Pharmacology & Therapeutics 204 (2019) 107406

Contents lists available at ScienceDirect

Pharmacology & Therapeutics

journal homepage: www.elsevier.com/locate/pharmthera

Targeting epigenetic machinery: Emerging novel allosteric inhibitors

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ARTICLE INFO

Available online 12 September 2019

Keywords:
Epigenetics
PRC2
MLL complex
SWI/SNF
DNMT1
allosteric inhibitors

ABSTRACT

Epigenetics has emerged as an extremely exciting fast-growing area of biomedical research in post genome era. Epigenetic dysfunction is tightly related with various diseases such as cancer and aging related degeneration, potentiating epigenetics modulators as important therapeutics targets. Indeed, inhibitors of histone deacetylase and DNA methyltransferase have been approved for treating blood tumor malignancies, whereas inhibitors of histone methyltransferase and histone acetyl-lysine recognizer bromodomain are in clinical stage. However, it remains a great challenge to discover potent and selective inhibitors by targeting catalytic site, as the same subfamily of epigenetic enzymes often share high sequence identity and very conserved catalytic core pocket. It is well known that epigenetic modifications are usually carried out by multi-protein complexes, and activation of catalytic subunit is often tightly regulated by other interactive protein component, especially in disease conditions. Therefore, it is not unusual that epigenetic complex machinery may exhibit allosteric regulation site induced by protein-protein interactions. Targeting allosteric site emerges as a compelling alternative strategy to develop epigenetic drugs with enhanced druggability and pharmacological profiles. In this review, we highlight recent progress in the development of allosteric inhibitors for epigenetic complexes through targeting protein–protein interactions. We also summarized the status of clinical applications of those inhibitors. Finally, we provide perspectives of future novel allosteric epigenetic machinery modulators emerging from otherwise undruggable single protein target.

Abbreviations: DNMT, DNA methytransferase; HDAC, histone deacetylase; TET, ten-eleven translocation; HAT, histone acetyltransferase; GABA, γ-aminobutyric acid; MLL, mixed lineage leukemia; CML, chronic myeloid leukaemia; PTP, protein tyrosine phosphatase; BPTF, bromodomain and PHD finger transcription factor; NURF, nucleosome remodeling actor; PHD, plant homeodomain; H3K4me3, trimethylation of histone H3 at lysine 4; PPI, protein-protein interaction; PRC, Polycomb repressive complex; HMT, histone methyltransferase; KMT, lysine methyltransferase; GAP, GTPase-activating protein; T-ALL, T cell acute lymphoblastic leukemia; MDS, myelodysplastic syndrome; EBD, EED binding domain; SRM, stimulation responsive motif; EM, electron microscopy; VEFS, VRN2-EMF2-FIS2-Su(z)12; HTH, helix-turn-helix motif; Zn, zinc finger; PCL1, polycomb-like homologs; ESC, embryonic stem cells; EH, extended homology; SAM, S-adenosylmethionine; DCR, disease control rate; BMI-1, B lymphoma Mo-MLV insertion region 1; LMS, Leiomyosarcoma; H2AK119ub, mono-ubiquitination of Lys119 of histone H2A; ALL, acute lymphoblastic leukemia; AML, acute myeloid leukemia; WIN, WDR5 interaction motif; C/EBPα, CCAAT/enhancer binding protein α; GOF, gain of-function; Men1, multiple endocrine neoplasia 1; BAF, BRG1-associated factor; PBAF, polybromo BRG1-associated factor; PDA, pancreatic ductal adenocarcinoma; MRT, malignant rhabdoid tumors; PDA, pancreatic ductal adenocarcinoma; HSA, helicase-SANT–associated; BET, bromodomain and extra-terminal; BRCA1, Breast cancer-associated gene 1; UHRF1, ubiquitin-like with PHD and ring finger domains 1; HSAN IE, hereditary sensory and autonomic neuropathy type IE; ADCA-DN, autosomal dominant cerebellar ataxia, deafness and narcolepsy; RFTS, replication foci targeting sequence; BAH, bromo-adjacent homology; CD, C-terminal methyltransferase domain; TRD, target recognition domain; MDS, myelodysplastic syndrome; MOA, mode of action; SRA, SET- and RING-associated; H3Ub, ubiquitinated H3; UBL, ubiquitin-like domain; USP7, ubiquitin specific peptidase 7; HAUSP, herpes virus associated ubiquitin specific protease; DEL, DNA-encoded chemical library.

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https://doi.org/10.1016/j.pharmthera.2019.107406

Contents

1. Introduction

"Epigenetics" was initially proposed by Conrad Waddington in the 1950s defining the stably heritable phenotype generating from chromosomes changes without any alternations of DNA sequence (Berger, Kouzarides, Shiekhattar, & Shilatifard, 2009). Epigenetics can be divided into three applications: DNA methylation, histone modification and non-coding RNA regulation. Covalent modifications on nucleosomes are highly dynamic and reversibly contribute to functional outcomes, significant for physiological and pathological processes. In the past decades, epigenetic mutations which are majorly represented by aberrant DNA methylation status, abnormal histone modification and chromatin structures have been found to play pivotal roles in variety of diseases, particularly in tumorigenesis (Pathania et al., 2015; Poli et al., 2018), tumor invasion and metastasis (Albrengues et al., 2015; Cock-Rada et al., 2012), immune evasion (Tomasi, Magner, & Khan, 2006; Topper et al., 2017; Tough, Tak, Tarakhovsky, & Prinjha, 2016) and drug resistance (Easwaran, Tsai, & Baylin, 2014; Glasspool, Teodoridis, & Brown, 2006; Stratikopoulos et al., 2015). Therefore, targeting epigenetic mutation has emerged as attractive therapeutic strategy in developing drugs for the treatment of diseases including cancer. Indeed, several remarkable achievements have been made due to the advancement of evolving technologies and an accumulative understanding of epigenetics regulation and disease pathogenesis. To date, seven epigenetic drugs have been approved by the FDA for cancer treatment. Two of these include DNA methytransferase (DNMT) inhibitors (Azacitidine; Decitabine); the other five are histone deacetylase (HDAC) inhibitors (Vorinostat; Romidepsin; Belinostat; Panobinostat; Chidamide).

DNA methylation is a major event responsible for silencing gene expression through epigenetic modification in vertebrates. DNA methylation is gene and tissue specific, and is crucial for differentiation and cell fate determination (Razin & Riggs, 1980). Methyl groups are covalently added by DNMT to the 5-position of cytosine nucleoside in CpG-islands which usually occur at or near the promoter site of genes essential for normal cell function (Bird, 1992; Robertson & Jones, 2000). Methylated DNA then potentially attracts specific methyl-CpG-binding proteins or/and chromatin remodeling factors to prevent transcription factors binding to, or leading to chromatin structure changes, thereby repressing gene expression. Hypermethylation in CpG-island promoters have been proved to be tightly associated with tumorigenesis (Esteller et al., 2000; Herman et al., 1994; Herman, Jen, Merlo, & Baylin, 1996; Stirzaker et al., 1997; Wang et al., 2009b). The methyl marker can be actively removed by DNA demethylation such as in the case of implementation of ten-eleven translocation (TET) family of dioxygenases. Interestingly, the only two approved DNMT inhibitors Azacitidine and its deoxy derivative, decitabine, are metabolized to 5-aza-2'-deoxycytidine-triphosphate, to subsequently incorporate into the newly synthesized DNA, which then form covalent interaction with DNMTs, leading to the degradation of enzymes and thereby the passive genomic demethylation during DNA replications (Stresemann & Lyko, 2008).

