Deacetylase inhibitors & Histone inheritance

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Chapter 7

Summary and future perspectives

Deacetylase inhibitors

The urgent need for new therapies to treat airway diseases is exemplified by the death of approximately 3 million patients suffering from chronic obstructive pulmonary disease (COPD) each year. Additionally, lung cancer is the most common cause of cancer death, causing over 1.5 million deaths per year. A promising direction for new therapies for these and other lung diseases, like asthma, comes from the notion that deranged intracellular signaling pathways in asthma, COPD and lung cancer are critically regulated by protein posttranslational modifications (PTMs). Acetylation and methylation are the quintessential PTMs, found on over a thousand proteins and influencing a diverse range of protein properties. Acetyl- and methyltransferases and deacetyl- and demethylases, the enzymes that control the dynamic process of acetylation and methylation, have consequently been recognized as important drug targets. Hence, inhibitors of these enzymes have been developed, which are currently being evaluated in preclinical models of asthma and COPD and in clinical trials of lung cancer. Significant progress has been made in this area, especially in the case of deacetylase inhibitors, with many promising results, but several challenges still need to be overcome to provide effective new therapies for these airway diseases.

One such challenge is the design of deacetylase inhibitors with unique selectivity profiles to unravel the roles of specific deacetylases in inflammation. Therefore, we explored *o*-aminobenzamide substituted five-membered heterocycles as zinc-binding group in deacetylase inhibitors. These inhibitors were evaluated for their inhibitory selectivity among histone deacetylase 1, 2 and 3 and their ability to influence inflammatory gene expression. An *o*-aminobenzamide substituted thiophene in the zinc binding core demonstrated potential for deacetylase inhibition. To investigate this further, a convenient synthetic route was developed to obtain 5-substituted 2-

nitro-3-aminothiophenes as precursors for the synthesis of deacetylase inhibitors with 5-substituted 2-amino-3-benzamide thiophenes as zinc-binding group. By employing alkynyl cyanides as starting materials, we developed a three-step, one-pot procedure that provided the desired products in reasonable yields. Using this method, a focused library of 5-substituted thiophenes was synthesized and investigated for selective inhibition among histone deacetylase 1, 2 and 3. Inhibitors with selectivity among histone deacetylase 1, 2 and 3 were employed in cell-based studies. We found that both histone deacetylase 1, 2 and 3 and histone deacetylase 1 and 2 selective inhibitors upregulated the NF-κB transcriptional activity in a reporter gene assay and upregulated the pro-inflammatory cytokine IL-6. In contrast, only the histone deacetylase 1, 2 and 3 selective inhibitor upregulated IL-10 expression, whereas the histone deacetylase 1 and 2 selective inhibitor did not. Altogether, these results provides insight in the use of non-selective inhibitors of histone deacetylase 1, 2 and 3 in diseases with an inflammatory component, such as asthma, COPD and lung cancer.

Future perspectives

One area of further research is to combine the features of isoform selective inhibitors to yield unique selectivity profiles that could potentially further improve their positive benefit-risk ratios. A uniquely selective HDAC3 and 6 inhibitor, for instance, would combine the attenuation of cytokine expression by inhibition of HDAC3 with the reduction of immune cell motility by inhibition of HDAC6, without eliciting the pleiotropic effects of HDAC1 and 2 inhibition. For instance, an HDAC3 and 6 inhibitor of unknown structure reproduced the anti-inflammatory effects of reduced expression of HDAC3 in an *in vitro* model of rheumatoid arthritis [68]. Other unique combinations left to explore are HDAC1, 2 and 6, HDAC1, 2 and 8 and HDAC3 and 8 inhibitors. A dual action HDAC6 and 8 inhibitor already exists, but has not been tested yet in inflammatory models [69].

Furthermore, the finding that HDACs exist in specific complexes has been used to design inhibitors for specific complexes, like the dual-warhead LSD-

1/HDAC1, 2 and 3 inhibitor corin directed to CoREST. The availability of structural information of the specific HDAC complexes will aid the discovery of comparable examples. Besides, further modification of *o*-aminoanilide-type HDACi might similarly yield complex-selective inhibitors as exemplified by Rodin-A.

