Ubiquitin Mass Spectrometry Kit: A Mass Spec Approach to Ubiquitomics

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BACKGROUND

The ubiquitin proteasome system (UPS) is a tightly regulated pathway that eukaryotic cells employ for myriad cellular functions. At the center of this system is ubiquitin (Ub), a small (8.5 kDa) protein that is conjugated to target proteins *via* its C-terminal glycine to a lysine residue of the protein of interest (POI). Additional Ub molecules can be attached to the conjugated Ub on the POI at its M1 methionine or any of its seven lysine residues (K6, K11, K27, K29, K33, K48, K63). This site-specificity gives rise to a coded language in the UPS and earmarks the protein for different fates. For example, the most well-studied UPS pathway concerns K48 chains, which flag proteins for 26s proteasomal degradation. (1) Another example is K6 chains, which have been associated with the DNA damage response and mitophagy. (2) Ubiquitylation is reversible – deubiquitinases (DUBs), which are also chain-specific, will cleave ubiquitin chains from target proteins and prevent them from being degraded. Thus, a balance is struck in the UPS wherein proteins are continually marked and unmarked for degradation depending on cellular needs and stimuli.

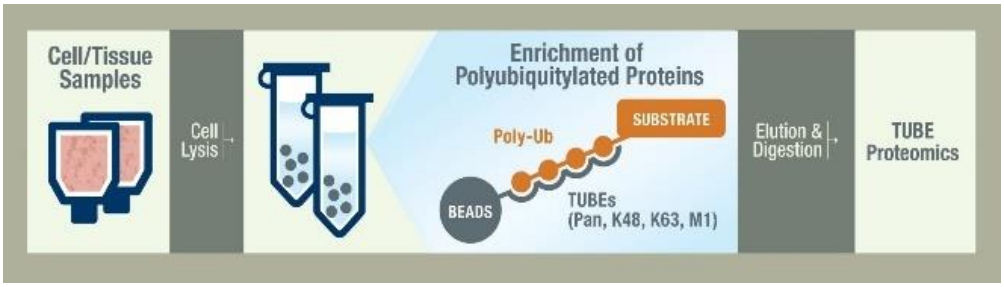

***Figure 1**: **Schematic representation ubiquitylated protein enrichment and proteomics workflow using TUBE technologies**. In general, cell lysates and tissue homogenates are incubated with beads coated in high-affinity, high-specificity TUBEs to capture poly-ubiquitylated targets, pulled-down, and submitted for proteomics analysis.*

In recent years, hijacking the UPS for targeted protein degradation or salvage has been a desirable target for small molecule drug discovery. These candidates will often bring the target protein in proximity to an E3 ligase for enhanced degradation (PROTAC) or deubiquitinase for protein stabilization (DUBTAC). Keeping with this trend is the need to identify ubiquitinated targets and the ubiquitin chains they harbor. “Ubiquitomics”, or the accounting of ubiquitylated proteins in a cellular environment, seems to be an emerging player in the drug discovery and therapeutic spaces. (3) However, knowing what proteins are ubiquitinated under certain disease states or stimuli is challenging; the dynamic nature of UPS signaling is a major obstacle to the isolation and functional characterization of polyubiquitinated proteins. For this reason, the ubiquitination state and identity of many proteins is unknown and poorly understood.

To address these concerns and to aid the research community, LifeSensors has developed UbiMS, a mass spec ubiquitomics kit. Designed to capture, enrich, and quantitatively profile ubiquitinated targets by mass spectrometry, the kit capitalizes on LifeSensors TUBE technology that effectively captures polyubiquitinated proteins with high efficiency ($K_D \leq 1$ nM). This kit enables the researcher to conduct a variety of experiments regarding ubiquitomics, including (but not limited to) the identification of ubiquitination sites, understanding protein degradation pathways, drug discovery and validation, and crosstalk between post-translational modifications.

