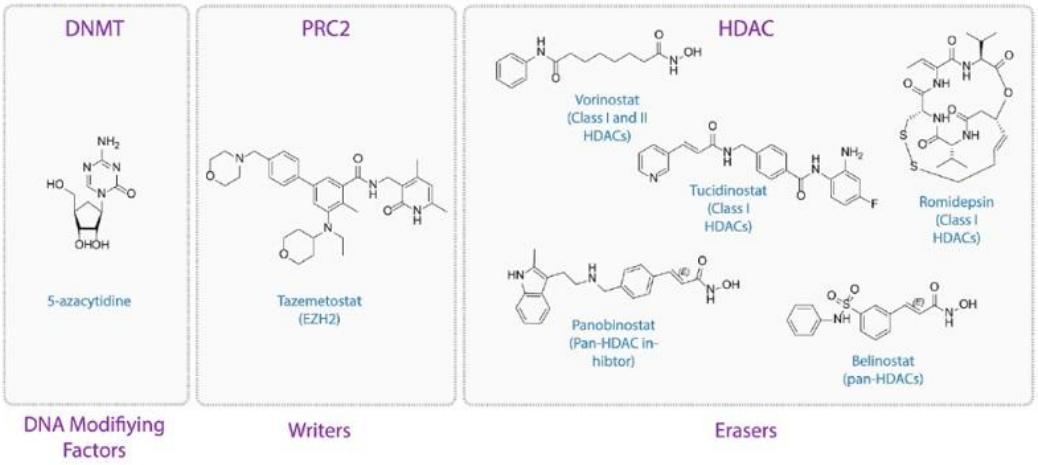
Future Perspective of PROTAC Combined With CRISPR In Anti-ancer Area

Targeted protein degradation therapy represented by PROTAC is one of the hot fields of new drug development. According to incomplete statistics, 24 targeted protein degradation therapies have entered the clinical development stage this year. Recently, the team of Professor Alessio Ciulli from the University of Dundee in the United Kingdom published a paper named Targeting epigenetic modulators using PROTAC degraders: Current status and future perspective. This article reviews the current status and future of using PROTAC therapy to target epigenetic targets. The article stated that the combination of CRISPR screening and PROTAC technology provides a powerful tool for the development of protein degradation therapies targeting epigenetic targets.

The role of epigenetics in disease pathology

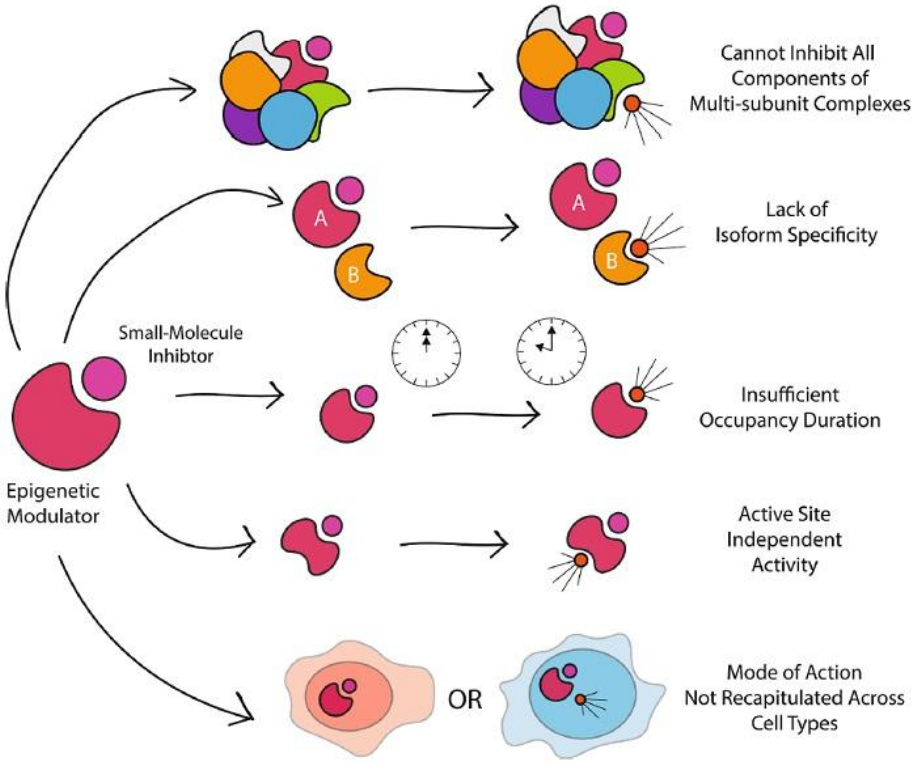
Epigenetics processes include modifications to DNA, DNA-associated proteins, and RNA that do not alter gene sequence, but affect gene expression. For example, DNA methylation is associated with gene silencing, and many histone modifications play important roles in transcriptional regulation, DNA replication and repair, RNA splicing, and chromosome condensation.

