Modular Production of Bispecific Antibodies via Sf-9 Cells

Introduction

Antibody drugs have become one of the most important classes of targeted cancer therapy because of their ability to specifically bind to receptors on tumor cells and activate downstream processes that disrupt their proliferation. Numerous large pharmaceutical and small biotech companies have invested a significant amount of effort in developing new and more effective platforms based on antibody therapeutics.[6] The majority of these approaches use monospecific monoclonal antibodies (mAbs), meaning that the antibody drugs recognize only one target. In contrast, Bispecific antibodies (bsAbs)– antibodies that bind to two different targets – are a new class of antibodies that have a distinctly different mode of action and therefore offer a greatly expanded therapeutic potential. The production of bsAbs however, remains challenging. My project focuses therefore on developing a more efficient modular method to produce bsAbs. Specifically, the project explores using enzyme-assisted assembly methods in conjunction with an insect cell expression platform to build bsAbs.

Background

Antibodies, which are Y-shaped proteins, identify foreign viruses or pathogens and kill them. Referring to Appendix A, an antibody is made of two light-and-heavy chain complexes bound together by disulfide bridges. The branch is called a Fab, and the stem is called the Fc region. The Fab's end regions are called variable regions, and different antibodies contain extremely diverse paratopes that are pathogen-specific. Additionally, the Fc region have glycosyl sugar chains. These glycosylated cabohydrates prevents the antibody clearance from the blood and significantly affects antibody activity.7 When confronted with a pathogen or a malignant cell, the paratope identifies and binds to a specific part of the target, called the antigen. Afterwards, Fc region interacts with the Fc receptors found on the surface of immune cells and stimulates the immune cells to kill the target via antibody-dependent cellular cytotoxity(ADCC). However, bsAbs have a different mechanism. By making one Fab bind to a malignant cell and the other bind to an immune cell, bsAbs create a synthetic immune synapse and activate the ADCC more efficiently than mAbs.

Currently, producing bsAbs is an inefficient process. Even using powerful recombinant DNA technology to produce antibodies with bispecificity, yield and purity of correctly assembled bsAbs is often poor.[5] These inefficiencies typically arise from the incorrect pairing of the antibody protein chains required to recognize each target independently. Briefly, each Fab contains one light chain and one heavy chain. Unlike monspecific antibody production, bsAb production requires selective pairing between the light chain and heavy chain for the same antigen. During expression of the antibody fragments in vivo, these chains have a high probability of encountering the wrong chain partners and this results in a scrambled Ab with loss of desired function. Recently, new breakthroughs have been made with addressing mis-pairing by Spiess et al. and Lewis et al.[5] Both strategies are based on the knob-in-hole method but have different approaches to correct pairing. The knob-in-hole method creates artificial 'knobs' that bulge out and 'holes' that bulge in by making gene mutations. The knob and hole selectively bind together. Spiess et al. engineered to E. Coli cells to only express half of an antibody, or one Fab. Two separate Fabs were produced and the Fc region was connected using knob-in-hole. Lewis et al. made knob-in-hole mutations on every light chain and heavy chain so that all four selectively bind correctly. (Appendix B) While these methods are powerful, they are not general insorfar as each requires significant effort in optimizing the antibody scaffold prior to production.

My study will work on developing a modular bsAbs production platform by applying enzyme-assisted assembly methods via insect cells. Spiess et al. cannot express individual Fab fragment in eukaryotes and, thus, is limited from making correct glycosylation patterns.

Lewis et al. requires extensive mutations for every heavy-light chain pairing. Expression in eukaryotes, such as insect cells, will provide correct glycosylation, and the enzyme-assisted assembly method eliminates the need for multiple mutations. Finding the correct mutation sites can limit the modularity of methods proposed by Lewis et al. because mutations can potentially affect the protein structure or compromise the protein's effectiveness.

Enzyme-assisted assembly is a modular protein assembly method developed in the Mrksich Lab.[4] This technology takes advantage of the rapid and selective reaction between an enzyme-protein fusions and small molecule linkers. By fusing Fab fragments to an enzyme assembly domain, heterologus fusion proteins can be specifically linked together via linkers to produce bsAbs. Unlike state-of-the-art methods, this production approach requires very little optimization prior to assembly.

Methodology

I will be working in Professor Milan Mrksich's laboratory. The production bsAbs can be divided into five phases: cloning/expression/purification, Fab fusion assembly, binding assays, immunohistochemistry, and cell sorting. Within this scheme, my project this summer will concentrate on phase one: cloning/expression/purification of Fab-enzyme fusion proteins. Phase one can be divided into four main intermediate goals: preparing fusion protein DNA constructs consisting of the Fab fragment and enzyme, culturing insects cells with the DNA-containing plasmid, inducing protein production from insect cells, and protein purification.

