Unraveling Ubiquitination Dependent Dynamics in Cell Signaling Networks by Quantitative Proteomics

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***Short Abstract* — Ubiquitination is a reversible post translational modification of proteins, recognized as major regulatory mechanism in numerous cellular processes, including proteins proteasomal degradation, endocytosis and vesicular trafficking, DNA repair. It also plays a significant role in growth factor signaling and it is required for efficient receptor down-regulation through endocytosis and degradation in lysosomes. We designed and utilized a quantitative mass spectrometry-based proteomics approach in order to identify the ubiquitination-dependent protein complexes in the epidermal growth factor receptor (EGFR) signaling network, as well as to profile their dynamic changes over time-course of ligand stimulation.**

***Keywords* — ubiquitination, quantitative proteomics, mass spectrometry, dynamics, cell signaling, receptor tyrosine kinases (RTK), protein networks.**

I. Introduction

THE ubiquitination is a post translational modification of proteins that has been recognized as a major regulatory mechanism in various cellular processes. Depending on the number and type of ubiquitin moieties attached to target proteins they can be mono-, multi- and polyubiquitinated making ubiquitination studies extremely challenging. This diversity of ubiquitin signals defines the stability, localization and activity of the taggeted protein.

Many growth factor receptors are known to undergo ubiquitination upon activation that is required for efficient receptor down-regulation through endocytosis and degradation in lysosomes [1]. These complex and dynamic processes involve many regulating components of the endosomal machinery, including different types of ubiquitinating and de-ubiquitinating enzymes, endocytic scaffold and adaptor proteins. The latter contain ubiquitin binding domains (UBDs) that are responsible for the recognition and sorting of the ubiquitinated receptors along the endocytic pathway [1, 2].

Here we describe a general strategy to study the complex and dynamic process of ubiquitination. Our aim is to identify the ubiquitination-dependent protein complexes in the epidermal growth factor receptor (EGFR) signaling network and to profile their dynamic changes over time-course of ligand stimulation. Our results may provide better understanding of the dynamic processes of ubiquitination-dependent signaling and help to elucidate the mechanisms of signal transduction regulation.

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II. Strategy and Results

A major challenge of such research work is to separate the ubiquitinated proteins functionally associated with EGFR signaling events from the much larger fraction of other ubiquitinated proteins and the free ubiquitin in the cell. We designed quantitative mass spectrometry (MS)-based proteomics approach using several stages of affinity purification and Stable Isotope Labeling by Amino acids in Cell culture (SILAC) [3]. Three populations of HeLa cells were differentially encoded using the triple-SILAC approach and then stimulated with EGF for three different time-points [4, 5]. Subsequently, cell lysates were mixed and incubated with recombinant Ubiquitin-binding domains (UBDs) from different endocytic adaptor proteins. Precipitated ubiquitinated complexes were separated by gel electrophoresis, digested with trypsin and quantitatively analyzed by high-accuracy mass spectrometry (LC-MS/MS). Repeating the experiment for another two triplets of time-points and having a common time point for all three sets of experiment, resulted in detailed seven time-points dynamic profile of the ubiquitination-dependent EGFR signaling network. Different categories of proteins of the EGF receptor endocytic pathway displayed distinct intensities and ubiquitination dynamics reflecting their unique roles in this complex process.

This general SILAC-based proteomics approach resulted in the quantitative characterization of the ubiquitination dependent dynamics upon growth factor stimulation and it should be easily applicable to other ubiquitination systems as well.

References

[1] Haglund K, Dikic I. (2005) *EMBO J*. **24**, 3353–3359.
[2] Hoeller D, Crosetto N, Blagoev B et al. (2006) *Nat Cell Biol*. **8**, 163-169.
[3] Blagoev B, Kratchmarova I, Ong SE et al. (2003) *Nat Biotechnol*. **21**, 315-318.
[4] Blagoev B, Ong SE, Kratchmarova I, Mann M. (2004) *Nat Biotechnol*. **22**, 1139-1145.
[5] Dengjel J, Akimov V, Olsen JV et al. (2007) *Nat Biotechnol*. **25**, 566-568