Tandem Ubiquitin Binding Entities (TUBEs)

TUBEs are protein-based technologies that capitalize on the enhanced avidity of ubiquitin binding domains (UBDs). In short, they are multiple copies of a single UBD (ca. 5-15 kDa) that are strung together by a linker chain. These species are optimized for both the UBD monomer and the chain-linker to ultimately give high-binding affinities, and in some cases, chain-selectivity. These tools are comparable to antibodies with respect to binding affinities and specificity but offer multiple advantages in downstream applications such as mass spec proteomics and pull-down assays. (4-8)

The UbiMS Kit comes with a pan-selective TUBE, but is interchangeable with other chain-selective, magnetic TUBEs, depending on the end-users needs. In summary, cell lysates are prepared, incubated with magnetic beads coated with TUBEs, the ubiqutylated proteins are enriched and eluted off the beads, and prepared for mass spec analysis (**Figure 1**). The digested proteins are identified against a database and quantified, providing an accurate reading of how much of the target is present in the ubiquitome of the sample. This kit enables robust and sensitive detection of ubiquitylated proteins with a high degree of sensitivity. Herein, we describe some recent advances in this MS approach to ubiquitomics and showcase its applications in various biological studies.

APPLICATIONS

Identification of an E3 Ligase and its Substrate in Systemic Sclerosis

Systemic scleroderma (SSc) is an autoimmune disease that results in dysfunctional connective tissues *via* excessive profibrotic signaling. This adversely affects numerous tissues, particularly in the lungs. Recent studies have shown profibrotic signaling by transforming growth factor-β (TGF-β) is directly linked to SSc, and that this signaling pathway is controlled in part by E3 ubiquitin ligases/UPS. Lear *et al.* showed by MS ubiquitomics that a connection exists between an E3 ligase, Kelch-like protein 42 (KLHL42), and SSc lung fibroblasts. (9)

