Online Proceedings

SESSION 1M

THERAPEUTIC VULNERABILITIES OF CANCER

Session Moderator: Rodney Ho, Pharmaceutics
MGH 389
12:30 PM to 2:15 PM

* Note: Titles in order of presentation.

Engineering M2 Macrophage-Targeting Peptide (M2pep) for Improved Serum Stability

Julio M. (Julio) Pineda, Senior, Mathematics, Bioengineering
Mary Gates Scholar, UW Honors Program
Mentor: Suzie Pun, Bioengineering

Macrophages are broadly classified as pro-inflammatory M1 and anti-inflammatory M2 macrophages. The majority of tumor-associated macrophages (TAMs) in tumors are known to express M2 phenotypes (M2-TAMs) which secrete cytokines and growth factors to promote cancer cell proliferation, tumor angiogenesis, and metastasis. Hence, depletion of the M2-TAMs is a promising adjuvant therapy to the current cancer treatments. Our lab has previously developed M2 macrophage-binding peptide (M2pep) which can bind to both M2 macrophages and M2-TAMs. However, in vivo targeted delivery of cytotoxic drugs to M2-TAMs using M2pep as a targeting ligand was limited by poor serum stability of M2pep. Previously, we engineered and tested different linear variants of M2pep; however, none of these was able to improve serum stability while retaining M2 macrophage-binding activity. In this study, we investigated cyclization of M2pep as a strategy to improve its serum stability since cyclic peptides are usually more serum resistant than their linear counterparts due to their higher structural rigidity. To synthesize a cyclic M2pep variant, the original M2pep was synthesized with two flanking cysteines on each terminus. These cysteines were then oxidized to form a disulfide bond yielding cyclic M2pep. The serum stability of cyclic M2pep was evaluated by incubating the peptide in serum and withdrawing aliquots of the serum at various time points for analysis by mass spectrometry. Compared to linear M2pep which was rapidly degraded in serum within 4 hours, the cyclic variant was detected even after incubation in serum for more than 24 hours. In conclusion, we have developed an optimized cyclic M2pep which exhibits enhanced serum stability while retaining M2-TAM binding activity. Our peptide can be utilized as a more effective ligand for targeted delivery of cytotoxic drugs to M2-TAMs. Future development of M2pep-based TAM-depleting therapeutics would serve as an effective adjuvant platform to potentiate current cancer therapies.

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Optimizing Peptides for Targeting Tumor-Associated Macrophages

Jonathan Lik Wing (Jonathan) Yu, Senior, Bioengineering
Mary Gates Scholar, UW Honors Program
Mentor: Suzie Pun, Bioengineering
Mentor: Gary W. Liu

Macrophages are cells of the immune system that play key roles in surveillance, destruction of pathogens, and tissue repair. In the context of cancer, macrophages can infiltrate solid tumors and adopt an anti-inflammatory profile resembling that of the "M2" macrophage subclass. These M2-like tumor-associated macrophages (TAMs) promote tumor growth and metastasis by suppressing the immune response. Therefore, selective destruction of TAMs using a biomolecule that targets and binds to TAMs may have therapeutic benefit in cancer. We have previously identified a novel peptide, M2pep, that selectively binds to M2 macrophages over other immune system cells. In other experiments, a specific pattern of amino acids, named the M2pep motif, was observed to be critical for peptide binding to TAMs. This project seeks to improve M2pep binding and selectivity to TAMs by constructing and screening a library of peptides containing the M2pep motif for binding to M2 macrophages. The library was constructed by genetically engineering bacteriophages to display all possible peptides containing the M2pep motif. This was achieved by: *(1)* designing DNA sequences encoding for peptides with certain amino acid positions "fixed" with the M2pep motif, and the other positions randomized; *(2)* inserting these se-

quences into bacteriophage DNA; and *(3)* incorporating the engineered DNA into bacteria to generate bacteriophages that display the peptide library on their outer surface. Then, the peptide library was screened for binding to M2 macrophages, and the peptides that bound were sequenced. Several candidates were evaluated for M2 macrophage-binding and compared with M2pep to identify sequences with improved binding and selectivity. This method of peptide library construction and screening using a bacteriophage platform can be a powerful tool for future drug development, and enable further refinement of binding molecules with established binding motifs.

