CHAPTER 6: Summary
Functional proteomics provides a powerful method for monitoring the global molecular responses following activation of signal transduction pathways, reporting altered protein post translational modifications and expression provides a complementary and potentially more comprehensive approach to the analysis of signaling mechanism by resolving the expressed proteins of the cell ("proteome") followed by protein sequencing and identification (Alizadeh et al., 2000; Shepard et al., 2000). Changes in protein profiles during signaling events can be monitored using two-dimensional (2D) gel electrophoresis (Gorg et al., 1988; O'Farrell, 1975). Furthermore, identification of proteins in subpicomolar quantities via mass spectrometry is performed, and posttranslational modifications can be detected and mapped (Wilm et al., 1996). Thus, in the same experiment, targets of signaling pathways can be identified by changes in transcriptional and posttranscriptional regulation, providing novel mechanistic insight into how signaling events elicit complex biological responses.

We have demonstrated by this study that quantitative phosphoproteomics is a streamlined and is a generic tool that can be broadly applied to study phosphoproteomics. Our screen is based on creating cell populations distinguishable by MS using SILAC, phosphoprotein enrichment and incorporates the latest advances in phosphopeptide enrichment by SCX fractionation, employs
the rapid, automated and highly accurate mass spectrometry, and finally makes optimal use of the data through the software based analysis.

Phosphorylation is a critical modification for various signal transduction events in normal as well as transformed cells and selective inhibition of the crucial kinases or phosphatases has been proven to be a useful strategy for correcting aberrant enzyme activities. But since last few decades, scaffold proteins have also emerged as important modulator of signaling and potent target for drug development. Because these kinases regulate various cellular functions, inhibiting their activity is likely to affect multiple processes, some not linked to the pathophysiology of the disease being targeted.

This work represents proof-of-principle that the combination of large-scale phosphoproteomics and a loss-of-function approach can contribute significantly to elucidating the role of key players in phosphorylation-dependent signaling; still there are chances of improvement. The incorporation of technological advances in mass spectrometry and the application of novel protein and peptide enrichment techniques will increase the sensitivity and accuracy of detecting phosphoproteins and identifying phosphorylation sites. Such advancement has already been made (Olsen et al., 2006; Shu et al., 2004). The degree of phosphorylation measured site specifically rather than for the protein as a whole in order to obtain accurate and functionally relevant understanding of activation kinetics would elucidate the molecular mechanism of such altered pathways. This goal can be accomplished by performing phosphate loss or dephosphorylation study, followed by manual inspection and use of bioinformatics based software tools to prevent false positives. Thus, the obtained data could be easily interpreted by modeling the
system by various available online GO functional annotation tools and involving systems biology. These high throughput phosphoproteomic have the potential to monitor information flow through large portions of signaling networks that ultimately control the overall response of a cell to changes in its environment.