Histone modification is also critical in transcription regulation to influence biological processes (Kouzarides, 2007). Chromatin is the complex of macromolecules consisting of DNA, protein and RNA, and its basic element is nucleosome. The nucleosome comprises 147 base pairs of DNA wrapping around a histone octamer containing dimer of four core histones (H3, H4, H2A, H2B), respectively (Luger, Mader, Richmond, Sargent, & Richmond, 1997). Different types of histone modifications have been found, such as acetylation, methylation, phosphorylation, ubiquitylation, and sumoylation. These modifications often occur in the structurally flexible and positively charged N-terminal tails of histones (Luger et al., 1997). Among them, the most well studied modifications are acetylation and methylation. Generally, histone acetylation maintains a dynamic equilibrium through the opposite effects of histone acetyltransferases (HATs) and histone deacetylases (HDACs). It has been found that dysregulations and mutations of HDACs are involved in malignant diseases especially cancers, such as hematological malignancies (Bradbury et al., 2005; Marquard et al., 2008; Marquard et al., 2009; Moreno et al., 2010), ovarian cancer (Hayashi et al., 2010; Weichert, Denkert et al., 2008), lung adenocarcinoma (Minamiya et al., 2011), gastric cancer (Weichert, Röske et al., 2008), prostate cancer (Weichert, Roske et al., 2008), pancreatic cancer (Fritsche et al., 2009). All five approved HDAC inhibitors such as vorinostat and romidepsin directly bind to the active site of histone deacetylases to processes the inhibition activity, and often are pan-HDAC inhibitors. Interestingly, all of these drugs are only approved for blood malignant tumors. Their applications in solid tumor are yet to be proved in clinical studies. Most of the selective HDAC inhibitors toward improved efficacy and safety profiles are still in the preclinical stage.

In the past two decades, great efforts have been taken on the drug discovery of targeting epigenetic machinery. However, discovering molecules with high selectivity has been proven challenging due to the evolutionally conserved active sites shared by many epigenetic enzyme families. Nowadays, allosteric modulators are prevailing in the field of epigenetics drugs discovery due to their potential efficient efficacy, less adverse clinical effects and unprecedented opportunities for otherwise undruggable targets.

The concept of allosterism was introduced in 1965 to describe the regulation of protein activity, confirmation and function induced by the binding of a ligand at a so-called allosteric site which is distinctive from the endogenous ligand binding orthosteric site. Compared with protein orthosteric sites, allosteric sites are relatively less evolutionarily conserved and thereby allosteric binding drugs possess greater selectivity and less off-target side effects. Additionally, it is quite common that disease treatment by orthosteric site binding drug could induce acquired mutation that often lead to drug resistance in patients. It is well known that the majority ATP competitive kinase inhibitors could quickly lose their efficacy due to kinase mutations, and therefore remain a huge need in developing next generation kinase inhibitors with efficacy sustainable and less mutation frequent properties. Furthermore, many targets are clinically attractive, yet are undruggable because of lacking appropriate defined binding site for chemical molecules.

Allosteric site created by conformational change such as through protein-protein interactions could provide unique opportunity to turn these undruggable protein target into attractive therapeutics. Lastly, it is also worth to note that orthosteric drugs which generally compete with endogenous ligands, may require high dose in order to achieve the balance between pharmaceutical effect and pharmacovigilance, whereas allosteric drugs are commonly saturable to enable safer dosing with improved physiochemical and drug metabolism/pharmacokinetics properties (Christopoulos, 2002).

Since the approval of the first allosteric inhibitor benzodiazepines which binds to a distal site of neurotransmitter γ-aminobutyric acid (GABA) receptor to overcome the deadly effects of orthosteric-acting $GABA_A$ agonists, many allosteric modulators have been discovered and approved for various disease treatment including rapamycin of mTOR1 inhibitor for malignant cancers and cinacalcet of calcium-sensing receptor for renal hyperparathyroidism. Brexanolone, an allosteric agonist of $GABA_A$ receptor has been approved for treating postpartum depression, a terrible disease that affect many women worldwide. In addition, numerous allosteric drug candidates are in clinical trials or waiting for FDA approval (Table 1). Taken for example, the revolutionary drug imatinib has transformed the devastating chronic myeloid leukaemia (CML) into a chronical manageable disease, yet many patients still encountered imatinib resistant due to acquired mutations (such as T315I) in the BCR–ABL1 tyrosine kinase domain. Second-generation inhibitor ABL001 (asciminib), discovered as an allosteric myristate pocket of BCR-ABL binder distinct from ATP pocket, maintains great activity against ATP-site resistant mutations while with very well tolerated safety profiles in patients (Schoepfer et al., 2018; Zhang et al., 2010). This inhibitor is now in phase III clinical trials for CML. In addition, phosphatases such as protein tyrosine phosphatase (PTP) have long been linked to biological implications in diseases such as diabetes and cancers. However, many efforts toward discovery of potent and selective inhibitors were very challenging, primarily because of the charged nature of the substrate pocket. It was until recently that a highly potent and selective inhibitor SHP099 for non-receptor protein tyrosine phosphatase SHP2 (encoded by gene PTPN11) (Chen et al., 2016). This compound binds to the interface of the SHP2 domains and functions as a glue to lock the protein into an auto-inhibition conformation therefore shutting down its activation through an unprecedent allosteric mechanism. Importantly, this compound demonstrated remarkable potency in inhibiting tumor growth of SHP2 dependent human cancer cell and mouse xerograph models. TNO155, a closed derivative of SHP099 is now in phase I clinical trial for advanced solid tumors (NCT03114319). Concurrently, Three other SHP2 allosteric inhibitors, RMC-4630, IFB-088 and JAB-3068 are in phase I clinical trials as well (Mullard, 2018).

Overall, drug candidates derived from allosteric mechanisms are indeed accumulating to significantly influent the future drug discovery and disease treatment, yet there are few well-established allosteric modulators up to date. Challenges to identify allosteric sites on target protein of interest is the main hurdle hindering the development of allosteric modulators. Theoretically, allosteric sites are different binding pockets on protein surface that distinct from endogenous orthosteric sites. However, detecting allosteric sites on a specific protein is not an easy task because allosteric sites can only be defined by ligand-bound crystal structures and are usually serendipitously discovered during high-throughput screening.

Epigenetic complexes especially histone modifying enzymes are significant regulators of gene expression both in normal and disease processes and thus drug investment on those complexes have gained ever-increasing momentum in the past decades. Classical drug discovery strategies targeting orthosteric sites are still constantly evolving, while novel strategies targeting allosteric sites have gained intensive attention with a series of advantages mentioned above. The activity of chromatin is largely determined by a set of covalent histone marks which are edited by enzymes called "writer", recognize by proteins called "readers", removed by enzymes called "erasers". These modifications are dynamic and act in a highly regulated manner. Knowing the mechanism of histone modification might provide fundamental information of why it is common that epigenetic complex exhibit allosteric sites. Generally, there are two well-known mechanisms. The first is disrupting contacts between nucleosomes while the second is regulating the recruitment of nonhistone proteins to chromatin (Kouzarides, 2007). During histone modification, a serial of well-organized nonhistone proteins carrying distinct enzymatic activities are attracted to bind chromatin or other proteins and this is because histone modification is not a "quick fix" but instead it needs several steps to be accomplished. For example, bromodomain and PHD finger transcription factor (BPTF) being a subunit of nucleosome remodeling actor (NURF) is capable to recognize trimethylation of histone H3 at lysine 4 (H3K4me3) through plant homeodomain (PHD). The direct association between BPTF and methylated H3 tail can target SNF2L ATPase to the promoter of HOXC8 gene activating the expression of HOXC8 (Wysocka et al., 2006). Hence, in addition to the endogenous catalytic pockets, intricate protein-protein interaction (PPI) in epigenetic regulation enables some proteins to evolve specific allosteric sites facilitating PPI involved in chromatin modification. Furthermore, a protein may obtain multiple allosteric sites compatible with its functional diversity. In this review, we summarized recent progress in allosteric modulator development targeting several emerging epigenetic complex machineries, namely polycomb repressive complex (PRC), mixed lineage leukemia (MLL) complex, SWI/SNF, and DNMT1/UHRF1 which are important in disease pathogenesis, and highlighted their potential applications in cancer treatment.

2. PRC2 complex machinery

2.1. PRC2 function

Histones can be methylated on the side chains of arginine, lysine, and histidine residues. Lysine residuals may be mono-, di-, or trimethylated, while arginine can be symmetrically or asymmetrically methylated. Moreover, different types of methylation in different sites of lysine associate with different functions. For example, the monomethylations of H3K27, H3K9, H3K79 are marks for gene activation, while trimethylations of H3K27, H3K9, and H3K79 are instead correlated to gene repression (Barski et al., 2007). Histone methylation is operated by histone methyltransferases (HMT). Based on different substrates, HMT can be further classified into histone-lysine N-methyltransferases (KMT, such as EZH1, EZH2, MLL1, MLL2) and histone-arginine N-methyltransferase (such as PRMTs).