The role of epigenetics in disease pathology has also been well established, as histone deacetylases (HDACs) are highly expressed in cancer. Loss-of-function or gain-of-function mutations in epigenetic proteins also frequently drive disease pathology. Historically, the development of inhibitors targeting epigenetics has also been successful. At present, **7 inhibitors targeting epigenetic proteins have been approved by the US FDA for marketing**. A recently approved new drug is tazemetostat, the first inhibitor targeting the histone lysine methyltransferase EZH2.



▲ FDA-approved inhibitors targeting epigenetic proteins (Image source: Reference [1])

However, the development of inhibitors targeting epigenetic proteins faces multiple challenges. One of the main limitations is that the catalytic protein domains or protein-protein interaction domains of many epigenetic protein families are highly structurally conserved, which makes the development of targeting specific inhibitors of isoforms become very difficult, a typical example is the BET protein. Another limitation limiting the use of inhibitors is that epigenetic proteins often contain multiple protein domains and are often part of protein complexes composed of multiple subunits. These features imply that the use of inhibitors to block a single catalytic activity or one interaction is not sufficient to alter the functional outcome of the complex, as there are many other activities or interactions that are not disturbed by inhibitors.



▲ Challenges in developing small molecule inhibitors to modulate epigenetic processes (Image source: Reference [1])

Breakthroughs in Genetic Screening

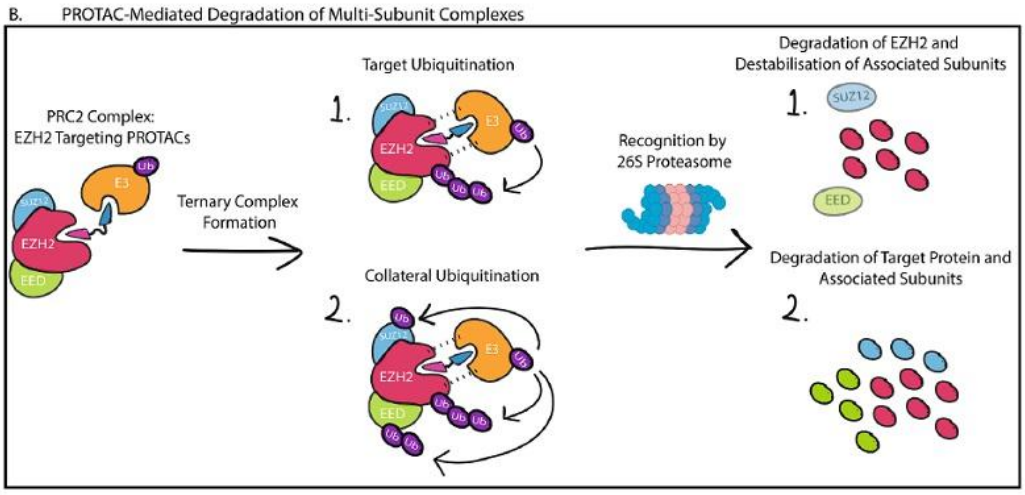
Genetic screens based on RNA interference (RNAi) and CRISPR gene editing technologies have revolutionized epigenetic target discovery, allowing scientists to systematically knock down or knock out individual genes in the genome expression, thereby discovering the role of epigenetic protein loss and establishing which diseases are particularly sensitive to epigenetic protein loss. For example, an RNAi screen has uncovered a critical dependence of acute myeloid leukemia cells on the activity of the BET protein Brd4. And recent CRISPR screens may offer lower off-target effects and a wider range of applications than RNAi. Furthermore, CRISPR screens can be designed to target specific subsets of genes, such as epigenetic factors.

