Bioengineering of Polypeptides and Nanoscale Assemblies for Cancer Cell Destruction

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My graduate research involved the chemoenzymatic syntheses and parallel screening of bioseparation ligands (displacers for displacement chromatography), DNA-binding ligands, and polymers for non-viral gene delivery. Information from Quantitative Structure-Activity Relationship (QSAR) models resulted in insights into the physicochemical phenomena influencing polyamine-DNA binding, displacer efficacy, and protein adsorption thermodynamics.

My postdoctoral research involves the use of recombinant DNA technology and protein engineering to develop fusion peptides, antibody-peptide conjugates, and quantum dot-polypeptide assemblies as anti-prostate cancer therapeutics. Prostate cancer is the most common malignancy affecting men and is the second-leading cause of cancer death in men in the United States after lung cancer. Our overall aims for this endeavor were to (1) design, express, purify, and characterize recombinant polypeptides for the selective targeting and ablation of prostate cancer cells, (2) generate supra-molecular (quantum dot-peptide) assemblies designed to manipulate cellular and molecular targets in prostate cancer, and (3) evaluate the cytotoxic efficacies these polypeptides and quantum dot-peptide assemblies using in-vitro screens.

The Prostate-Specific Membrane Antigen (PSMA) is a type II membrane glycoprotein which is over-expressed in all stages of human prostate cancer disease. The increased expression of the receptor in cases of hormone-refractory disease and metastasis makes it an attractive target for anti-prostate cancer therapy. Coiled-coil motifs are ubiquitous in biology and form the basis of molecular recognition and assembly in a variety of natural systems including fibrous proteins (e.g. keratin), transcription factors (e.g. yeast GCN4 leucine zipper), and viral proteins (e.g. gp41 of HIV). We employed a parallel coiled-coil motif for the epitope display of bivalent analogs of PSMA-targeting peptides (PTPs). Different mutants, generated using deletion/insertion site-directed mutagenesis of the coiled-coil core, were expressed, purified and characterized.

We then investigated the in vitro efficacies of an apoptotic peptide (AP) and PTP-AP fusion peptides. Different cell lines including, hormone-dependent (LNCaP) and hormone-refractory (PC3) prostate cancer cell lines, normal primary human prostate epithelia, and primary human fibroblasts were used in the investigation. The energy dependent cellular uptake and therefore, the cell penetrating abilities, of these peptides were examined. In addition, liposome disruption / leakage experiments were carried out in order to investigate the membrane permeabilization activity of the peptides. Next, we examined cancer cell death mechanisms upon exposure to these peptides using fluorescence microscopy and flow cytometry (FACS). We asked whether the cells were undergoing apoptosis or necrosis and whether the caspase-mediated apoptotic pathway played a role in cell death. Finally, we examined the effect of the peptides on depolarizing the potential across the mitochondrial membrane in treated cells.

The interfacing of biological therapeutic agents with nanoparticles has tremendous potential in diagnostic cancer imaging and therapy. The simultaneous display of multiple peptides on a scaffold is known to result in higher efficacies owing to polyvalent display. We explored the use of fluorescent quantum dots for the dual purpose of polyvalent peptide display and imaging. Peptides were self-assembled on ZnS coated CdSe quantum dots via polyhistidine tags. We are currently using this ‘bottom-up' approach to load individual peptide components on quantum dots leading to multi-functional constructs that possess targeting, apoptotic and imaging capabilities on a single platform.

My future work will involve the application of protein, cellular, and tissue engineering to anti-cancer therapy. Protein engineering tools will be employed to design novel apoptosis-inducing polypeptides

and targeted supra-molecular (nanoscale) therapeutics. The intracellular levels of key proteins involved in apoptotic pathways will be manipulated (e.g. knockdown of anti-apoptotic proteins including Bcl-2 and c-FLIP using RNA interference) and the apoptotic efficacy of the above polypeptides will be investigated using in vitro screens. Finally, tissue engineering models of cancer disease, that mimic the in vivo tumor microenvironment, will be developed for a more realistic in vitro evaluation of these peptides. Information from these tissue models will be employed to investigate the kinetics of tumor penetration and transport of polypeptide / supramolecular therapeutics.