In mammals, there are two main forms of polycomb group complexes—PRC1 and PRC2. PRC2 has gained intensive attention in recent years because of its tight association in cell fate determination and cancer development. The PRC2 core which is highly conserved from Drosophila to mammals comprises four components: EZH1/2, SUZ12, EED and RBAP46/48 (Fig. 1). EZH1/2 is the catalytic subunit that catalyzes the transfer of methyl groups to Lysine 27 of histone H3 (H3K27me2/3). The catalytic subunit EZH1/2 itself is inactive, the activation of the PRC2 complex requires the minimal binding of EED and SUZ12 to EZH1/2. Although EZH1 and EZH2 both can constitute PRC2 complexes, they maintain gene repression through different mechanisms. In contrast to PRC2-EZH2, PRC2-EZH1 exhibits lower HMT activity but rather higher efficiency in preventing transcription, which may pertain to PRC2-EZH1-mediated chromatin compaction and more abundant expression in non-proliferative adult organs of EZH1 (Margueron et al., 2008). Latest data suggested that PRC2-EZH2 and PRC2-EZH1 display similar basal activity, however, the latter is less responsive to H3K27me3-mediated enzyme stimulation (Lee et al., 2018). All the results indicate that repressive role of PRC2-EZH2 may involve other cofactors. Besides the catalytic subunit, the zinc-finger protein SUZ12, the WD40 repeat protein EED and its accessory subunits such as

Table 1
List of representative allosteric binding drugs in discovery and clinical trials.

Drug Name	Target	Originator Company	Disease Indications	Highest Status	References
CIP-137401	Allosteric MEK inhibitor	Cheminpharma	Cancer; Myocardial disease	pre-clinical	
MAK683	EED binding PRC2 inhibitor	Novartis	Diffuse Large B-cell Lymphoma	Phase I/II Clinical	(Qi et al., 2017)
GSK-1264	HIV integrase inhibitor	GlaxoSmithKline	HIV infection	Discovery	(Gupta et al., 2014)
MRL-248	Selective allosteric inhibitor of ROR-gamma-t	Merck	Autoimmune disease	Discovery	(McNeil et al., 2016)
RMC-4550	Selective allosteric SHP2 inhibitor	REVOLUTION Medicines Inc	Cancer	Discovery	(Nichols et al., *2018*)
DF-2593A	C5aR inhibitors, Dompe	Dompe SpA	Pain	Discovery	(Moriconi et al., 2014)
BILB-1941	Allosteric NS5b polymerase inhibitors	Boehringer Ingelheim	Hepatitis C virus infection	Phase I/II Clinical	(Beaulieu et al., 2012)
MLT-827	Selective allosteric MALT-1 inhibitors	Novartis	Diffuse large B-cell lymphoma	Discovery	(Bardet et al., 2018)
ADX-71743	Selective negative allosteric modulator of metabotropic glutamate receptor 7 (mGluR7)	Addex Therapeutics	Depression; Generalized anxiety disorder; Post traumatic stress disorder	Discovery	(Kalinichev et al., 2013)
JG-98	Allosteric inhibitor of HSP70/Bag-3 interaction	UCSF	Cancer; Viral infection	Discovery	(Li et al., 2015)
ADX-92639	Negative allosteric modulator of metabotropic glutamate receptor 2	Addex Therapeutics	Alzheimers disease; Depression; Stroke	Discovery	(Motolese et al., 2015)
AIK-3	MAP kinase inhibitor	Allinky Biopharma	Inflammatory disease	Discovery	(Gomez-Gutierrez, Campos, Vega, & Perez, 2016)
TNO-155	Allosteric inhibitors of SHP2 phosphatase	Novartis AG	Solid tumors	Phase I Clinical	(Chen et al., 2016)
BPN-14770	PDE4D allosteric inhibitors	Tetra Discovery Partners LLC	Alzheimers disease; Fragile X syndrome; Mild cognitive impairment	Phase II Clinical	(Zhang, Xu et al., 2018)
BNC-210	Negative allosteric modulator of alpha7 nicotinic acetylcholine receptor	Bionomics Ltd	Agitation; Anxiety disorder; Depression; Panic disorder	Phase II Clinical	
MSC-1936369B,pimasertib	Highly selective, potent, ATP non-competitive allostericMEK1/2 inhibitor	Serono Pharmaceutical Research Institute SA	Ovary tumor	Phase II Clinical	(Kim et al., 2010)
LGD-6972; MB-11262; RVT-1502	An allosteric glucagon antagonist	Metabasis Therapeutics Inc	Insulin dependent diabetes; Non-insulin dependent diabetes	Phase II Clinical	(Vajda et al., 2017)
CGP-36742; DVD-742; Lu-AE58479; SGS-742;	Selective GABA-B receptor antagonist	Ciba-Geigy AG	Autosomal recessive disorder	Phase II Clinical	
ABL-001; asciminib	Allosteric Bcr-Abl inhibitor	Novartis AG	Acute lymphoblastic leukemia; Chronic myelocytic leukemia	Phase III Clinical	(Wylie et al., 2017)
BMS-791325; BMS-821095; beclabuvir	Allosteric inhibitor of the hepatitis C virus (HCV) NS5B RNA-dependent RNA polymerase	Bristol-Myers Squibb Co	Hepatitis C virus infection	Phase III Clinical	(Esposito, Marciano, & Trinks, 2018)
Ronopterin	Allosteric inhibitor of nitric oxide synthase	vasopharm GmbH	Traumatic brain injury	Phase III Clinical	(Ott et al., 2019)
CVC; TAK-652; TBR-652; cenicriviroc	Dual allosteric antagonist of CCR5 and CCR2	Takeda Pharmaceutical Co Ltd	Liver fibrosis; Liver injury; Non alcoholic fatty liver disease; Non-alcoholic steatohepatitis; Primary sclerosing cholangitis	Phase III Clinical	(Tacke, 2018)

JARID2 and AEBP2 also play crucial parts in the functional activity of PRC2 complex. EED can bind to the H3K27me3 mark by its aromatic cage, and this binding will facilitate the allosteric activation of the PRC2 methyltransferase activity (Margueron et al., 2009). Biochemistry technologies have revealed that existence of SUZ12 is important for the methylation of H3K27 and the silence of *Hox* gene (Cao & Zhang, 2004).

2.2. PRC2 dysfunction in cancer

Dysregulation of EZH2 is additionally frequently observed in many cancer types and has gained wide research attentions in these years. Growing data have proved that PRC2 has diverse properties in cancers that PRC2 can serve as either oncogene or tumor-suppressor with the observations of both gain-of-function and loss-of function mutations of PRC2 being found in different human tumors (Fig. 1a).

Gain-of-function mutations (mainly Y641) occurred in EZH2 can promote catalytic activity of SET domain, leading to the hypermethylation of H3K27 (McCabe, Graves et al., 2012; Sneeringer et al., 2010; Yap et al., 2011). Overexpression of EZH2 has been found to be associated with progress and poor prognosis of many cancers. EZH2, EED and SUZ12 are all found as the targeted genes regulated by pRB-E2F pathway. Among them, EZH2 and EED are indispensable for cancer cells' proliferation. High level of EZH2 stimulates breast tumor initiating cell expansion and cancer progression by downregulation of RAD51 and thus resulting in disrupted DNA damage repair along with activation of RAF1-β-catenin signaling pathway (Chang et al., 2011). In metastatic prostate cancer, EZH2 was found to be a driver of metastasis by silencing DAB2IP which is a Ras GTPase-activating protein (GAP) that acts as a tumor suppressor. Such inactivation simultaneously activates Ras and NF-κB leading to metastasis and tumor progression (Min et al., 2010). Interfering expression level of PRC2 complex core components especially EZH2 using siRNA or small molecule inhibitors can effectively attenuate tumor cell proliferation or tumor growth rate in murine xenograft models (Amatangelo et al., 2013; Bracken et al., 2003; Cebria et al., 2002; Iliopoulos et al., 2010; Neff et al., 2012; Wilson et al., 2010), which highlights the promising future amplifying PRC2 inhibitors in cancer therapy.

On the other hand, loss-of-function mutations of PRC2 complex are frequently observed in tumor progression. NOTCH1-dependent loss-of-function mutations of EZH2 and SUZ12 are found in nearly 25% T cell acute lymphoblastic leukemia (T-ALL) patients, suggesting PRC2 may act as a tumor suppressor in T-ALL (Ntziachristos et al., 2012). In myelodysplastic syndromes (MDS), deletions on chromosome 7 or 7q leading to the inactivation mutation of EZH2 directly impair the normal function of PRC2 and results in the abnormal activation of oncogenic pathway. In this context, EZH2 is identified to play a tumor suppressor role (Ernst et al., 2010; Nikoloski et al., 2010). Additionally, frequent deletions of JARID2 were found to be correlated to the leukemic transformation of chronic myeloid malignancies which implied that the necessity of JARID2 in myelopathy (Puda et al., 2012).

