Development of a Novel Tandem Peptide for Delivery of siRNA for treatment of Glioblastoma Multiforme

Jessica Boulos, Jorrian Abadeer, Tyler Willis, Dr. Angela Alexander-Bryant
Clemson University

Statement of Purpose: RNAi has demonstrated promise for treating many diseases, including cancer, however, significant barriers to delivery hinder clinical translation of RNAi therapies. These barriers include non-specific delivery, insufficient cellular uptake, and lack of endosomal escape. There are three classes of peptides that have demonstrated the ability to overcome these specific barriers: targeting, cell-penetrating, and fusogenic peptides, respecitively.[1,2,3] Although these peptides have been studied individually, few studies have evaluated the effects of combining peptides to overcome multiple delivery barriers. Thus, the purpose of this study is to design a novel tandem peptide for delivery of siRNAs to treat glioblastoma multiforme. The tandem peptide is a novel combination of fusogenic peptide, 599, and EGFR-targeting peptide, GE11.[4,5] A cationic tail creates a positive charge for electrostatic complexation with negatively charged siRNA. STAT3, the target gene, is an oncogene that plays a role in cell proliferation, differentiation, apoptosis, and angiogenesis in GBM.[6] We characterize the tandem peptide and evaluated its ability to delivery bioactive siRNAs compared to the individual peptides alone.

Methods: Dynamic Light Scattering (DLS) was used to determine size, PDI, and zeta potential of the peptides complexed with siRNA at a 60:1 ratio. Furthermore, gel shift assays were used to evaluate complexation efficiency for the tandem peptide with siRNA at varying N:P ratios from 10:1-80:1. To determine the siRNA protection efficiency of the complexes at N:P ratios varying from 50:1-80:1, complexes were incubated in RNaseA for 1 hour, dissociated with an SDS solution, and electrophoresed on an agarose gel. MTS assays were used to evaluate cytotoxicity of the peptide complexes on U118 GBM cell lines. Western Blot experiments were conducted to confirm basal expression of EGFR and STAT3 in GBM cell lines.

Results: Agarose gel electrophoresis was used to evaluate the ability of the tandem peptide to complex free siRNA at an array of N:P ratios, and complete complexation was observed at ratios of 50-80:1 (Fig. 1b). Dynamic light scattering analysis revealed the average diameter of tandem peptide/siRNA complexes was approximately 102 nm with a polydispersity index of 0.23, indicating the formation of uniform, monodisperse nanoparticles. The zeta potential was 27.3 mV, which is favorable for interactions with the negatively charged cell membrane (Figure 2a). To ensure clinical translatability, the ability of the tandem peptide nanoparticles to protect siRNA from degradation was evaluated by incubating tandem/siRNA complexes with RNaseA. Following incubation, the complexes were dissociated to release siRNA, revealing intact siRNA indicating the stability of the complexes. (Figure 2c). An MTS assay showed no difference in viability between GBM cells treated with the tandem peptide compared to untreated cells, indicating minimal toxicity. To determine the potential for receptor-mediated internalization via the EGFR targeting sequence, western blots confirmed high EGFR expression in the U118 and U87 GBM cell lines. Furthermore, western blots showed high expression of the target oncogene, STAT3, in the U118 and U87 GBM cell lines.

(a)

Tandem DLS		
Size	**PDI**	**Zeta Potential**
101.9 ± 1.71 nm	0.23	27.3 ± 1.67 mV

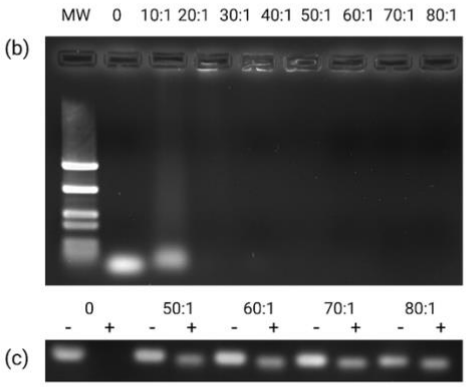

Figure 1. (a) Characterization of the tandem peptide complexed with siRNA at a 60:1 ratio through dynamic light scattering experiments. **(b)** Complexation of the tandem peptide with siRNA at varying N:P ratios. **(c)** Protection analysis of siRNA complexed with the tandem peptides at varying N:P ratios after incubation with(-) or without (+) RNaseA for 1 hour at 37° C.

Conclusions: Characterization studies have confirmed the tandem peptide's ability to complex and protect siRNA from degradation. These nanoparticles are positively charged, monodisperse, and biocompatible. Based on the results, it is hypothesized that the tandem peptide will enhance EGFR-specific uptake of STAT3 siRNA into glioblastoma cells for mediating endosomal escape, resulting in more effective gene silencing compared to the 599 and GE11 peptides alone. Future studies include immunofluorescence for uptake and endosomal escape, gene silencing studies (Western Blot and qPCR assays), and evaluation of the downstream effect of STAT3 silencing through migration and invasion assays.

References: [1] Mousavizadeh, A. *et al. Colloids Surf. B* 158 (2017) [2] Copolovici, D. *et al. ACS Nano* 8,3 (2014) [3] Oliveira, S. *et al. Int. J. Pharm.* 331,2 (2006) [4] Li, Z. *et al. FASEB* 19 (2005) [5] Cantini, L. *et al. PLOS ONE* 8(9) (2013) [6] Kamran, M *et al. Biomed Res Int* (2013)