CHAPTER 6

CONCLUSIONS

6.1 CONCLUSIONS

This study comprehensively details the identification of signaling molecules in the EGFR signaling pathway that have been identified using a high throughput mass spectrometric approach. SILAC was used to label cells that were treated with the growth factor EGF and the drug Erlotinib. Using a 3-plex SILAC method, we have identified several signaling molecules that are activated on EGF and dephosphorylated on Erlotinib that are key molecules that could be targeted as drug targets or as promising markers. We have been able to show certain important signaling pathways that are modulated again reason for further biomarker research. This is a comprehensive phosphorylation profile of the H3255 lung adenocarcinoma cell line for identifying possible markers as indicators of sensitivity to the reversible tyrosine kinase inhibitor Erlotinib. We have used phospho RTK and MAPK arrays that show modulation of certain key kinases that are modulated with the drug. Again because of only pan antibodies that are available for certain molecules site specific validation was not comprehensive for all possible targets that we hoped to investigate.

We have reported several molecules that have been implicated in EGF signaling that could play a very vital role in this pathway. Among some of the molecules that were identified, we identified several members of the ERBB family and observed change in phosphorylation dynamics of signaling molecules that are differentially regulated in the PI3/AKT/mTOR pathway. We for the first time have been able to show change in phosphorylation of certain residues that are activated on EGF and are dephosphorylated on Erlotinib. Identification of these molecules is vital in order of identifying novel molecules in the EGF pathway. Signaling molecules that constitute the ERFGF, VEGF and Ephrin pathways have also been identified and either by mass spectrometric or by antibody arrays, we have been able to show change in phosphorylation on treatment with EGF and deactivation followed by treatment with Erlotinib. We report also for the first time, certain kinases, phosphatases, autophagy proteins, transcription and translation proteins that are modulated and are key molecules that are part of the larger EGF signaling pathway.

The bigger question that we hope to address is the development of resistance in lung adenocarcinoma cells that on exposure to Erlotinib develop resistance. This project hopes to identify the phenotype of only the L858R mutation. Ongoing experiments with different cell lines form our lab that was done to investigate the phosphorylation status of signaling molecules using the H3255 cell harbouring the L858R mutation. In understanding the change with treatment with the inhibitor, we have also identified key pathways that are modulated in EGFR L858R mutant cell line. In exploring the phospho dynamics, we have indicated a series of molecules that are vital substrates on which the Erlotinib acts. This again in the first report that catalogues all critical molecules in EGF signaling in the lung adenocarcinoma cell line H3255.
We postulate that on treating the cells with Erlotinib, the cells undergo apoptosis. This is with regard to the PI3K/AKT/mTOR pathways that are activated and also from several of the autophagy related molecules that show drastic changes on treatment with the drug. More experiments though need to be done to determine additional key regulatory elements of the pathway.