanced uptake of siRNAs into ovarian cancer cells will be evaluated.

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