The reason why PRC2 complex acts distinct roles in different tumor types remains to be investigated. However, some reports have proposed the hypothesis that this context-dependent role of PRC2 is depending on the origin of tumor cell and the developmental alternations that may occurred during individual tumor formation (Beguelin et al., 2013; Comet, Riising, Leblanc, & Helin, 2016; McCabe, Ott et al., 2012).

2.3. PRC2 structure

Understanding the spatial organization of PRC2 is indispensable for exploring the underlying mechanism of PRC2 modulation. Han et al. showed that the binding groove with hydrophobic residues from the WD-repeat domain of EED facilitates the recognition of EED binding domain (EBD) of EZH2 (Han et al., 2007). However, it remains unknown how EZH2, the catalytic unit of PRC2, undergoes conformational alternation and signaling transmission after receiving stimulating signals. In 2015, Jiao et al. published crystal structures of activated and basal states of *Chaetomium thermophilum* EZH2-EED-SUZ12 three-member complex with and without peptide H3K27me3, shedding new light on the activation mechanism of PRC2. They showed an allosteric regulation pathway based on the stimulation responsive motif (SRM) of EZH2. In the activated state, H3K27me3 was sandwiched between EED and SRM. In accordance with the previous reports, the side chain of H3K27me3 inserted into the aromatic cage of EED and residues surrounding the methyl-lysine mark interacts SRM motif through hydrogen bonds. Moreover, with the presence of H3K27me3, the catalytic moiety is headed counterclockwise toward SRM rather than that of the basal state. SRM can then transmit stimulating signals resulting from its contacts with both EED and SUZ12 (Jiao & Liu, 2015).

In addition to the interaction among the subunits of PRC2, the interaction between PRC2 and its accessory factors is also important. For example, the presence of AEBP2 significantly increases the HMT activity of minimal active EED-EZH2-SUZ12 subcomplex (Cao & Zhang, 2004). The specific architecture of interaction between human PRC2 components and AEBP2 was firstly revealed with the help of electron microscopy (EM) and MS-coupled cross-linking technology. Region of AEBP2 (K_{95}-K_{116}) where containing last two Zinc fingers (Zn) simultaneously interacts with the WD40 domain ($K_{423/425}$) of EED, SET domain of EZH2 (K_{568}-K_{740}) as well as the Zn finger domain of SUZ12(K_{414}-K_{514}). At the same time, SET domain also interplays with the SUZ12's Zn finger domain. Moreover, the C-terminal region of AEBP2 interplays with both RBAP48 and the middle parts of SUZ12 (Ciferri et al., 2012). On the other hand, JARID2 which contains Jumonji- and ARID-domain is necessary for the proper differentiation and development of mouse embryonic stem cells (Pasini et al., 2010). However, JARID2 lacks histone demethylase activity and this probably owes to the deficiency of two conserved histidine residues in the HXD/EXnH metal-binding motif of the JmjC domain. Sanulli et al. illustrated that JARID2 which acts as the cofactor of PRC2 can be di- and tri-methylated *in vivo* at K116 by PRC2 (Sanulli et al., 2015). Particularly, JARID2-K116 methylated peptide also binds to EED, which essentially resembles to the binding manner of H3K27me3, with the exception of forming hydrophobic interaction between the phenylalanine F117 of JARID2 and aromatic pocket residues of EED (Sanulli et al., 2015). Such interaction exhibits higher affinity than that of H3K27me3 *in vitro*, and functionally mimics the allosteric activation of PRC2 when targets to the chromatin region lacking H3K27me3 (Sanulli et al., 2015).

Recently, a cryo-electron microscopy (EM) structure of human PRC2-JARID2-AEBP2 complex has been reported (Kasinath et al., 2018). The structure provided direct evidence of the model that methylation of JARID2 at K116 is recognized by the aromatic cage of EED and then accelerates the allosteric activation of PRC2 (Fig. 1b). Interestingly, they found that AEBP2 imitates the binding of unmodified N-terminal histone H3 to RBAP48. The binding of unmodified H3K4 to RBAP48 has been suggested to be involved in stimulating PRC2 activity, such binding could be allosterically prevented by H3K4me3 modification and consequently inhibits the HMT activity of PRC2 with decreased methylation level of H3K27 (Schmitges et al., 2011). However, how RBAP48 and histone tail interactions mechanistically affects the PRC2 activity remains indistinct. Of note, the new cryo-EM structures showed that N-terminal Zn domains of AEBP2 may serves as the "bridge" connecting RBAP48 and SET domain to promote the allosteric coupling between RBAP48 and SET domain (Fig. 1b). The structures clearly draw the conclusion that the respective binding of JARID2 and AEBP2 to PRC2 mimics the modified and unmodified histone tail interaction with the complex, and these significant interactions between JARID2-EED and AEBP2-RBAP48 could exert vital effects on the allosteric activation of PRC2. Hence, the binding interface between JARID2-EED as well as AEBP2-RBAP48 may potentiate novel allosteric pockets targeting PRC2.

As a component of PRC2 complex, the minimal SUZ12 critical for PRC2 activity is its C-terminal VEFS (VRN2-EMF2-FIS2-Su(z)12) domain

