Structural analysis of MDM2-E2~ubiquitin interaction in p53 regulation

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Post-translational modification by ubiquitin (Ub) regulates diverse cellular processes. Ubiquitin ligases (E3s) catalyze the final step of Ub transfer from E2 ubiquitin-conjugating enzyme thioesterified with Ub (E2~Ub) to a lysine side chain of substrate. RING-type E3s are the largest family of E3s with approximately 600 members in humans. RING E3s function by recruiting E2~Ub via the RING domain and promote direct transfer of Ub from E2 to the substrate lysine.

MDM2 is a RING E3 that plays a critical role in down-regulation of the tumour suppressor protein p53. Like other RING E3, MDM2 simultaneously recruits an E2~Ub and a substrate protein to promote the transfer of Ub to the lysine on the substrate. Homodimerization of MDM2's C-terminal RING domain is essential for its ligase activity. Alternatively, MDM2 can heterodimerize with an inactive RING E3, MDMX, to form an active ligase. How MDM2 or MDM2-MDMX complex recruits E2~Ub remains elusive. We determined a 2.4 Å crystal structure of MDM2-MDMX RING dimer bound to an E2 covalently linked to Ub. The structure reveals the mechanism of E2~Ub activation by MDM2-MDMX heterodimer and provides a rationale for how MDM2 homodimer activates E2~Ub. Guided by the crystal structure, we generated specific point mutations that prevent E2~ubiquitin binding without altering the RING domain structure. These mutants have lost E3 activity and are unable to ubiquitinate and degrade p53. Interestingly, they retain some ability to limit p53's transcription activity, thus revealing a ligase-independent role of MDM2 in p53 regulation. I will discuss these results in this meeting.