However, while CRISPR and RNAi screens can discover and validate targets of interest, inhibitors targeting these targets often fail to replicate the target knockout phenotype. Because inhibition of target protein activity and knockdown of target protein expression have different mechanisms of action. This opens up opportunities for targeted protein degradation therapies.

Targeted protein degradation (TPD) is an important tool for epigenetic targets

PROTAC protein degradation therapy utilizes the cellular ubiquitin-proteasome system to specifically degrade target proteins. These molecules lead to ubiquitination of the target protein by pulling the target protein closer to the E3 ligase and forming a ternary complex, which is subsequently recognized and degraded by the proteasome. Past studies have shown that PROTAC-mediated protein degradation can replicate the phenotype of multiple epigenetic targets identified through genetic knockout or knockdown screens, while inhibitors often fail to do so.

Epigenetic proteins often form multi-subunit complexes that allow specific modifications to the genome. Targeting protein degradation is a powerful strategy for targeting epigenetic targets because, for proteins containing multiple protein domains, it can target the protein domain that is most prone to ubiquitin addition, even if this protein domain is not active function, can also lead to the degradation of the entire protein. In terms of targeting multi-subunit protein complexes, it can be used to target the subunit that is most easily degraded, and the removal of this subunit may cause instability and degradation of the entire complex. Targeting protein degraders also possibly mediates E3 ligase addition of ubiquitin chains to non-target proteins in the complex, leading to degradation of the entire complex.



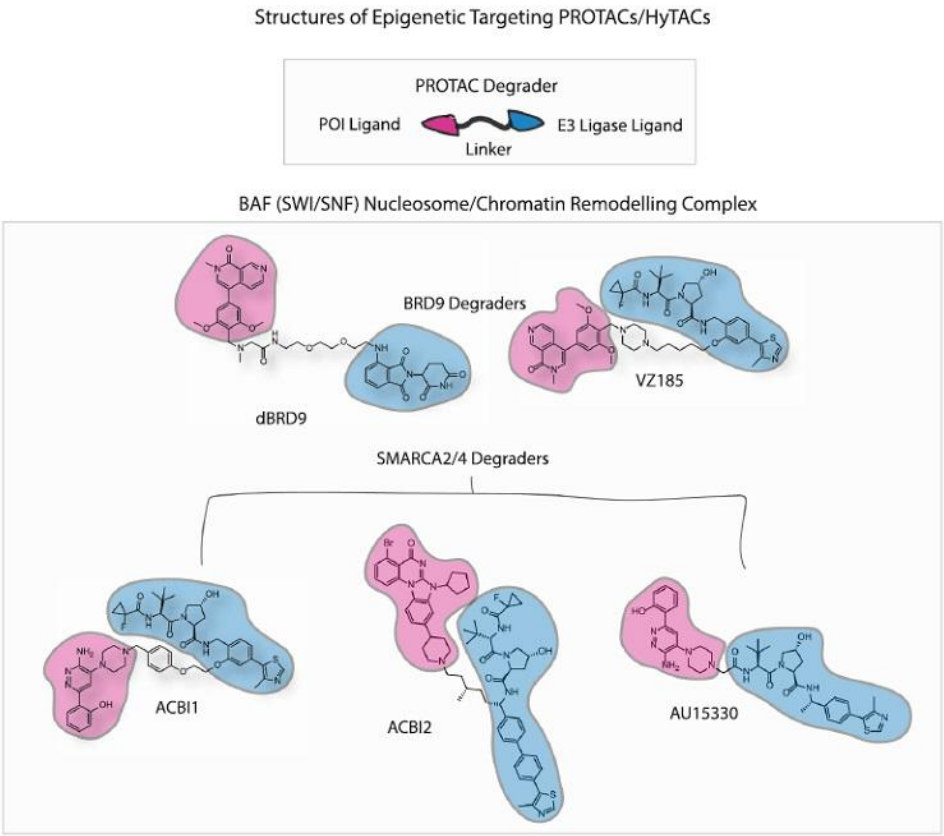
▲ Degradation of multi-subunit complex mediated by PROTAC (Image source: Reference [1])

