CHAPTER 2
AIMS AND OBJECTIVES

2.1 INTRODUCTION

Lung cancer is the leading cause of constituting 8.73% of relative proportion of all cancers in Bangalore (NCRP, 2008). The development of specific tyrosine kinase inhibitors (TKIs) such as Erlotinib against the EGF Receptor has been promising in targeted therapy of lung cancers. Specific driver mutations in EGFR kinase domain are responsible for determining sensitivity or resistance to TKIs. More than 50% of the mutations have a leucine to arginine substitution at position 858 (L858R) that confers sensitivity to Erlotinib. The goal of this study was to identify the sites of phosphorylation of proteins and quantify the degree of phosphorylation upon ligand stimulation or TKI inhibition of a lung adenocarcinoma cell line harboring a TKI-sensitizing mutant EGFR. This section describes in detail the aims and objectives to identify potential mechanisms of drug sensitivity and new targets for drug treatment

2.2 AIM 1

IDENTIFICATION OF DIFFERENTIALLY PHOSPHORYLATED MOLECULES IN THE EGFR SIGNAL TRANSDUCTION PATHWAY USING A SILAC PHOSPHOPROTEOMIC APPROACH

Hypothesis: Investigation of global phosphorylation kinetics in a model cell line system using quantitative proteomics will help identify signaling molecules that are regulated by the EGFR tyrosine kinase

Rationale: EGFR is a tyrosine kinase which either by autophosphorylation or phosphorylation of downstream molecules transduces signals from the surface of cells to mediate control on gene expression by effecting transcription and translation factors.

Methodology: Using SILAC we hope to quantify these interactions downstream of the EGFR pathway by specifically enriching these phosphorylated proteins by a dual enrichment strategy; using antibody enrichment for phosphotyrosine peptides and a TiO₂
based enrichment for the serine and threonine residues that are phosphorylated on proteins. We hope to identify these molecules using a high throughput mass spectrometric approach.

2.3 AIM 2:

IDENTIFICATION OF SIGNALING PATHWAYS THAT ARE DIFFERENTIALLY REGULATED ON TREATMENT WITH ERLOTINIB IN H3255 AN EGFR MUTANT CELL LINE

Hypothesis: Phosphorylation of tyrosine/serine/threonine residues decreases on treatment with the tyrosine kinase inhibitor Erlotinib and identifying signaling molecules that are dephosphorylated one can identify true substrates of the inhibitor with respect to EGF signaling post activation.

Rationale: In identifying true substrates, an inhibitor based approach helps to compare cells treated only with the growth factor with cells treated with the inhibitor and the growth factor to identify molecules that are integrally part of the EGFR signaling cascade. In using a mutant cell line that harbors the EGFR mutant we hope to apply this knowledge of signaling to understand proteins that could be responsible for determining sensitivity in NSCLC adenocarcinomas.

Methodology: A three state SILAC with untreated cells labeled with light amino acid, cells treated with EGF labeled with medium amino acids and cells treated with Erlotinib and EGF labeled as heavy. Using such an experimental design we hope to identify molecules and later using bioinformatics analysis to narrow down pathways that are critically regulated by EGF and Erlotinib
2.4 Aim 3:

Validation of the Phosphorylation Status of Key Molecules in Signaling Pathways to Verify Mass Spectrometric Findings

Hypothesis: Targeted validations using phospho specific antibody based strategies validate mass spectrometric observations by confirming sites and physical change in phosphorylation status.

Rationale: Increase or decrease in phosphorylation can be viewed distinctly using Western blot to confirm mass spectrometric observations at the protein level.

Methodology: Antibodies and kinase (MAPK and RTK) phospho antibody arrays will be used in tandem with conventional Western blots to investigate cell lysates to confirm mass spectrometric identifications to avoid false positives