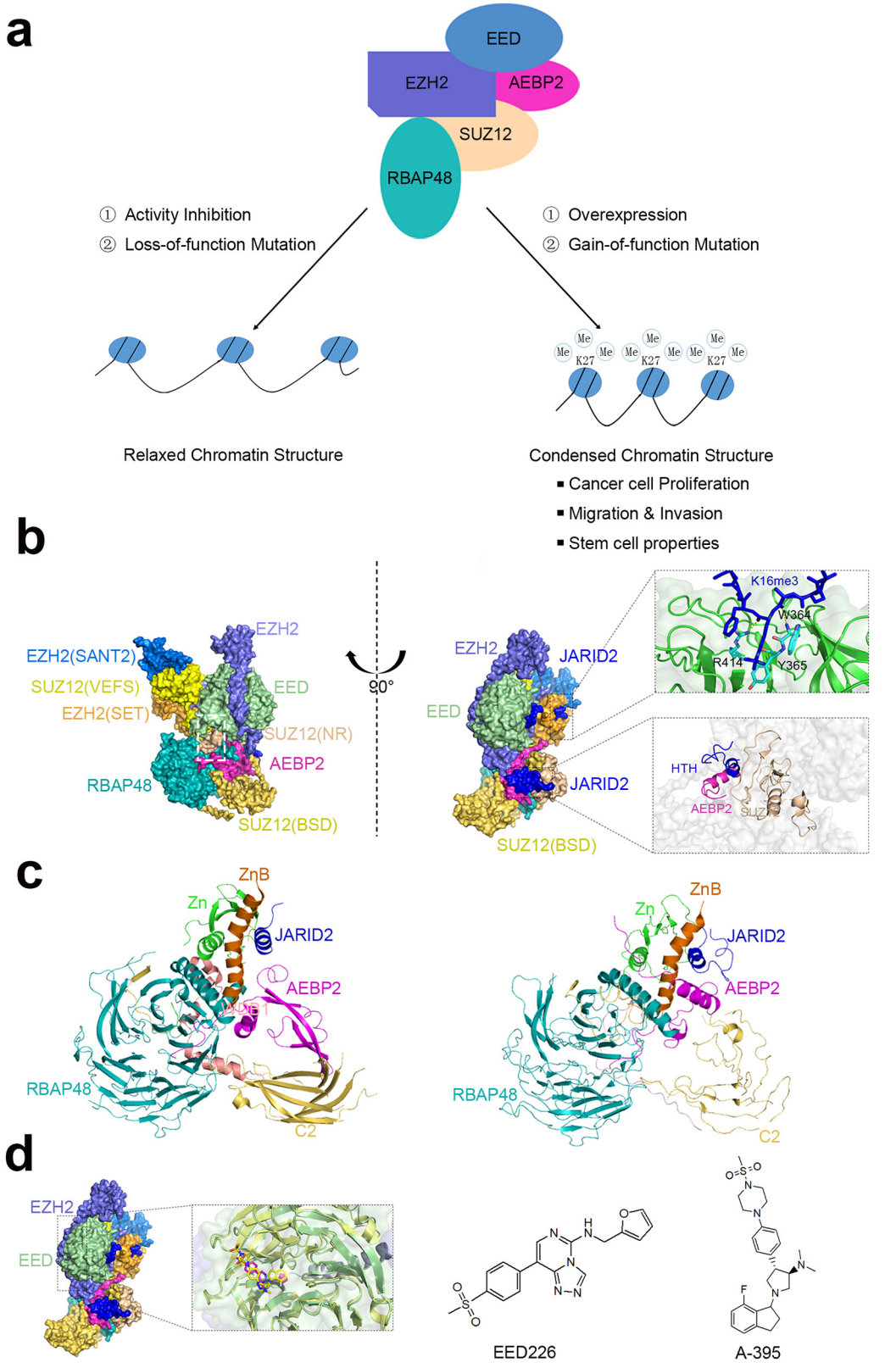

Fig. 1. PRC2 structure, function and potential allosteric sites. (a). Schematic diagram showing the core of PRC2 complex and the two main scenarios of dysfunction in cancer. (b). Structure of PRC2 complex and potential allosteric sites critical for PRC2 activities. (Upper lane) Cryo-EM structure of PRC2 bound to cofactors AEBP2 and JARID2 in the extended active state (PDB: 6C24), with EED in palegreen; EZH2 in salte (SANT1, SBD, BAM, and SRM), marine (SANT2), bright orange (SET); SUZ12 in yellow orange (BSD), yellow (VEFS), wheat (NR); RBAP48 in teal; AEBP2 in light magentas; JARID2 in blue. Dashed circle highlights the central position of neck region (SUZ12) locating at the whole PRC2 complex, while AEBP2 connects SET domain and RBAP48. (Lower lane) Potential allosteric sites: JARID2 K116me3 (blue) interacts with EED (green) by forming hydrogen bond with W364 and R414. Three-element junction between HTH of JARID2, AEBP2 (residual 246-266) and SUZ12. (c). (Left) Crystal structure of SUZ12-RBBP4 (RBAP48)-JARID2-AEBP2 heterotetrameric complex (PDB: 5WAI): RBAP48 in teal, SUZ12 with C2 domain (yellow), WDB1 domain (pink), Zn domain (green), ZnB domain (orange), JARID2 (blue), AEBP2 (magenta). (Right) Corresponding domains in PRC2 Cryo-EM structure (PD 6C24) (d). Structures of allosteric inhibitors of PRC2 complex. EED226 and A-395 targets H3K27me3 binding site of EED.

which sandwiched between the EZH2 SET domain and EED to glue the complex tightly. Importantly, the EM structures illustrated that SUZ12 is critical for maintaining the integrity of the full complex through interactions with all four subunits of PRC2 and two cofactors AEBP2 and JARID2. In particular, "neck" region (residuals 426 to 528) of SUZ12, located in the center of the whole PRC2 complex, interacts with the SAL motif of EZH2, EED as well as the VEFS domain of SUZ12 through intensive hydrophobic interactions (Fig. 1b). Additionally, this neck region was found to facilitate AEBP2 "bridging" between RBAP48 and SET domain of EZH2 by interacting with AEBP2. Moreover, the helix-turn-helix motif (HTH) of JARID2 (residuals 140 to 166) was found wedging between AEBP2 and a β-sheet region in neck region of SUZ12, which forming a three-element junction and thereby stabilizing the interaction between SUZ12 and AEBP2 (Fig. 1b). Although there have been no reports illustrated the importance of this three-way junction, it may be possible that three-way conjunction involving both cofactors AEBP2 and JARID2 could be another new allosteric inhibitory pocket of PRC2. This proposal is supported by the evidence that the stability of PRC2 complex is drastically reduced when AEBP2 and JARID2 is devoid (Ciferri et al., 2012).

Another cryo-EM structure of PRC2 in complex with dinucleosomes indicated that near the SET domain, there is a positively charged pocket of the EZH2 CXC domain which fits DNA strands via the electrostatic interaction with the DNA phosphodiester backbone. It was also observed that the PRC2 can adapt to variable DNA linker lengths between the two nucleosomes, and such function may potentially link to the highly flexible first zinc cluster of CXC. The conformational change of nucleosome-binding domain CXC may lead to the change of catalytic unit SET domain activity when exposing to different chromatin environment (Poepsel, Kasinath, & Nogales, 2018). Although the precise mechanism of how PRC2 accommodates itself to different chromatin contexts remains unclear, this study suggests that disrupting the chromatin-PRC2 interaction around the CXC domain may provide another novel allosteric site in modulating PRC2 function.

Meanwhile, Chen et al reported the crystal structure of SUZ12-RBBP4 (RBAP48) - JARID2-AEBP2 heterotetrameric complex (Chen, Jiao, Shubbar, Yang, & Liu, 2018), in which EZH2-EED-SUZ12 (VEFS) are missing. Interestingly, the crystal and cryo-EM structures do not match with each other (Fig. 1c). The SUZ12 fragment (residuals 78 to 532) becomes much better ordered in the crystal structure, especially the C2 domain (residue 151-370) which adopts an eight-strand β sandwich structure and is closely associated with AEBP2. Additionally, the C2 domain of SUZ12 sits on an edge of the top surface of RBBP48, and prevents histone H3K4 binding to RBAP48 in the core PRC2 complex. The ZnB helix (residue 78 to 109) forms an intramolecular complex with the zinc finger (Zn) domain (residue 420 to 500), which provides a composite concave surface for JARID2 binding. Deletion or mutation of the ZnB helix significantly diminished JARID2 binding to the PRC2 complex, suggesting the importance of JARID2-ZnB interface. The AEBP2 adopts distinctly different conformation in two structures, which spans from the ZnB to the C2 domain and ends on the top surface of RBAP48 in the crystal structure. Taken together, the crystal structure, together with biochemical data, imply that the interface between C2-AEBP2 as well as JARID2-ZnB may serve as potential allosteric pockets for PRC2 inhibition.

Other accessory components such as the Polycomb-like homologs PHF1 (PCL1), MTF2 (PCL2), PHF19 (PCL3) have also been found to be associated with the HMT activity modulation and recruitment to targeted genomic loci of PRC2 (Casanova et al., 2011; Hunkapiller et al., 2012; Sarma, Margueron, Ivanov, Pirrotta, & Reinberg, 2008; Walker et al., 2010). Polycomb-mediated modulation is well known to be significant in tumorigenesis and cellular reprogramming. In mouse embryonic stem cells (ESC), abundantly expressed MTF2 is enriched in the PRC2 complex, while knockdown of MTF2 triggers enhanced self-renewal, impaired differentiation capacity as well as altered H3K27me3 level (Casanova et al., 2011; Kloet et al., 2016; Walker et al., 2010). Moreover, MTF2 was observed to make interactions with core components of PRC2 in mouse ESCs in a mutually exclusive manner against Jarid2 or C17 or f96 (Casanova et al., 2011; Kloet et al., 2016). In 2017, Li et al. first reported the crystal structure of MTF2 bound to CpG-containing DNA highlighting extended homology (EH) domain within MTF2 which is dispensable for recruitment of PRC2 to chromatin, highlighting it might potentially serve as a novel CpG-recognizing domain (Li et al., 2017). Furthermore, Perino et al. hypothesized that MTF2 distinguishes targeted CpG islands through "DNA-shape-reading" ability (Perino et al., 2018). Therefore, considering the critical interplay between MTF2 and PRC2 complex, it is of great significance to further investigate whether additional allosteric sites may exist within MTF2 PRC2 interactions.

