Regulation of target proteins by small ubiquitin-like modifiers

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Summary

Genomic instability serves as both a distinguishing character of tumor cells and a primary contributing factor to carcinogenesis. To ensure genome stability, eukaryotic cells harbor a global signal transduction cascade, termed the DNA damage response (DDR). Modification of chromatin and DDR machinery components by rapid and reversible posttranslational protein modifications (PTMs) including by Small Ubiquitin-like Modifiers (SUMOs) plays important roles in orchestrating cellular responses to DNA damage. Our lab has developed a generic lysine-deficient SUMO purification strategy combined with mass spectrometry for the identification of these SUMO target proteins and their acceptor lysines. My primary goal was to employ this quantitative proteomics approach to perform unbiased, proteome-wide and site-specific analyses of SUMOylation to identify individual target proteins that are regulated by SUMOs during DNA damage responses. I reviewed the SUMOylation process, roles of SUMO in DNA damage responses, cross talk among SUMO and ubiquitin, SUMO and phosphorylation, and technical developments in SUMO proteomics **(Chapter 1)**.

SUMOs are known to play a key role in counteracting replication stress caused by genotoxic agents. In order to identify individual target proteins that are regulated by SUMOs in response to DNA replication stalling induced by DNA damage, we identified SUMO-2 targets as well as SUMO-2 modified sites from cells exposed to DNA damage due to DNA replication stress caused by Hydroxyurea (HU), using the lysine-deficient SUMO-2 purification approach coupled to mass spectrometry as developed in our lab. We found sets of SUMO-2 targets up-regulated or down-regulated as well as SUMO-2 modified acceptor lysines in response to short (two hours) or long (twenty-four hours) periods of HU treatment. Our results show that SUMO-2 target proteins are functionally connected as groups to ensure genome stability during replication stress. We also found that SUMOylation regulates sets of other factors related to DNA damage response **(Chapter 2)**.

DNA replication is vital for eukaryotic organisms, and it is constantly threatened by endogenous and exogenous damages. To ensure efficient responses to genotoxic insults and maintain genomic integrity, different PTMs precisely coordinate with each other. Phosphorylation is widely known to be a key player in response to DNA damage caused by replication stress. In later years, SUMO has also been shown to play an essential role in the DNA damage response. In order to investigate cross-talk between phosphorylation and SUMOylation in response to DNA replication stress-induced DNA damage, we have employed complementary proteomics strategies and identified 3300 phosphorylation sites and 1400 SUMOylation acceptor lysines upon mitomycin C (MMC) and hydroxyurea (HU) treatment and found that a set of proteins are co-regulated by both SUMOylation and phosphorylation. We further proved that TOPBP1, a major partner and co-activator of ATR, is SUMOylated upon MMC

treatment-induced replication stress. This indicates that SUMOylated TOPBP1 together with ATR and ATRIP, may play a key role in the ATR dependent checkpoint signal transduction **(Chapter 3)**.

The SUMO-Targeted Ubiquitin Ligase (STUBLs) RNF4 is known to ubiquitylate SUMOylated proteins upon DNA damage. In order to gain a global view of RNF4 regulated proteins and better understand the function of RNF4 in DNA damage repair, we have optimized methods to identify direct substrates and indirect interactors of ubiquitin E3 ligases. We named this method: TULIP (Targets for Ubiquitin Ligases Identified by Proteomics).

Our study showed that the SUMO E2 Ubc9 and five SUMO E3 ligases (ZNF451, NSMCE2, PIAS1, PIAS2 and PIAS3) are direct RNF4 substrates. This indicates a role for RNF4 in balancing SUMO signal transduction. Moreover, we identified the tumor suppressor ubiquitin E3 ligase BARD1, the partner of BRCA1, as an indirect RNF4 substrate. We propose that the ubiquitin E3 ligase RNF4 targets autoSUMOylated Ubc9 and SUMO E3 ligases to balance SUMO signal transduction. More importantly, the TULIP technology we developed is able to distinguish covalently bound substrates and non-covalent binding interactors and it is applicable for identifying targets for other E3 ligases **(Chapter 4)**.

UFM1is a recently identified ubiquitin-like protein that covalently modifies lysine residues of its substrates through a three component enzymatic cascade. We applied our lysine-deficient Ubiquitin-like (Ubl) purification strategy coupled to mass spectrometry as developed in our lab to study UFMylation in a site-specific manner in Hela cells. We showed that RPL26, RPL26L1, TUBA1B, MCM5, SLC26A7, SCYL2, WDR63 are covalently modified by UFM1. We further confirmed RPL26 as a key UFM1 target and the UFMylated form of RPL26 can efficiently interact with SRPR. We propose that RPL26 is targeted by UFM1 and serves as a platform for ribosome associated protein interaction **(Chapter 5)**.

In the last chapter, I discussed results obtained in this thesis. The SUMO-2 site-specific proteomics method is applicable to identify targets for other Ubls such as UFM1 as well as their acceptor lysines, which provides important resources to decipher the complex protein networks regulated by these PTMs. However, to fully understand the role of PTMs in the cells, more functional work is needed. It is also of great interest to note that SUMOylation is involved in coordinating with other post translational modifications such as ubiquitination and phosphorylation. The TULIP method developed in our lab is not only helpful for us to better understand the role of the STUBL RNF4 in regulating the SUMO conjugation machinery to maintain genomic stability but also provides a strong tool for identification of targets for E3 ligases in other regulatory pathways **(Chapter 6)**.