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Savannah Seely

Dustin P. Patterson

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Investigating Programming of a Virus Like Particle for Cell Specific Chemotherapy

Savannah Seely¹, Dustin P. Patterson¹
¹Department of Chemistry & Biochemistry, The University of Texas at Tyler, Tyler, TX 75799, USA

Introduction
Cell specific drug delivery is a major challenge in chemotherapeutic strategies targeting, particularly in cancer strategies. Side effects for non-specific uptake of chemotherapeutic drugs by normal healthy cells include hair loss, vomiting, nausea, and other negative symptoms. The use of nanoparticles such as those derived from protein cage structures present potentially useful agents for drug delivery by encapsulation of drugs on the interior of the protein cage nanoparticle. However, while strategies for encapsulation of drugs are plentiful, methods for programming site/cell specific delivery of nanoparticles are needed. One potential protein cage platform for drug delivery is the virus-like particle (VLP) derived from the bacteriophage HK97 (97VLP). The HK97 VLP self assembles from 420 copies of the GPS coat protein to form a hollow icosahedral 56nm structure. HK97 is an interesting protein because it has been observed to require cell targeting moieties to be added to the exterior for entry into cells.

The research presented here looks to examine a proof of concept strategy for localizing HK97 VLPs based on chemosensitive programming of the exterior. The approach looks at exploiting the property of cancer cells to upregulate metalloproteases in the cancer microenvironment. We hypothesize that peptides can be designed to prevent assembly of poly-lysine peptide sequence to the GPS protein C-terminus. Treatment with N-aspartate protease, a “model” protease, is expected to lead to VLP self-assembly and aggregation in support of our proposed hypothesis.

Properties of the HK97 VLP Ideal for Drug Delivery
- Large hollow interior for drug loading.
- Drugs can diffuse through 2 nm sized pores in the cage.
- Exposed C-terminus as an attachment site on exterior (Figure 2).
- Highly stable for delivery in vivo.
- Ideal nanoparticle size for accumulation in cancerous tissue (EPR effect).

Manipulating HK97 VLP For Encapsulation and Self Assembly
- Engineer the interior surface for allowing localization of therapeutics.
- Engineer exterior surface for attachment of cell targeting moieties.
- Combining internal drug packaging with external modification for cell targeting will produce “smart bomb” drug delivery nanoparticles.

Targeting Metalloproteases
- Cancer microenvironments are known to contain proteases, which are proteins that digest other proteins at specific amino acids.
- The VLP exterior can be programmed with peptides that are modified removed by metalloproteases that are in the microenvironment of the cancer, but are not found elsewhere.
- Designing the peptides to cause self-assembly upon digestion/cutting by the protease is expected to localize the drug delivery VLP.

Exploiting Self Assembling Peptides
- The de novo design of peptides that self-assemble through non-covalent interactions into higher order structures is well studied.

Incorporating Site for Targeting Moieties
- C-terminus exposed to exterior permits genetic addition of peptides to the exterior of the HK97 VLP.
- Plasmid was constructed enabling peptide sequences to be added in a modular fashion using KpnI and NotI restriction enzymes (Figure 4).
- Peptides appended could be used directly for cell targeting or as molecular handles to attach targeting moieties.

Colonies for Gene Insertion
- DNA was extracted from E. coli colonies
- The DNA underwent Polymerase Chain Reaction (PCR) and then was run on a gel
- The gel is visualized in Figure 7 and includes a standard protein ladder
- Based on the GPS coat protein, colonies that had the gene inserted in the plasmid would have bands at approximately 1200 base pairs.
- The colonies with the bolded bands at approximately 1200 BP indicated by the red arrow were selected for further testing.

Sequencing of Gene Inserts
- DNA sequencing was performed on the colonies using sequencing primer pairs for the GPS gene
- DNA sequencing performed by Eurofins Operon sequencing service
- All colonies showed correct sequence expected for attachment of the FFFDKKKKK gene at the C-terminus of GPS gene

Summary and Future Directions
- Gene encoding FFFDKKKKK has been successfully incorporated into the expression vector for GPS
- Trials are underway to evaluate the expression of the modified HK97 VLP (Gene encoding FFFDKKKKK).
- Modified HK97 VLPs will be characterized to determine their ability to self-assemble after N-Aspartate Protease treatment
- Designs of peptides that will allow targeting of metalloproteases in cancer cell microenvironments are being examined in the case that our proof of concept studies are successful.

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