2.4. Allosteric inhibitor(s) of PRC2

It has been reported that overexpression of PRC2 components is found in various cancers including melanoma, lymphoma, breast and prostate cancer and is an attractive therapeutic target. Therefore, many pharmaceutical companies and institutions around the world have been developing small-molecule inhibitors of PRC2 for cancer treatment. The first widely used PRC2 inhibitor is DZNep, which is an AdoHcy hydrolase inhibitor that induces apoptosis in breast cancer cells through interference with AdoMet–AdoHcy reaction, thus indirectly leading to reduced methyltransferase activity of PRC2. The majority of previously reported PRC2 inhibitors are S-adenosylmethionine (SAM) competitive compounds directly binds to the catalytic site of EZH2. To name a few, GSK126 is a highly selective EZH2 inhibitor ($IC_{50} = 9.9$ nM versus that of EZH1 $IC_{50} = 680$ nM) blocking the proliferation of EZH2 mutant DLBCL cell lines and the growth of EZH2 mutant DLBCL xenografts in mice. EPZ6438 is another orally bioavailable selective inhibitor of EZH2. It blocks H3K27 methylation in both wild-type and mutant EZH2 lymphoma cells and has been shown to regress SMARCB1-deleted malignant rhabdoid tumor xenograft in mice. CPI-1205 is a third orally bioavailable, highly potent and selective inhibitor of EZH2, EPZ-6438 is currently in clinical trials against multiple tumor types including DLBCL, NHL, INI1-negative tumors and synovial sarcoma. The most advanced progress is in phase II clinical trial for Epithelioid sarcoma, a rare and difficult to treat soft tissue sarcoma. EPZ-6438 as a single agent has showed a confirmed response rate of 13% and a DCR (disease control rate) of 24% with reasonable safety profiles in patients. Additionally, EPZ-6438 as a monotherapy showed a remarkable overall response rate of 71% to in Follicular lymphoma bearing EZH2 mutant in phase II clinical trial. Based on those data, EPZ-6438 was planned to be submitted for FDA approval for Epithelioid sarcoma treatment in Q2, 2019 and Follicular lymphoma treatment in Q4, 2019. If approved, it would be the first ever histone methyltransferase inhibitor marketed for cancer treatment. CPI-1205 is now in phase II clinical trial in combination with androgen receptor signaling inhibitor Abiraterone or Enzalutamide for the treatment of metastatic castration-resistant prostate cancer. Gene signature of EZH2 downstream genes in prostate cancer has shown poor prognosis, and importantly, CPI-1205 has synergistic effects with Enzalutamide in killing prostate cancer cells *in vitro*. The phase 1b data indicated that CPI-1205 is well tolerated and some patients showed improved metastatic disease and reduction of PSA. Additionally, three other EZH2 inhibitors, PF-06821497 (Kung et al., 2018), DS-3201b and SHR2554 are in phase clinical trials for leukemia, lymphoma or small cell lung cancer treatments (Table 2; PF-06821497:NCT03460977; DS-3201b: NCT03879798, NCT02732275,NCT03110354; SHR2554: NCT03603951). GSK126 has been registered for phase I clinical trial (NCT02082977) in multiple tumors, however, no further progress has been reported so far.

Similar to kinase inhibitors binding to orthosteric ATP cofactor sites that would induce acquired mutations in patients, treatment of EZH2 inhibitors binding to SAM cofactor pocket also led to resistance in cancer

Table 2
Epigenetics drug candidates in clinical trial stage

Target	Modulators	Stage	Disease
EZH2	GSK126	Phase I	diffuse large B-cell lymphoma (DLBCL)
	EPZ-6438	Phase II	follicular lymphoma (FL)
	CPI-1205	Phase II	SNF5/INI-1/SMARCB1 genetically defined solid tumors
	PF-06821497	Phase I	Cancer
	SHR2554	Phase I	Relapsed or Refractory Mature Lymphoid Neoplasms
	DS-3201	Phase I	T-cell leukemia/lymphoma, AML, small cell lung cancer
DOT1L	EPZ-5676	Phase I	Adults with Relapsed or Refractory Leukemia
	ORY1001/RG-6016	Phase I/II	AML
		Phase I	SCLC
LSD1	GSK2879552	Phase I	Relapsed/refractory SCLC, AML
	INCB059872	Phase I/II	Advanced malignancies
	SP-2577	Phase I	advanced solid tumors, Ewing Sarcoma
	IMG-7289	Phase II	myelofibrosis
HDAC	ACY-1215	Phase I/II	Lymphoma, Lymphoid Malignancies
	ACY 241	Phase I	Non-Small Cell Lung Cancer
	KA2507	Phase I	melanoma and/or other solid tumors
	Tinostamustine (EDO-S101)	Phase I/II	Hematologic malignancies, Small-cell Lung Cancer, Soft Tissue Sarcoma, Triple-negative Breast Cancer, Ovarian Cancer, Endometrial Cancer
	Abexinostat	Phase II	Follicular Lymphoma
BET	I-BET762 (GSK525762)	Phase II	Hematologic Malignancies, NUT Midline Carcinoma
	OTX015(MK-8628)	Phase I/II	Non-small Cell Lung Cancer, acute myeloid leukemia and diffuse large B-cell lymphoma, advanced solid tumors, glioblastoma multiforme
	CPI-0610	Phase I/II	progressive lymphoma, multiple myeloma, acute leukemia, myelodysplastic syndrome, myelodysplastic/ myeloproliferative neoplasms
	TEN-010	Phase I	acute myeloid leukemia and myelodysplastic therapeutics syndrome, solid tumors
	BMS-986158	Phase I	r pediatric solid tumors, lymphoma, or brain tumors
PRC1	PTC-596	Phase I	Solid tumors, mantle cell lymphoma
Menin-MLL	KO-539	Phase I	Acute leukemias
	SNDX-5613	Phase I/II	relapsed/refractory acute leukemias.

cells. Overcoming such resistance becomes an attractive therapeutic direction targeting PRC2 complex. It is of interest for targeting EZH2-EED interactions. One approach is to directly disrupt the interaction between the N-terminal α-helices of EZH2 and bottom side of the β-blade structure of EED, due to the key role of the EZH2-EED interaction in PRC2 activation. Another approach is to antagonize the interactions between H3K27me3 and the top side of the β-blade structure of EED, given the strong evidence that H3K27me3 peptide stimulates PRC2 activity and propagates repressive H3K27me3 mark in chromatin. The first inhibitor that disrupt EZH2-EED interaction is a string of cell permeable stabilized α-helix of EZH2 (SAH-EZH2) peptides with most potent one having binding affinity of 264nM to EED. This inhibitor not only selectively inhibits H3 Lys27 trimethylation by disrupting the EZH2/EED complex, but also inhibits the growth of PRC2 dependent MLL-AF9 leukemia cells. Using structure based virtual screening, we have discovered that the FDA-approved drug astemizole may be a potential inhibitor of the EZH2-EED interaction (Kong et al., 2014). Astemizole was supposedly directly competed with the N-terminal α-helices of EZH2 for EED binding. Based on the molecular docking and simulation analysis, we have predicted that the benzimidazole group of astemizole fits into a hydrophobic groove of EED occupied by the Phe42 of EZH2, a key interaction of EED-EZH2. Although Astemizole only showed an IC_{50} of 93 μM in inhibiting EED-EZH2 interaction, we have demonstrated it represses lymphomas cell growth mainly by the mechanism of disrupting PRC2 complex, suggesting a promising potential of optimizing astemizole as a more potent and selective EZH2-EED inhibitor. While EED-EZH2 disruptive inhibitors are still at early stage, a breakthrough progress has been made in developing PRC2 inhibitors by targeting H3K27me3 binding site of EED. We have discovered a small molecular compound named EED226 which is a highly selective and potent inhibitor of PRC2 (Fig. 1d). Remarkably, this inhibitor blocks not only the H3K27me3 stimulated activity but also the basal activity of PRC2 *in vitro*, and significantly reduces the H3K27me3 marks in repressive locus and upregulated target genes regulated by PRC2. EED226 demonstrated great efficacy in completely regress human lymphoma derived murine xenograft model. Importantly, EED226 is still sensitive to cancer cells harboring EZH2 mutant that are resistant to SAM-competitive inhibitors, paving promising applications for this new class of PRC2 inhibitor in treating cancers with acquired mutations of EZH2 (Qi et al., 2017). It is worth noting that the parental inhibitor of EED226 was discovered by a strategy of comprehensive high throughput screening. A series of biochemical and biophysical approaches have been on purposely implemented in the hit finding process to identify EED binding inhibitors. This eventually led to the discovery of quite few chemically attractive and active EED binders with PRC2 inhibition activities. EED226 binds to both EED (Kd = 82 nM) and PRC2 complex (Kd = 114 nM) with similar binding affinity. EED226 showed strong competition activity with H3K27me3 peptide for EED binding in biochemical and biophysical assays. This was unambiguously confirmed by co-crystal structure of EED with the compound, in which the inhibitor binds to the H3K27me3 pocket. Interestingly, the binding of EED226 induced a significant conformational change of EED in order to accommodate compound. Its triazolopyrimidine core forms intensive π–π interactions with the residues Y148, Y365, and F97 of EED aromatic cage, whereas the furan group of EED226 is deeply embedded into the inside of the pocket stabilized van der Waals and cation–π interactions with neighboring residues of EED. Thus, EED226 has been demonstrated a new class of PRC2 inhibitor by previously undefined allosteric mechanism through directly competing with H3K27me3 for EED binding. Meanwhile, another novel small-molecule inhibitor, A-395 was also reported. Similar to EED226, A-395 also inhibits PRC2 activity by an allosteric mechanism of disrupting H3K27me3 binding to EED (Fig. 1d).