PROTAC molecules targeting epigenetic complexes

Several PROTAC molecules targeting different epigenetic complexes are described in this review. A chromatin-remodeling complex called SWI/SNF is frequently mutated in tumors and neurological diseases. Scientists have developed a series of PROTAC molecules for different subunits of this complex. The discovery of VZ185, which selectively degrades BRD9 and BRD7, demonstrated that systematic iterative design of degradants can develop highly efficient protein-degrading molecules, even if the degradation characteristics of the initial molecule are poor, by continuously monitoring the cellular degradation capacity of the degradant and the thermodynamics of the terpolymer structure.

Studies on the development of inhibitors targeting the SmarCA2/4 subunit demonstrated that other subunits of the SWI/SNF complex were simultaneously eliminated by the degradation of SmarCa2/4. These studies highlight the feasibility of the development

strategy of targeting the subunits that are most easily degraded, leading to degradation of the entire complex.



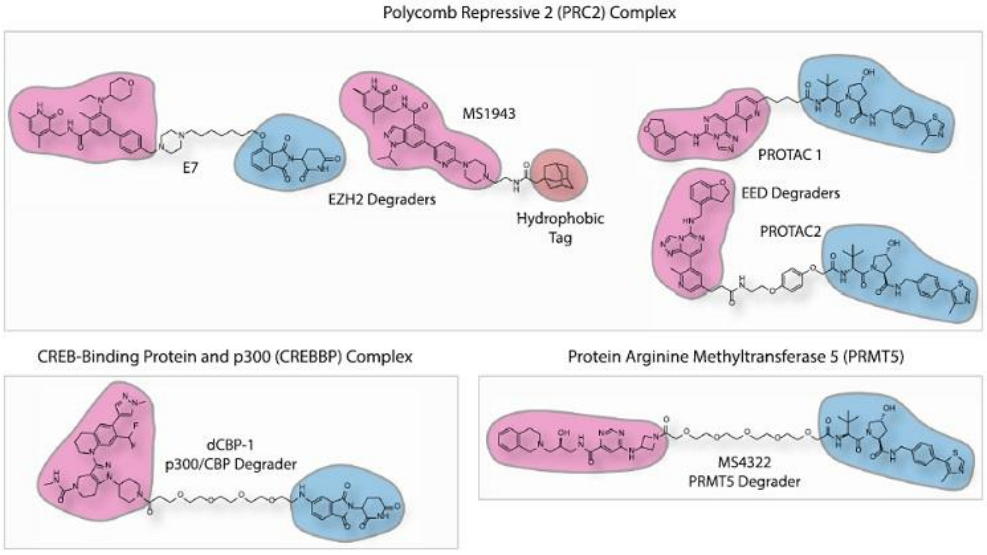
▲Various PROTAC molecules targeting SWI/SNF (Image source: Reference [1])

The PRC2 complex is a histone methyltransferase that catalyzes the methylation of H3K27. The PRC2 complex has attracted attention in recent years because scientists have found that its EZH2 subunit is associated with cancer progression. The FDA-approved EZH2 inhibitor Tazemetostat has been clinically validated for targeting EZH2 in in the treatment of cancer.

The PRC2 complex consists of four key subunits, SUZ12, RbAp46, EZH1/2 and EED. Several protein degraders targeting this complex have been developed, all of which have shown the ability to selectively modulate the expression of specific subunits, or to cause degradation of all PRC2 subunits. Among them, the EZH2-targeting PROTAC molecule

E7 not only increased the ubiquitination level of EZH2, but also increased the ubiquitination level of EED and SUZ12 subunits. This result shows that PROTAC molecules can lead to ubiquitination of untargeted subunits in the complex, resulting in the degradation of the entire complex.

