CHAPTER 5

BIOCHEMISTRY

Doctoral Theses

01. MATHUR (Kapil)
Development of Reagents for Detection of *Mycobacterium Tuberculosis* and a Novel System for Identification of Boimarkers.
Supervisor: Prof. Vijay K. Chaudhary
Th 23781

Contents

1. Introduction and review of literature 2. Production of recombinant 19 kDa antigen (Rv 3763, a cell surface molecule of M. tuberculosis) and use of anti-19k Da monoclonal antibodies in detecting M. tuberculosis cells 3. Cloning of DNA encoding functional variable domains 9Vl & Vh) of anti-19k Da MAbs. 4. Development of novel phage lambda display vectors for regulated display of proteins 5. Development of reagents to detect displayed proteins in lambda display system 6. Optimization of bio-panning conditions using lambda display libraries 7. Summary and conclusions. Appendices and publications

02. ROHILLA (Akshay)
Identification and Evaluation of Inhibitors against important Drug targets of Mycobacterium Tuberculosis.
Supervisors: Dr. Garima Khare and Prof. Anil K. T`yagi
Th 23671

Abstract
(Verified)

Tuberculosis remains one of the deadliest diseases in the world killing around 10.4 million people in 2015 worldwide. Long duration of the treatment by the frontline drugs often leads to non-compliance ultimately leading to the generation of drug resistant *Mycobacterium tuberculosis (M. tb)* strains. Thus,there is an urgent need to identify new inhibitory molecules to fill the pipeline. We targeted essential proteins involved in iron homeostasis inside *M. tb*. Virtual Screening, pharmacophore development and structure based similarity search lead to the identification of various inhibitory scaffolds against IdeR, an essential transcriptional regulator of *M. tb*. Compound I-108 exhibited IC of 24 µg/ml in EMSA, MIC of 17.5 µg/ml against the growth of *M. tb in vitro*, negligible cytotoxicity to various mammalian cell lines and intraphagosomal inhibition of *M. tb* at 125 µg/ml. We also targeted PptT (4' Phosphopantetheinyl tranferase) of *M. tb*. PptT carries out phosphopantetheinylation type post translational modification of various proteins in *M. tb*. Virtual screening and structure based similarity search led to the identification of two inhibitory scaffolds against PptT. N, N-diethyl-N`-(2- methylquinolin-8-yl) propane-1, 3 di amine emerged as one of the potent inhibitory scaffolds with 5 analogues exhibiting IC < 1 µg/ml. PS40 emerged as one of the most potent inhibitory molecule with IC of 0.25 µg/ml, MIC of 10 µg/ml, negligible cytotoxicity to various mammalian cell lines and intraphagosomal inhibition of *M. tb* at 40 µg/ml. We carried out homology based 3-Dimensional structure determination of MbtA, MbtB and MbtE (Mycobactin synthases) followed by docking studies. MIC studies led to the identification of few inhibitory molecules with negligible cytotoxicity against mammalian cell lines tested. Thus, *in silico,*

in vitro and *ex vivo* studies were carried out against IdeR, PptT, MbtA, MbtB and MbtE to identify various inhibitory molecules against *Mycobacterium tuberculosis.*

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1. Introduction 2. Review of literature 3. Aims and objectives 4. Summary and conclusions 5. Appendix

03. VERMA (Vaishali)

Strategies for Selection of Cognate Antigen- Antibody Pairs for *M. tuberculosis* Proteome.

Supervisors: Prof. Vijay K. Chaudhary

Th 23670

Abstract
(Verified)

Due to their exquisite specificity towards the target, antibodies find tremendous utility in basic research,diagnostics, and therapeutics. It was conceived that the availability of a comprehensive collection of monoclonal antibodies directed against the whole proteome of any organism can serve as an extremely valuable resource for studying localization, function, and quantification of the expression levels of different proteins and will facilitate numerous applications pertaining to both infectious and non-infectious diseases. The conventional approaches for antibody production allow selection of antibodies against one target at a given time. In addition, these approaches require purified preparation of the target protein, which may not be available for the whole proteome. In essence, to obtain specific monoclonal antibodies at genome-scale, an efficient strategy is required that circumvents the need for purification of every protein individually, and allows the selection of antibody library against antigen library, followed by identification of cognate antigen-antibody pairs in high-throughput format with minimal background. The work embodied in this thesis describes a streamlined and robust strategy to produce protein targets at genome-scale, selection of recombinant antibodies from a naïve human antibody library, and finally to decipher the target for each selected recombinant antibody in a high-throughput manner. The first part of the strategy involves the selection of specific binders from a naïve human phage-displayed antibody library available in the laboratory against a mixture of mono-biotinylated protein fragments obtained from an ORF-selected DNA fragment library. The final part of the strategy employs a combination of phage display and NGS technology for the determination of the specificity of individual antibodies selected against a mixture of proteins in a high-throughput format. The strategy has been successfully optimized using *M. tuberculosis* as the model, and is expected to enable rapid selection of specific monoclonal antibodies against a large number of targets in parallel.

Contents

1. Introduction and review of literature 2. Construction of an ORF-selected M. tuberculosis 30 gene fragment library using split Beta-lactamase complementation system 3. Characterization of a phage displayed naïve human antibody library 4. Development of vectors for soluble expression of protein and antibody fragements in E.coli 5. Development of system for high-throughput selection of human antibody fragments against M. tuberculosis protein fragments and identification of cognate antigen-antibody pairs 6. Summary and conclusions. Appendices and publications