Similarly, the co-crystal structure showed that binding of A-395 led to the rotamer changes in Trp364, Tyr365, and Arg367 disruptive the original aromatic cage of EED to facilitates the incorporation of the pyrrolidine core in A-395. A-395 reduces H3K27me3 level in cancer cells and showed antitumor activity in DLBCL cell lines as well as in murine xenograft model (He et al., 2017). In addition to those two inhibitors, Barnash et al. developed two peptidomimetic ligands UNC5114 and UNC5115 based on the parental peptide of Jarid2$_{114-118}$-K116me3 by the strategy of combinatorial chemistry and structure-based design (Barnash et al., 2017). Such a ligand only shows a single digital μM binding to EED and modest PRC2 inhibition in biochemical assays, limiting its further applications in cell activity and beyond. A closed compound of EED226, MAK683 from Novartis, demonstrated great potency, selectivity, pharmacological and safety profiles and tumor killing activities in animal studies, has been registered for clinical trials. The compound is now in phase I/II clinical trial for advanced malignancies including Diffuse Large B Cell Lymphoma, nasopharyngeal carcinoma or other solid tumors (NCT02900651). Collectively, targeting allosteric sites of PRC2 is emerging as a promising strategy for the treatment of cancer malignancies depending on PRC2 dysregulation.

3. PRC1 complex machinery

3.1. PRC1 function

In contrast to PRC2, the poly-comb repressive complex 1 (PRC1) with more intricate structure is relatively less studied. PRC1 is generally composed of different components, such as BMI-1, RING1A, RING1B, CBX (CBX2/4/6/7/8), PHC (PHC1/2/3) and RYBP/YAF2. Various assembly of these core subunits members can produce different PRC1 complexes with distinct binding domain and functional modifications (Gil & O'Loghlen, 2014).

Based on the existence of CBX, PRC1 is commonly divided into two types. With the presence of CBX, canonical PRC1 is generally comprised of four parts, including CBX, PCGF, HPH and RING. The canonical view suggested that PRC1 acts downstream of PRC2 from the observation that H3K27me3 can recruit PRC1 to chromatin through the conserved N-terminal chromodomain of CBX subunit. Specifically, PRC1 is recruited to chromatin through reading the H3K27me3 mark via the chromodomain of CBX7 (Fig. 2a). The RING domain of ubiquitin ligase RING1B will catalyze the histone H2A ubiquitylation at lysine 119 (Fig. 2a), and thereby disrupts the elongation of RNA pol II to repress gene transcription (Bernstein et al., 2006; Lee et al., 2007; Simon & Kingston, 2009). On the other hand, it has been reported that some genes can be suppressed by PRC1 in PRC2-indepently manners (Gao et al., 2012; Schoeftner et al., 2006), suggesting the complexity and diversity of the PRC1 functions. The precise mechanism of PRC1 on gene transcriptional silencing remains unclear. BMI-1 (B lymphoma Mo-MLV insertion region 1 homolog, also called PCGF4) was identified as a key component of PRC1 in 2004 (Grima, Chelot, Xia, & Rouyer, 2004) and is essential to keep multipotency of adult hematopoietic, peripheral and central nervous system neural stem cells and their ability of self-renewal (Fasano et al., 2007). BMI-1 and RING1B are heterodimerized via their N-terminal RING domains to form an active E3 ubiquitin ligase for H2A ubiquitylation at lysine 119. The crystal structure of BMI-1/RING1b RING–RING heterodimer and the E2 enzyme UbcH5c illustrated that the RING domain of BMI-1 not only stabilizes RING1B, but also binds to UbcH5c by its N-terminal α-helix and L4, L7 loops (Fig. 2b). Importantly, this minimal BMI-1/RING1B may be capable of directly binding to DNA on nucleosome which is vital for the ubiquitin ligase activity (Bentley et al., 2011). In agreement with this, the crystal structure of Ring1B–Bmi1–UbcH5c E3–E2 (McGinty, Henrici, & Tan, 2014) bound to its nucleosome core particle substrate shows that Ring1B–Bmi1 directly contacts DNA and histone proteins to specifically anchor the complex for histone ubiquitylation.

On the other hand, it has been reported that some genes can be suppressed by PRC1 noncanonically in PRC2-independnet manners (Gao et al., 2012; Schoeftner et al., 2006). There is existence of various composition of PRC1 lacking CBX, such as RYBP-PRC1 and L3MBTL2–containing PRC1 (Endoh et al., 2017; Gao et al., 2012; Qin et al., 2012; Schoeftner et al., 2006). Unlike canonical PRC1, the recruitment of those complexes to chromatin may be through distinctive mechanisms as they don't have CBX component to read H3K27me3 marker (Hisada et al., 2012). One of the typical examples is L3MBTL2–containing PRC1 which was found to be important in proliferation and maintaining pluripotency of mice ESCs (Qin et al., 2012). L3MBTL2 contains four MBT domains that bind to mono- and di-methylated histone H3 and H4 tails *in vitro*. The Zinc finger domain and the MBT domains in L3MBTL2 were essential for recruitment and binding with HDAC1, G9A, RING1B forming parts of the E2F6 repressive complex in somatic cells in a locus specific manner for gene expression silencing. Intriguingly, most of the genes targeted by L3MBT2 in ESCs are not regulated by canonical PRC2 and PRC1.

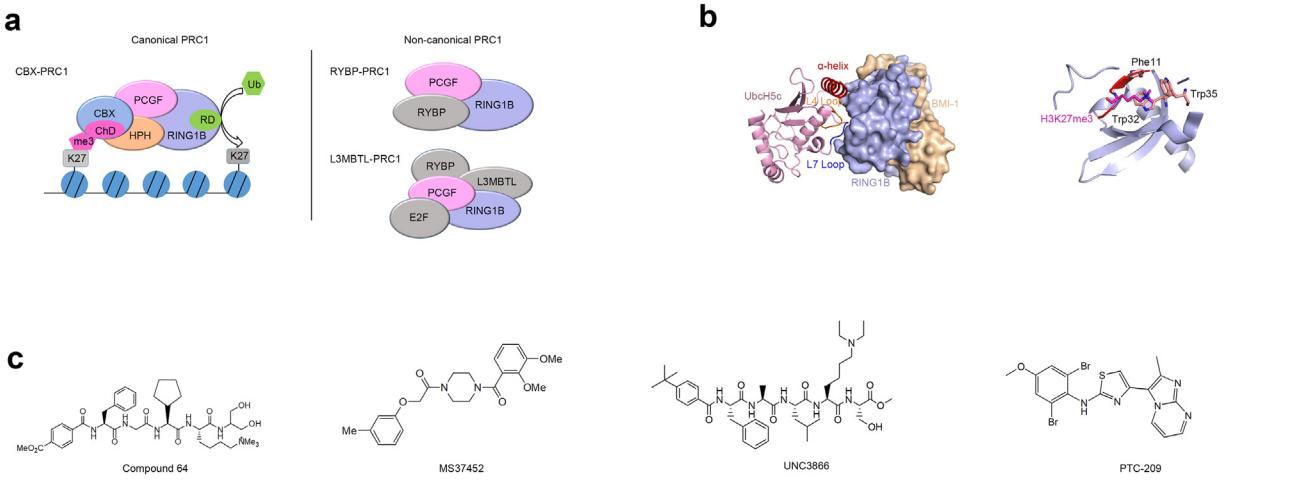

Fig. 2. PRC1 structure and allosteric inhibitors. (a). Classification of canonical and non-canonical PRC1 based on the presence or absence of CBX. (b). (Left) Structure of BMI-1/RING1b-UbcH5c ternary complex (PDB: 3RPG). UbcH5 shown in cartoon is in pink, red (N-terminal a-helix), yellow (L4 loop), and blue (L7 loop), while BMI-1 and RING1B shown in surface are in wheat and light blue respectively. (Right) Structure of CBX7ChD bound to H3K27me3 peptide (PDB: 4X3K). H3K27me3 is colored in magentas. The methyl-lysine binding pocket is formed by three aromatic residues Phe11, Trp32, Trp35 in CBX7 chromodomain. (c). BMI-1 inhibitors that attenuate PRC1 functions.

