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Mass spectrometry-based study of the relationship between ubiquitination and autophagy

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Autophagy primarily serves as a cytoprotective mechanism to maintain cell homeostasis; however, dysfunctional autophagy can lead to a number of human diseases. Ubiquitination playing multiple roles in the regulatio of autophagy. The recently developed Ubisite approach and StUbEx cell system were applied to systematically identify dynamic cellular ubiquitination targets, respectively. About 10,000 ubiquitinated sites were identified by peptide enrichment, compared between cells were treated with rapamycin plus and minus concanamycin A, 157 sites were significantly regulated. Two interesting candidates, TNIP1 and WBP2, were chosen for detailed follow-up studies.

TNIP1 is a key suppressor of inflammatory signaling. Under inflammatory conditions, TNIP1 was selectively degraded via autophagy further supporting inflammation. We characterized two canonical LC3-interacting regions (LIR) on TNIP1, mutation of these LIR motifs dramatically impaired TNIP1 lysosomal degradation. Our study revealed that selective autophagy plays a critical role in controlling inflammation.

WBP2 is an emerging oncogene in human cancers. We found that WBP2 is ubiquitinated and accumulated when lysosomal degradation is blocked. In the absence of WBP2, autophagosome formation was markedly reduced and abnormal autophagosome accumulated at the endoplasmic reticulum. By microscale thermophoresis (MST) assay and lipid flotation assay, we found that the GRAM domain of WBP2 binds to phosphoinositides. Thus, we concluded that WBP2 is functioning as a tether for vesicles which contain lipids required for autophagosome formation.

Taken together, the data presented in this thesis provide a valuable resource on ubiquitination events under autophagy induction. We studied the role of autophagy in TNIP1 degradation and inflammatory signaling, and the novel role of WBP2 in autophagy regulation. This may help to develop therapeutic strategies for TNIP1-related autoimmune diseases and WBP2-related cancers.

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