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Phosphoproteomics and gene expression profiling reveals systems-wide changes in response to EGF receptor activation

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Introduction: The epidermal growth factor receptor (EGFR) is a receptor tyrosine kinase that plays a major role in regulating cellular processes including proliferation, differentiation and migration. Binding of its ligand leads to the activation of intracellular signaling pathways, which is commonly achieved through protein phosphorylation. Effectors are often transcription factors that promote increased RNA synthesis. Deregulation of these receptors has been found to be associated with a number of diseases such as cancer and diabetes. Because of this signal transduction through EGFR has been studied intensively, but the molecular mechanisms of the regulation of cellular behavior through the receptor are still not fully understood. To gain a comprehensive picture of how these signal pathways ultimately lead to changes in gene expression and cell phenotype after EGFR activation we have performed the first global study using phosphoproteomics, transcript profiling and phenotypic analyses to identify how EGFR mediated signaling networks regulate transcriptional activity, gene expression and cellular behavior on a global level.

Material and Methods: HEK293 cells overexpressing EGFR were stimulated with EGF for different amounts of time. For the proteomic analysis proteins were trypsin-digested and the peptides labeled with iTRAQ for quantitation. Phosphorylated peptides were enriched using an anti-phosphotyrosine immunoprecipitation and iron-activated IMAC. Peptides were analyzed with LC-MSMS on an LTQ Orbitrap Velos mass spectrometer. Proteins were identified using SEQUEST, and quantification of the iTRAQ reporter signal was performed with Libra. Similarly, RNA was isolated from cells stimulated with EGF and generation of libraries and sequencing was done using the Illumina pipeline. The sequences were mapped using Tophat and quantitation of transcripts was done with DEseq. Phenotypic assays were done using real time contrast-phase, and fluorescence microscopy inside our tissue culture incubator.

Results: Using phosphopeptide enrichment strategies and high resolution mass spectrometry, around 5500 phosphosites have been identified. Of these about 400 are phosphotyrosines which are the main means of signal transduction by receptor tyrosin kinases such as EGFR. The peptides were quantified over the course of 2 hours using isobaric tags (iTRAQ) which allow the precise calculation of their relative concentrations. This quantitation shows that the levels of phosphopeptides change over time in response to EGFR stimulation. Several of these identified proteins are known effectors of EGFR signaling such as several MAP kinases, Ras and mTOR. In addition several novel proteins that show phosphorylation in response to EGF stimulation have been identified. Using RNAseq we identified 14743 transcripts. 1310 of these transcripts changed significantly over a 24 h time course. The biggest change with respect to the control occurred 12 h after stimulation with 736 changed transcripts. Analysis of the immediate response after EGFR stimulation (3 h) and later time points (12 and 24 h) shows that there are distinct sets of transcript that represent early and late changes in the cell. Gene ontology analysis of upregulated transcripts shows that a large number are involved in DNA binding and transcriptional regulation. To identify the transcription factors involved in causing these changes upregulated transcripts were assayed for the presence of 445 known transcription factor motifs. Of these 255 motifs were found to be enriched within our dataset including binding motifs for transcription factors known to be regulated by the EGFR signaling pathways such as Myc, Jun and Fos. In addition assays have been performed measuring cellular behavior (proliferation, apoptosis) in response to EGFR activation. Integration of the data obtained from the three experiments will allow us to create network models. These models will highlight critical nodes of the signaling networks which can be exploited as potential therapeutic targets and they will enable us to predict cellular behavior.

Conclusion: In this comprehensive approach proteomics, transcription profiling and phenotypic assays are used together for the first time to increase our understanding of the signaling events after EGFR activation which will help us gain better understanding of EGFR mediated biology as well as identify new potential therapeutic targets or diagnostic tools.

Biography

Alejandro Wolf-Yadlin obtained his Ph.D. from the Massachusetts Institute of Technology Bioengineering Division and completed his postdoctoral training at Harvard University Chemistry Department. He is an Assistant Professor at the University of Washington Department of Genome Sciences. He has published more than 10 papers in reputed journals and serves as reviewer for many others. He works on the development and application of new high throughput proteomic tools for the study of cellular signaling networks. He lectures yearly at Cold Spring Harbor Laboratory Proteomics Course and is a member of the study panel for the Russian Federation's Skolkovo Institute of Technology.

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