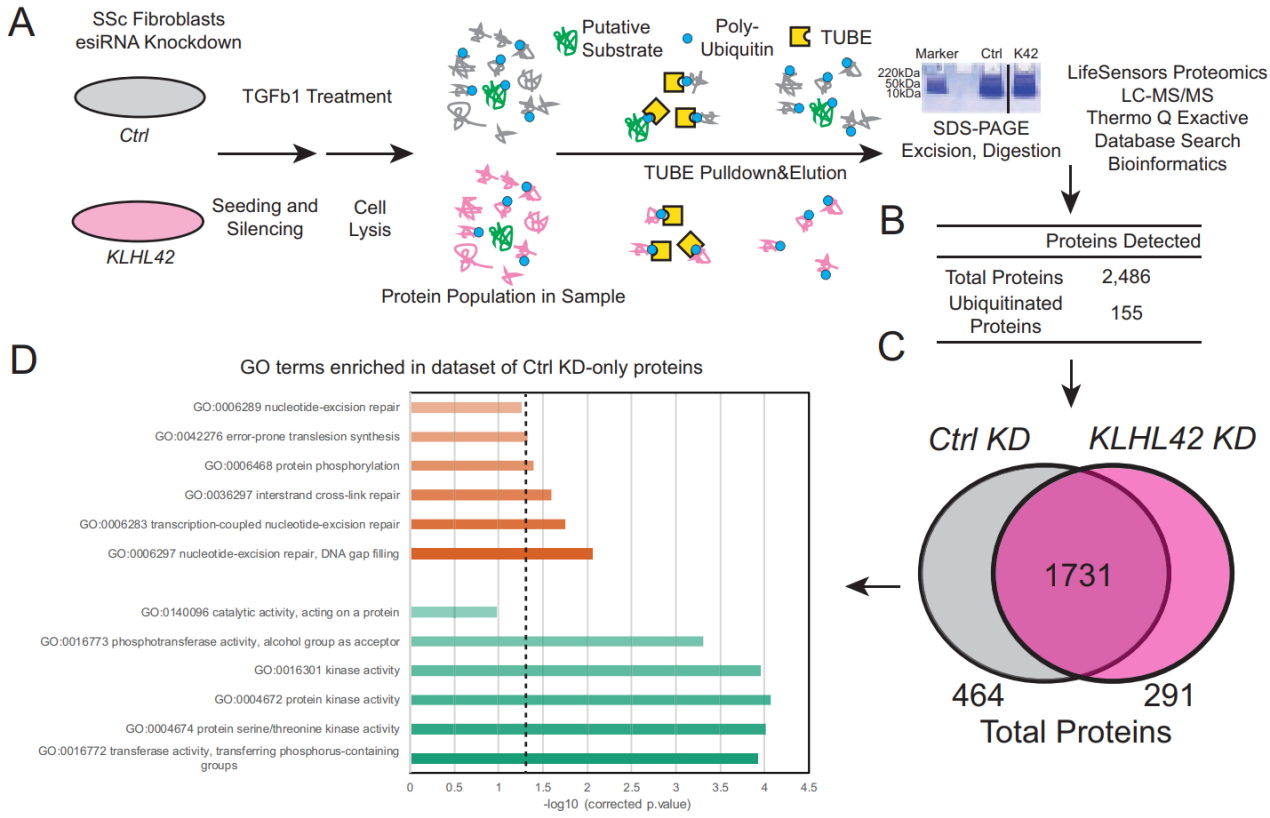

***Figure 2*: Ubiquitin proteomics screening reveals PPP2R5ε as a substrate for KLHL42.** *Workflow schematic of ubiquitin proteomics performed on SSc control fibroblasts and KLHL42 knockdown mutants. TUBE enrichment was performed on cellular lysates, separated by SDS-PAGE, excised, and trypsin digested prior to MS analysis. PPP2R5ε was detectable in the control KD-only proteome and directly detected to be ubiquitinated. Adapted from Reference (9).*

Initial RNAi screening of E3 targets in SSc patient lung cell samples revealed a knockdown of KLHL42 impairs TGF-β activation. In their MS ubiquitomics experiment, the authors compare the ubiquitylated proteomes of WT control cells with the KLHK42 knockdown cells. Silencing the KLHL42 ligase would presumably reduce the degree of ubiquitylation of any substrates. Using TUBE-base pulldown/enrichment on the KLHL42 knockdown, the substrate for KLHL42 was indeed revealed to be phosphatase 2 regulatory subunit B'ε (PPP2R5ε) by LC-MS/MS (**Figure 2**). Subsequent knockdown experiments of PPP2R5ε led to pronounced TGF-β-mediated profibrotic signaling. Together these findings suggest a role between KLHL42/PPP2R5ε and in profibrotic signaling in SSc fibroblasts. Notably, there are no approved drug candidates to manage lung SSc. This research lays foundational groundwork for drug discovery and development for this disease.

Other Application of UbiMS

Other application of UbiMS include (but are not limited to):

- Identification of new E3 ligases and their substrates
- Mechanistic studies of E3 Ligases and substrates (e.g. ubiquitination sites, degree of ubiquitylation, etc.)
- Efficient screening of small-molecule drug candidates (e.g. PROTACs & molecular glues)
- Diagnostic/clinical biomarker discovery

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About LifeSensors, Inc.

LifeSensors is a biotechnology company located 35 miles west of Philadelphia, Pennsylvania, USA. Founded in 1996, LifeSensors has developed a number of innovative protein expression technologies that enable efficient translation of the genome into proteome.

LifeSensors is well-known for its innovations in an important family of proteins consisting of ubiquitin and ubiquitin-like proteins (UBL) such as SUMO (Small Ubiquitin-like MOdifier).

LifeSensors has been granted several patents to cover the use of SUMO and other UBLs as gene fusion tags to improve the expression and purification of recombinant proteins. Additional patent applications are in various stages of review. Currently, LifeSensors is expanding its protein production capabilities and is developing a protein micro array for drug discovery and diagnostics.

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