The significant portion of summer will be spent culturing insect cells to produce fusion proteins and purifying them. The premade fusion protein DNA constructs will be intregrated into a plasmid carrying an essential gene for survival. The plasmid will be loaded inside a baculovirus in order to infect Sf-9 cells from Spodoptera frugipderda.[3] Sf-9 cells with stable infections will be selected and stored for culture. After growth conditions and growth media are optimized for maximal growth, the cells will be induced with methanol to trigger antibody production. The products are isolated and purified by a series of chromatography. Data for this lab will consist of diagnostic checkpoints. Data will include gel electrophoresis pictures that confirm that the correct DNA sequence is integrated and western blot pictures that identify if the correct protein was made. These Fab fusion proteins will then be assembled by treating with small molecule crosslinkers, purified using preparatory chromatography and then assessed for correct function via various biochemical methods such as ELISA and Biacore; and also via cell-based assays.

Preparation

During the previous summer, I participated in Northwestern's iGEM (International Genetically Engineered Machine) competition and learned DNA design and cloning. Additionally, I took or would complete various courses that will assist me. I took ChemE375: Biochemical Engineering where I learned how to design culture conditions for biochemical protein synthesis and BME315 Application of Genetic Engineering to Immunochemistry. Spring quarter I plan on taking BME395 Engineering Design of Therapeutic Antibodies. Both of these classes will provide me with an in-depth understanding of how antibodies work and how to design them.Additionally, I have been doing preparatory work for this project since I have joined the Mrksich lab in the fall of 2013. I designed the DNA cloning vector for effective integration of antibody DNA into the plasmid. During the winter of 2014, I have started cloning DNA sequences for the baculovirus. During the spring of 2013, I expect to get an early start with infecting Sf-9 cells.

Conclusion

Through this summer project, I hope to gain experiences in cell culture and protein purification as part of my academic pursuit in recombinant protein pharmaceuticals as well as pursuing a PhD. I expect to continue with this research after the summer in order to obtain a broader and deeper insight into antibody conjugated drugs (ACD).

References

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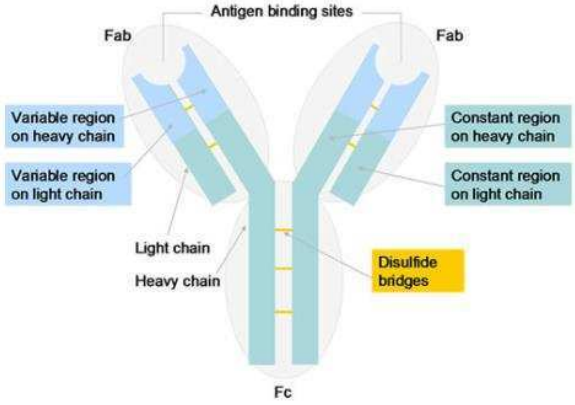
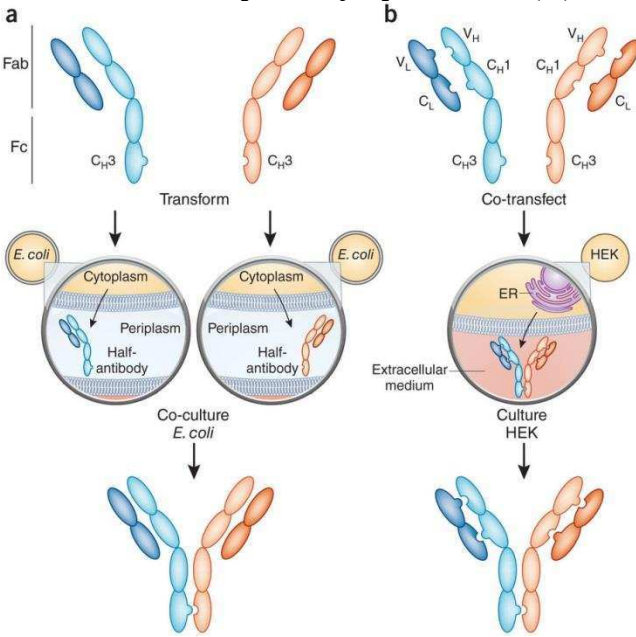
Appendix A: Antibody Structure



Appendix B: Schematic of Methods Proposed by Spieess et al.(A) and Lewis et al. (B)



Appendix C: Enzyme Assisted Protein Assembly for Fab-enzyme Fusion Proteins