Abstract

In the cell, tubulin undergoes post-translational modifications that create functionally distinct microtubules and mark them for specialized functions. Acetylation of Lys40 of α-tubulin is one of such post-translational modifications controlled by the activity of histone deacetylase 6 (HDAC6). The Lys40 acetylation is a hallmark of stable microtubules, it protects them from mechanical aging, influences cell motility as well as axonal branching and maintenance of neuronal processes. Tubulin stands out as the most prominent physiological substrate for HDAC6. Being a multidomain cytosolic protein, HDAC6 is involved in the myriad of cellular processes and is a promising target for the treatment of cancer and neurodegenerative diseases. The understanding of the mechanisms of HDAC6 interactions with its substrates, especially with tubulin, can open avenues for the development of new treatment strategies exploiting highly selective HDAC6 inhibitors.

In this thesis, we have investigated the molecular basis of tubulin recognition by HDAC6. We provided a detailed kinetic analysis showing the HDAC6 deacetylation rate of free tubulin is 1500-fold faster than microtubules. Additionally, we have shown that amino acids of the flexible Lys40 loop (except P1 and P-1) make a minor contribution to the substrate recognition by HDAC6, while the more important role can be assigned to residues at the longitudinal and lateral interactions between tubulin dimers. Moreover, we visualized the direct binding of HDAC6 to microtubules and qualitatively/quantitatively mapped HDAC6 binding to microtubules. Here we identified the N-terminus of HDAC6 to be the microtubule-binding domain (MBD), showing that HDAC6/tubulin (microtubules) interactions are driven by ionic (electrostatic) forces. Interestingly, while HDAC6 binding to microtubules is not dependent on its deacetylation activity, the presence of MBD enhances tubulin deacetylation more that 100-folds. Our results thus provide mechanistic underpinnings on the recognition of tubulin/microtubules by HDAC6.

Using our biochemical and X-ray crystallography expertise, we have also contributed to the development of SS-208, an HDAC6-specific inhibitor harboring an isoxazole moiety as a zinc-binding group. This inhibitor is highly specific for HDAC6 modestly potent against HDAC1. *In vivo* studies revealed that SS-208 impairs tumor growth by mediating immune-related tumor destruction rather than by the direct cytotoxic effect on tumor cells.