ABSTRACT

Lung cancer is the leading cause of mortality in India with cancers of the lung 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 Epidermal growth factor receptor (EGFR) 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. EGFR activation at the membrane triggers a series of tyrosine and serine/threonine phosphorylation cascades. 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. H3255, a lung adenocarcinoma cell line harboring L858R EGFR was SILAC (Stable isotope labeling with amino acids in cell culture) labeled and phosphorylated serine/threonine/tyrosine peptides were enriched using a titanium dioxide based chemical method and also an antibody enrichment method (Phosphoscan). A 3-plex SILAC labeling method where control cells were labeled “light”, cells treated with EGF labeled “medium” and cells treated with EGF and erlotinib were labeled “heavy” was used. From the Phosphoscan enrichment protocol we could identify a total of 460 tyrosine (pY), 64 serine (pS) and 49 threonine sites (pT) that represents 207 proteins while in tandem, in the TiO2 enrichment protocol we could identify of a total of 3586 phosphosites (3167 phosphoserine sites, 395 phosphothreonine sites and 24 phosphotyrosine sites) that represents 1434 proteins. The higher counts for the serine and threonine residues are representative of the biological stoichiometry where serine/threonine residues are more abundant than tyrosine phosphorylated residues. With EGF activation, nearly 11% of peptides were hyperphosphorylated, 72% remained unchanged while 17% were dephosphorylated. Looking at how each of these peptides behaved on treatment with Erlotinib, the majority of the sites 62% remain unchanged on EGF and Erlotinib treatment which is evident of specific signaling molecules that are phospho regulated. A very small percentage of sites are actually activated on Erlotinib treatment (5.2%). We do not know currently as to why these molecules are hyperphosphorylated. This class of molecules could be important but more studies need to be done to confirm the same. Molecules that are of significant importance to this study is a fine niche of 15% of all peptides that were identified are those that are activated on EGF but dephosphorylated on treatment with Erlotinib. These changes could be because of direct or indirect action of the drug. We identified changes in phosphorylation of several key molecules including several kinases, adapter molecules, transcription and translation factors, autophagy molecules, ERBB, VEGF and PI3K-AKT-mTOR pathway members that are regulated on treatment with erlotinib. These approaches have the potential to identify mechanisms of drug sensitivity and new targets for drug treatment.