POSTER SESSION 2

Commons East, Easel 72

1:00 PM to 2:30 PM

Reducible Comb-Like Polymers for Gene Delivery

Binhan Pham, Senior, Bioengineering
Mary Gates Scholar, UW Honors Program
Mentor: Suzie Pun, Bioengineering
Mentor: Kevin Tan, Bioengineering

Gene delivery has successfully been accomplished with the use of polycations, both in vivo and in vitro. However, a major problem in this category is the inherent cytotoxicity associated with a high density of positive charges. Polymers that can be degraded within the cells are typically better tolerated compared to their non-degradable counterparts. This research studies the use of disulfide bonds to reversibly connect cationic polymer moieties to a stable polymer backbone. Reducible and non-reducible, statistical co-polymers of 2-aminoethyl methacrylate (AEMA) and oligo(ethylene glycol) monomethyl ether methacrylate (OEGMA) were synthesized and evaluated for a comb-like, backbone chain. These monomers have been chosen due to their low cytotoxicity as well as their high degree of polymerization control in reversible addition-fragmentation chain transfer (RAFT) polymerization. Backbones containing 50 and 100 monomers per polymer have been successfully synthesized. The current phase of the project is to functionalize AEMA with a unique biomolecule called dibromomaelimide that contains two points for positively charged moieties. This final polymer will be used to transfect cells with genetic material to test its feasibility for gene delivery. Success of this project will create a well-defined polymer containing positively charged cations that are easily dispersed upon internalization by cells.

POSTER SESSION 2

Commons East, Easel 73

1:00 PM to 2:30 PM

Identification of Peptide Targeting Ligands for Cancer Immunotherapy by Phage Display Technology

Emi Alexandra Lutz, Senior, Bioengineering
Amgen Scholar, Mary Gates Scholar, UW Honors Program
Mentor: Suzie Pun, Bioengineering
Mentor: Brynn Olden, Bioengineering

Cancer cells can grow into life-threatening tumors and metastases partly because of their ability to evade the immune system. Immunotherapies combat cancer by boosting the natural immune response towards cancer cells. Immunotherapies are often more successful than standard treatments like chemotherapy because they are more targeted and have longer lasting effects with milder side-effects. A key component of immunotherapy is the identification of ligands that selectively target immune cells for drug delivery or immunomodulation. Peptide ligands are of special interest because they are inexpensive to synthesize and can be identified by a library screening technique called phage display. The purpose of this project is to identify peptide ligands to address two needs in cancer immunotherapy. The first need is to enable selective depletion of M2 tumor-associated macrophages, which promote tumor growth and suppress the adaptive immune response. We successfully identified 6 peptide ligands that preferentially bind murine M2 macrophages over M1 macrophages by using phage display and Illumina next-generation sequencing. The second need is to develop technologies for T-cell based immunotherapies. T-cells are an effective target for immunotherapies because they kill pathogens and regulate short-term and long-term immune responses. The identification of inexpensive peptide ligands to specific surface receptors on T-cells will be useful in increasing accessibility and efficacy of these treatments. We applied the successful next-generation sequencing methods from M2 macrophage ligand identification to target the CD28 and CD3e surface receptors on T-cells.

POSTER SESSION 2

Commons East, Easel 70

1:00 PM to 2:30 PM

The Impact of Polymer Size and Architecture on Tumor Penetration

Nick Tan, Senior, Bioengineering, Biochemistry
Mary Gates Scholar, UW Honors Program
Mentor: Suzie Pun, Bioengineering
Mentor: Christine Wang, Bioengineering

Current chemotherapies exploit the larger pore size of tumor vasculature to reach cancer cells. However, this rudimentary form of targeting offers low selectivity and carries the risk of severe systemic toxicity. In addition, chemotherapy distribution to tumors is limited by the large distance

between blood vessels in tumors, increased interstitial fluid pressure, and physiological resistance by the extracellular matrix (ECM). With the goal of addressing these limitations, researchers have developed drug-bearing polymers of various sizes and architectures that selectively target tumor cells. The Pun lab previously produced a novel drug delivery polymer, the sunflower polymer, that can be utilized for anticancer drug-delivery. The sunflower polymer is a macrocyclic brush polymer that can be functionalized with a folic acid targeting ligand and an anticancer drug, doxorubicin, that is released in the acidic tumor microenvironment. In order to maximize the potential of anti-cancer drug-delivery polymers such as this, the impact of polymer size and architecture on tumor penetration must be better understood. This is because the efficacy of any anticancer drug, regardless of its mechanism of action, is limited by its ability to navigate the tumor microenvironment and reach all viable cells in a tumor. This project seeks to discover how polymer size and architecture impacts tumor penetration by using a 3D *in vitro* perfusion chip model of the tumor microenvironment. The model consists of microfluidic channels seeded with KB carcinoma cells and ECM, and has been optimized using model polymers of polyethylene glycol (PEG) and dextran. Tumor penetration is studied by perfusing fluorescently labelled polymers of various morphologies through the seeded microfluidic channels. The information obtained from this project could potentially be used to optimize the size and architecture of drug-delivery polymers for tumor penetration. In turn, these optimizations could present a significant contribution to the development of anticancer drug-delivery polymers.