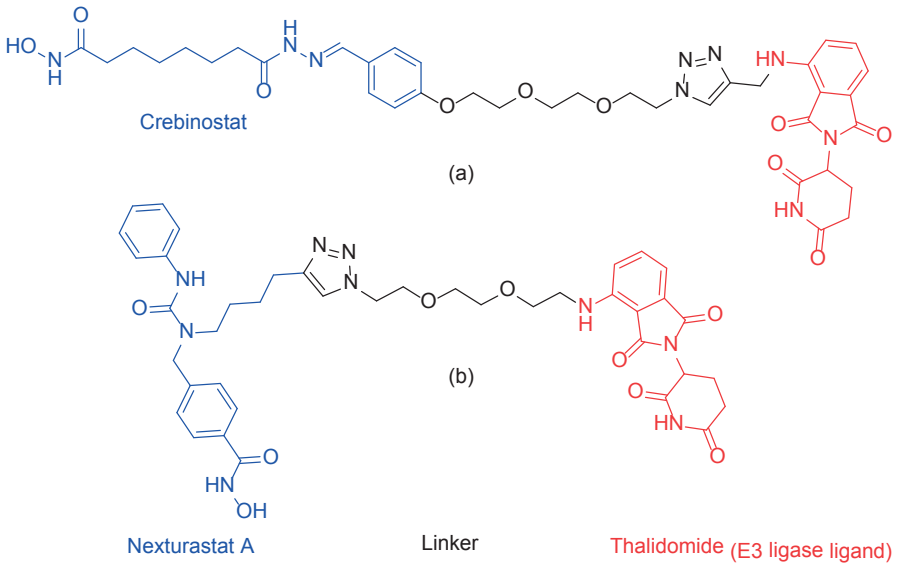

Figure 1. Structures of histone deacetylase 6 selective proteolysis targeting chimera's. A histone deacetylase inhibitor is connected to the E3 ligase ligand thalidomide with a hydrophilic linker. (A) Compound 9c, that contains the histone deacetylase inhibitor crebinostat. (B) Compound NP8, that contains the histone deacetylase inhibitor nexturastat A.

Lastly, due to the important structural role of HDACs in protein complexes, prevention of HDAC complex formation by protein-protein interaction inhibitors is an interesting option. A more novel but related approach is to target enzymes for degradation using the proteolysis targeting chimera (PROTAC) technique [70]. In this respect, PROTAC molecules for the selective degradation of HDAC6 (Figure 1) have recently been developed [71, 72]. The compounds consist of an HDAC inhibitor directed to the HDAC catalytic site and the E3 targeting ligand thalidomide, to bind to an E3

ubiquitin ligase complex, coupled together with a linker [73, 74]. It is interesting to note that while compound **9c** (Figure 1A) contains the non-selective inhibitor crebinostat [75], it is reported to selectively degrade HDAC6. This may be the result of the formation of a stable ternary complex between the E3 ligase and HDAC6 and not with the other HDACs [76], but further research is needed to confirm this. Compound Np8 (Figure 1B) contains the HDAC6 selective inhibitor nexturastat A [77]. Finally, small-molecules that selectively degrade HDAC1, 2 or 3 have not yet been developed and this is therefore an interesting area for further research. PROTACs for specific complexes may even be developed by adding ubiquitin ligase functionalities to the described complex-specific inhibitors. With these new strategies, the validation of HDACs as targets for improved therapies for asthma and COPD enters an exciting new era.

Histone inheritance

Following DNA replication, equal amounts of histones are distributed over sister chromatids by re-deposition of parental histones and deposition of newly synthesized histones. Molecular mechanisms balancing the allocation of new and old histones remain largely unknown. Here, we studied the genome-wide distribution of new histones relative to parental DNA template strands and replication origins using the newly developed method double-click-seq. In control conditions, new histones were preferentially found on DNA replicated by the lagging strand machinery, which was more pronounced in AT-rich regions. PARP inhibition increased this bias in agreement with PARP's necessity in lagging strand synthesis. Strikingly, replication stress induced by hydroxyurea or curaxin treatment, inhibition of ATR or p53 inactivation inverted the observed histone deposition bias to the leading strand, which was also most pronounced in AT-rich regions. We propose that asymmetric deposition of newly synthesized histones onto sister chromatids reflects uncoupling of leading or lagging strand DNA synthesis from helicase activity and speculate that this could be an important driver of epigenetic instability in normal and malignant cells.

Future perspectives

Replication fork directionality has been shown to be co-directional with transcription, from which it may be inferred that, with similar transcription profiles during S phase, this directionality will not change across cell cycles. Replication stress also does not change the orientation of the leading and lagging strands, as it has been shown that HU treatment of hRPE-1 cells mostly stimulates already active origins and does not change the overall pattern of replication origins. This implies that asymmetric histone deposition, in connection with partial restoration of histone PTMS, could accumulate over multiple cell cycles and provides a mechanistic explanation for epigenetic changes in cancer. The double-click-seq method is uniquely

suited to investigate whether new histone deposition asymmetry accumulates over multiple cell cycles, because labeled histones can be tracked for their entire lifetime. In theory, the experimental conditions would only require an adaptation of the culturing methods to accommodate pulse labeling of new histones followed by a chase period over several cell cycles.

Additionally, the finding that asymmetry in new histone deposition is especially pronounced around replication origins may indicate that epigenetic instability is most likely to arise around replication origins. Therefore, future research could determine whether there is an association between replication origins and areas of epigenetic instability in various cancer cells. Ultimately, this would allow us to predict which cancer cells are prone to undergo epigenetic changes and at which locations along the genome this is most likely to occur, providing a deeper understanding of the disease.