PRMT5 is a class of arginine methyltransferases responsible for the methylation of histone substrates in mammals. Uncontrolled PRMT5 expression is associated with multiple cancer types with poor prognosis, including lung, breast, and hepatocellular carcinoma. The dependence of these cancer types on PRMT5 has been demonstrated using tissue-specific knockdown experiments. At present, a number of small-molecule inhibitors targeting PRMT5 have entered clinical trials, including GSK, Janssen and Amgen's investigational therapies. However, although current PRMT5 inhibitors can inhibit the methyltransferase activity of PRMT5, there is no evidence that they can inhibit the backbone protein function of PRMT5. PROTAC molecules may simultaneously reduce the skeleton protein function of PRMT5 and block the oncogenic effect of PRMT5 more effectively.



▲Protein degradation molecules targeting other epigenetic complexes (Image source: Reference [1])

Summary and future perspective

The review authors note that the link between epigenetic dysregulation and disease pathology has been demonstrated, especially for cancer types with poor prognosis. Genetic screening in recent years has found that many specific cancer types are particularly sensitive to the loss of epigenetic regulators, yet inhibitors targeting these targets often fail to replicate the knockout or knockdown phenotype. PROTAC technology can better replicate the target phenotype found by CRISPR or RNAi screening by degrading the target protein.

Degradation of other subunits of the complex by targeting one subunit of the complex is a common phenomenon. Moreover, PROTAC molecules can exhibit higher selectivity for on-target degradation than would be expected from binary binding, implying that degraders may be more selective than inhibitors, thereby extending the therapeutic window and improving the safety of the drug.

The link between epigenetic dysregulation and disease pathophysiology has been demonstrated, especially for cancer types with poor prognosis. Genetic screening in recent years has revealed that a number of specific cancer types are particularly sensitive to loss of epigenetic regulators, yet inhibitors targeting these targets generally fail to replicate the knockout or knockdown phenotype. PROTAC technology is able to better replicate the target phenotype identified by CRISPR or RNAi screening by degrading target proteins.

It is a common phenomenon that targeting one subunit of the complex causes degradation of other subunits of the complex. Furthermore, PROTAC molecules can show a higher selectivity for targeted degradation than would be expected based on binary binding, which means that the inhibitor may be more selective than the inhibitor, thus expanding the therapeutic window and improving the safety of the drug.

The authors note that the PROTAC molecules identified in the current study have not undergone extensive pharmacochemical optimization. With the biological mechanisms of PROTAC and the advantages of targeting epigenetic targets well established, the authors believe that initiating more extensive drug chemistry work will be the next step in translating this treatment model into clinical candidate therapies.

Currently, CFT8634 (C4 Therapeutics) and HFD-609 (Foghorn Therapeutics), both PROTAC inhibitors targeting epigenetic target Brd9, are on the way or have already entered the clinical stage. The authors say that in the coming years, there will undoubtedly be more PROTAC degraders targeting more epigenetic targets.

At present, the PROTAC degraders CFT8634 (C4 Therapeutics) and FHD-609 (Foghorn Therapeutics) targeting the epigenetic target Brd9 are about to enter the clinic or have already entered the clinic. The authors say that in the coming years, there will undoubtedly be more PROTAC degraders targeting more epigenetic targets.

The commonly used linkers in the development of PROTACs are PEGs, Alkyl-Chain and Alkyl/ether. As a reliable PEG derivatives supplier, **Biopharma PEG** provides multi functionalized PEG derivatives as PROTAC linkers. The following products can act as PROTAC linkers.

mPEG-mal (MW 2000), CAS NO.: 99126-64-4

N3-PEG3-CH2COOH, CAS：172531-37-2

Boc-NH-PEG3-OH, CAS：139115-92-7

NH2-PEG3-OH, CAS NO.: 6338-55-2

Reference:

[1] Targeting epigenetic modulators using PROTAC degraders: Current statusand future perspective.

Related articles:

[1] Four Major Trends In The Development of PROTAC

[2] PROTAC And Other Protein Degradation Technology

[3] PROTACs VS. Tranditional Small Molecule Inhibitors

[4] Peptide PROTAC in Drug Development