Investigating protein acetylation in health and diseases with chemical tools

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Acetylation of the Nε-amino group of lysine side chains is a posttranslational modification (PTM) of proteins found in all kingdoms of life. On the level of chromatin, this PTM serves as a molecular switch for activation of gene expression. In addition, acetylated lysine residues also constitute binding sites for bromodomains (Brds) and these epigenetic readers play key roles in chromatin remodeling and transcriptional regulation. Dysregulation of these epigenetic mechanisms can lead to several diseases such as cancer. A prominent example is the NUT carcinoma. This type of cancer is driven by the translocation of the genes encoding NUT and the Brds of the proteins BRD3 and BRD4, leading to aberrant gene expression. The so-called BET-Brds of BRD3 and BRD4 are of major biomedical interest and represent promising targets for drug discovery. A first generation of synthetic small-molecule ligands has already been developed, that bind tightly to the acetyllysine binding cleft of BET-Brds.

The reversibility of PTMs renders their investigation highly challenging. By transplanting a triazole as structural motif of the BET-inhibitor JQ1 onto an amino acid scaffold, a set of potential acetyl-lysine mimicking amino acids was developed as non-reversible surrogates of this PTM. The development process started with the establishment of a synthesis route to such triazole-containing amino acids, which were then incorporated into peptide probes and tested for BRD binding in pulldown experiments. These experiments revealed ApmTri as the most potent and selective acetyl-lysine mimicry. The binding mode of ApmTri peptide ligands and Brds was investigated on molecular level by structural elucidation and the binding affinities were quantified. These investigations confirmed that ApmTri is an efficient acetyl-lysine mimicry. By expanding the genetic code of mammalian cells using the amber suppression technique, ApmTri was incorporated into whole proteins of living cells. Co-expression of BRD3(2) and ApmTri-containing proteins demonstrated efficient binding even in a cellular context.

ApmTri as a new epigenetic tool can be used in various future application. The generation of full proteins containing ApmTri allows the investigation of BET-Brds and their interaction partners as well as potentially new insights into the pathology of the NUT carcinoma. Finally, the exploration of ApmTri in the context of peptide-based inhibitors of BET-Brds is a further exciting perspective.