Proteomics and Functional Investigation of SUMO and Ubiquitin E3 ligases

Salas Lloret, D.

Summary

In this thesis, we focus on the post-translational modification of proteins (PTM) with special interest in Ubiquitination and SUMOylation. We describe both the Ubiquitin and SUMO E3 ligase families, showing not only their different mechanisms to conjugate Ubiquitin or SUMO respectively to a substrate protein, but also the signaling pathways they are involved in. E3s can conjugate one or several moieties to a substrate protein giving rise to polymers. Depending on the chain type and polymer morphology, the substrate protein will lead to different cellular outcomes. At the end of chapter 1, we analyze current Mass-Spectrometry (MS) approaches to identify both Ubiquitin and SUMO conjugates with their advantages and limitations.

In chapter 2, we review recent literature about the DNA damage repair (DDR) and how it is orchestrated by different E3 ligases. Among these E3s, we focus on BRCA1-BARD1 as one of the main coordinators of the DDR and its implications in breast and ovarian cancers. The BRCA1-BARD1 heterodimer is formed by the interaction of BRCA1 and BARD1 through their Really Interesting New Gene (RING) domains. The formation of this heterodimer confers its only known intrinsic enzymatic activity, with only one well-defined ubiquitination target, the histone H2A. After more than 30 years studying BRCA1 protein, its E3 ligase activity remains unclear and its role in homologous recombination (HR) repair pathway and its tumor suppression role is still unresolved. We discuss the controversy about this heterodimer and the current directions in the field. To finish this chapter, we disclose several ways to target the ubiquitination machinery and control the DDR in order to tackle cancer.

In chapter 3, we developed a Mass-Spectrometry methodology that we termed Targets for Ubiquitin Ligases Identified by Proteomics 2 (TULIP2). There are many diseases including neurodegenerative disorders and cancers that come from dysregulation of E3 ligases. TULIP2 allows the identification of specific targets for these E3 ligases using MS and offers new possibilities to target a particular disease. Compared to previous methodologies, TULIP2 increases more than 50-fold the purification yield of ubiquitination conjugates and 8-fold the signal of the E3 ligase under study. Overall, this chapter offers a clear and detailed methodology that can be implemented in any laboratory interested in the identification of targets for an E3 ligase of interest.

TULIP2 methodology can be modified to trap SUMO conjugates by changing the fused ubiquitin by a SUMO moiety. In chapter 4, we developed SUMO Activated Target Traps (SATTs), which allowed the identification of an E3-specific SUMO proteome. Employing eight different SUMO E3 ligases (PIAS1, PIAS2, PIAS3, PIAS4, NSMCE2, ZNF451, LAZSUL (ZNF451-3) and ZMIZ2) we identified 427 SUMO1 and 961 SUMO2/3 potential targets in an E3-specific manner. Although we found redundancy between E3 ligases, we also found high specificity, even at the substrate isoform level. To browse the dataset in an user-

friendly way, we developed the Polar Volcano plots online web app, which is freely accessible (https://amsterdamstudygroup.shinyapps.io/polarVolcaNoseR_revised/).

Next, we employed TULIP2 technology to study the BRCA1-BARD1 E3 ligase. In chapter 5, we generated both BRCA1-TULIP2 and BARD1-TULIP2 to search BRCA1-BARD1-specific ubiquitination targets. BRCA1 and BARD1 germline mutations are associated with high risk of developing breast and ovarian cancer. Finding BRCA1-BARD1 ubiquitination targets is crucial to understand its E3 activity and therefore, new possibilities for cancer treatment. Performing TULIP2, we found that BARD1 preferentially directs the ubiquitination of targets differing from BRCA1. In addition to its well-known target H2A, we found that the macro H2A (mH2A) variant is a specific target, and PCNA seems to be the main ubiquitination target during normal grow conditions. We report that BARD1-mediated PCNA ubiquitination is involved in the prevention of single stranded DNA (ssDNA) accumulation in contrast to RAD18-mediated PCNA ubiquitination, which is involved in UV-induced damage repair. Additionally, we found that the BRCA1 catalytic dead mutant is not phosphorylated at S114, leading to a compromised replication fork protection. Overall, we conclude that BRCA1-BARD1 E3 activity is important to keep genetic stability by controlling replication fork homeostasis instead of being involved in HR pathway, which opens new therapeutic opportunities.

In chapter 6, we aimed to find specific targets for an E2 conjugating enzyme, UBE2D3. This E2 has been reported to interact with BRCA1-BARD1 at DNA damage sites and with RNF8 for PCNA ubiquitination. Commonly, it has been used as promiscuous E2 for *in vitro* ubiquitination. However, the *in vivo* role has not been defined yet. In chapter 6, we performed SILAC-based and label-free quantitative ubiquitin diGly proteomics, together with TULIP2 technology, to study global proteome and ubiquitinome changes associated with UBE2D3 depletion. The ubiquitin proteome completely changed after UBE2D3 depletion, having a prominent effect on molecular pathways related to mRNA translation. With UBE2D3-TULIP2 methodology we were able to detect, in addition to PCNA as already known target, two ribosomal proteins (RPS10 and RPS20) as specific targets, which are critical for ribosome-associated protein quality control (RQC). Ubiquitination of RPS10 and RPS20 was also UBE2D3-dependent in SILAC and diGly proteomics, revealing a new *in vivo* role for UBE2D3.

In the last chapter, we discuss about different MS approaches in shotgun or bottom-up proteomics regarding sample complexity and challenges in finding E3 specific targets. Then, we explain the potential and limitations of the TULIP2 technology developed in this thesis and discuss how all our findings can be of potential use in the clinic. Finally, we contrast our findings on the SUMO field with published literature to show how a previously believed small post-translational modification network could become very complex and sophisticated.