Post-translational modification with ubiquitin (Ub) initiated by sequential actions of Ub-activating enzyme (E1), Ub-conjugating enzyme (E2) and Ub ligase (E3) regulates diverse cellular processes, including signal transduction, cell cycle progression, apoptosis and gene transcription. Deregulation in the Ub pathway is often associated with human pathogenesis, including cancer. Our group uses structural biology and biochemical approaches to study the enzymes in the Ub pathway to understand their regulation, mechanistic function and mutation-induced deregulation. We anticipate that the knowledge gained from our structural studies will assist in the development of selective therapeutic targets within the Ub pathway.

Ubiquitin conjugation cascade

Covalent attachment of Ub involves three key enzymes, namely E1, E2 and E3 (Figure 1). E1 adenylates Ub to C-terminus in the presence of Mg²⁺ and ATP, which is then activated by E1 in a covalent thioester intermediate with Ub. E1 then transfers an E2 and transfers the thioesterified Ub to the E2’s catalytic cysteine, forming an E2–Ub thioester intermediate (~ indicates the thioester bond). E3 generally consists of an E2-binding module (HECT, RING, RBR or U-box domain) and a protein–protein interaction domain that can recruit the substrate directly or indirectly. With this configuration, E3 recruits E2–Ub and the substrate to promote Ub transfer from the E2 to a lysine side chain on the substrate. In humans, there are ~600 RING E3s, and we are interested in uncovering their regulation and function and exploring the Ub system for cancer therapeutics.

Deregulation in CBL ubiquitin ligase

CBL proteins (CBLs) are RING E3s that negatively regulate receptor tyrosine kinases, tyrosine kinases and other proteins by promoting their ubiquitination and degradation by the proteasome or lysosome. Mutations in CBL have been observed in human patients with myeloproliferative diseases. Investigating the mechanism by which CBL mutants exert oncogenicity, we showed that CBL mutants inactivated E3 activity, thereby functioning as an adaptor to recruit other proteins such as CIN85 to elicit oncogenic signalling. Mechanistically, CBL mutants bound to receptor tyrosine kinases such as EGFR, which led to phosphorylation of CBL mutants’ C-terminal tyrosines. Phosphorylated tyrosines induced conformational changes that enabled CBL mutant–CIN85 interaction. CBL mutants could not ubiquitinate CIN85, leading to deregulated CBL–CIN85 signalling which altered transcriptome landscape, that in turn upregulated p14ARF, AKT signalling cascade to drive oncogenesis (Ahmed et al., 2021, Oncogene). Our ongoing work is aiming to develop therapeutics targeting CBL mutant–EGFR interaction and thereby reducing the oncogenic property of CBL mutant.

Molecular mechanism of CBL mutants

CBL mutations lead to accumulation of oncogenic signalling that can promote oncogenesis. We showed that CBL mutants could disrupt the formation of MDM2–p53–CBL complex, which ultimately led to reduced p53 activity. This was the result of the interaction of CBL mutants with the RING domain of MDM2, which is known to promote ubiquitination and degradation by the proteasome. We also showed that CBL mutants could recruit the substrate directly or indirectly, leading to the formation of a covalent thioester intermediate (~ indicates the thioester bond). E3 generally consists of an E2-binding module (HECT, RING, RBR or U-box domain) and a protein–protein interaction domain that can recruit the substrate directly or indirectly. With this configuration, E3 recruits E2–Ub and the substrate to promote Ub transfer from the E2 to a lysine side chain on the substrate. In humans, there are ~600 RING E3s, and we are interested in uncovering their regulation and function and exploring the Ub system for cancer therapeutics.

In conclusion, our work has elucidated the molecular mechanisms by which CBL mutants promote oncogenesis, and our findings have implications for the development of therapeutic strategies targeting CBL mutants.

UBIQUITIN SIGNALLING