Recently, multiple studies have demonstrated tight interplay between PRC1 and PRC2. Mono-ubiquitination of Lys119 of histone H2A (H2AK119ub), the catalytic product of non-canonical PRC1 (RYBP-PRC1), could facilitate recruitment of PRC2 through its accessory protein (i.e., JARID2) interaction with H2AK119ub (Blackledge et al., 2014; Cooper et al., 2016). PRC2 catalyzes the generation of H3K27me3, which in turn recruits canonical PRC1 (CBX- PRC1). Canonical PRC1 exhibits an intrinsic activity for chromatin compaction and a relatively low activity for the catalysis of H2AK119ub (Brockdorff, 2017; Cooper et al., 2016; Kahn et al., 2016; Yu, Lee, Oksuz, Stafford, & Reinberg, 2019). The cross talk between PRC1 and PRC2 may synergistically contribute to the generation of facultative heterochromatin.

3.2. Allosteric inhibitor(s) of PRC1

Unlike PRC2, allosteric modulation of PRC1 remains elusive, partially due to diversity of subunit composition of different PRC1 complexes. Yet, efforts have been taken to identify inhibitors that can intervene PRC1 activities. Several compounds targeting CBX proteins in canonical PRC1 have been reported (Fig. 2c). As mentioned above, CBX family proteins (CBX 1-8) are critical for targeting PRC1 to chromatin. Different CBX proteins have distinct and nonoverlapping functions. Among them, CBX7 is the most studied component to date in CBX family because of its important roles in the regulation of cell proliferation and cancer progression. Like other CBX proteins, CBX7 contains a N-terminal chromodomain responsible for recognition of both repressive markers, H3K9me3 and H3K27me3 (Bernstein et al., 2006; Vincenz & Kerppola, 2008). Importantly, multiple evidence have suggested that CBX7 is highly involved in maintaining stemness of normal and cancer stem cells such as gastric cancer cell, hematopoietic stem cell, breast and pancreatic cancer stem cell mainly through its H3K27me3 binding (Klauke et al., 2013; Ni et al., 2018; van Vlerken et al., 2013) (Fig. 2b). Hence, antagonizing H3K27me3 binding activity for chromodomain of CBX7 may serve as a new therapeutic target in allosterically abrogating canonical PRC1 activities. The first peptidomimetics chemical inhibitor derived from methylated histone peptide was reported (Simhadri et al., 2014). Compound 64 has a Kd of 200 nM to CBX7 with selectivity over CBX8 and CBX1. Ren et al. discovered the first small-molecule inhibitor, MS37452 with binding affinity of 29 μM to the CBX7 chromodomain (Kaustov et al., 2011; Ren et al., 2015). Co-crystal structure clearly illustrated that MS37452 directly binds to CBX7 chromodomain through hydrophobic interactions between its dimethoxylbenzene and three aromatic residues (Phe11, Trp32, and Trp35) in CBX7 chromodomain. MS37452 could reduce suppressive transcription of PRC1 target gene p16/CDKN2A specifically through blocking binding of CBX7 to the INK4A/ARF locus in prostate cancer cells. Interestingly, suramin, an essential medicine used to treat African sleeping sickness and river blindness, also was found to bind to CBX7 chromodomain (Kaustov et al., 2011; Ren et al., 2015). It displaced the SETDB1-K1170me3 binding, however, with a different mechanism of dimerizing two CBX7 chromodomain than directing binding to methyl-lysing binding site. Suramin is also known to bind to several different proteins such as thrombin and pyruvate kinase, therefore, this interaction might be one of the mechanism of action for Suramin, which requires further investigation. Perhaps one of the most exciting findings is the discovery of UNC3866, a peptide-derived chemical probe (Stuckey et al., 2016). UNC3866 is a potent antagonist against CBX7 and CBX4 chromodomain with 6- to 18-fold selectivity as against other CBX and CDY chromodomains. UNC3866 antagonizes engagement of PRC2 to chromatin and is effective in inhibiting proliferation of PC3 prostate cell (Bernard et al., 2005). However, the compound showed low bioavailability and modest clearance in mice, suggesting optimization is necessary to improve its pharmacological profiles in order to test its anti-cancer activities in more meaningful models.

In addition to CBX, targeting BMI-1 is another approach to allosterically modulate PRC1 activity. Importantly, Overexpression of BMI-1 has been reported in various human cancers and has been associated with tumorigenesis and cancer progression such as prostate cancer and colorectal cancer (Jacobs, Kieboom, Marino, DePinho, & van Lohuizen, 1999; Lukacs, Memarzadeh, Wu, & Witte, 2010; Vlantis et al., 2011; Warner et al., 2005). PTC-209 was discovered as a BMI-1 small-molecule inhibitor by high throughput screening measuring the reduction of BMI-1 transcript levels. PTC-209 selectively inhibits the self-renewal of human cancer-initiating cells and further suppresses the tumor growth in murine model (Kreso et al., 2014). PTC-209 has been tested in many types of tumors in cancer cells and in animal models since then, however, it has not been submitted to clinical trials duel to its limited potency and poor pharmacokinetic properties. PTC-596 is another BMI-1 inhibitor disrupting BMI-1 within cancer stem cells by inducing hyper-phosphorylation and thus degradation of BMI-1. PTC-596 has potently inhibited BMI-1 function in multiple tumor cell lines and showed good efficacy in various humor tumor animal models. PTC-596 has registered for clinical trials for children with diffuse intrinsic pontine glioma and high grade glioma (NCT03605550), and ovarian cancer (NCT03206645). It is now completed phase I clinical trial in patients with advanced solid tumors (NCT02404480).

4. MLL complex machinery

4.1. MLL complex function

Histone H3 lysine 4 (H3K4) methylation is mainly catalyzed by MLL family of proteins (also known as KMT2 family) sharing a conserved SET domain. In mammals, six MLL family members have been characterized: MLL1–MLL4, SET1A and SET1B (Ansari & Mandal, 2010; Ruthenburg, Allis, & Wysocka, 2007; Shilatifard, 2008). MLL proteins contain multiple functional domains for the assembly of multiprotein complexes that are required for the proper H3K4 methylation activity (Fig. 3a). By associating with WDR5, ASH2L and RbBP5, MLL proteins could catalyze mono-, di- and trimethylation of H3K4 through its SET domain (Fig. 3a-b). MLL1, also called KMT2A, has been extensively studied because the rearrangements of the MLL1 gene are the cause of aggressive acute lymphoblastic and myeloid (Krivtsov & Armstrong, 2007). MLL1 drives the gene expression programs that control stem cell functions by regulating the transcription of specific target genes, including many of the HOX genes and associated cofactors (e.g., *Hoxa9* and *Meis-1*) (Brockdorff, 2017; Lim et al., 2009). Thus MLL is crucial in the development of the axial skeleton and hematopoietic systems in mammals (Yu et al., 2019).

4.2. MLL complex dysfunction in cancer

MLL1 undergoes several types of rearrangements, which are related to leukemogenesis. The most MLL1 rearrangements are balanced translocations on one allele, which is truncated and fused in frame with over 70 translocation partners (Vedadi et al., 2017). Translocations of MLL1 occur in approximately 5% of acute lymphoblastic leukemia (ALL) and 5–10% of acute myeloid leukemia (AML) cases in adults as well as in more than 70% of infant ALL and 35–50% of infant AML patients (Chen & Armstrong, 2015). MLL1-rearranged leukemia has been shown to be associated with high expression of the homeobox (HoxA) cluster genes, which specify cell identity during hematopoiesis and favor immortalization of leukemic cells (Alharbi, Pettengell, Pandha, & Morgan, 2013). MLL1 fusions cause persistent activation of HoxA9 and its cofactor MEIS1 which are very important for the normal development of hematopoietic function (Zeisig et al., 2004). The translocation of MLL1 gene resulted in the formation of oncogenic MLL1 fusion proteins, such as MLL1-AF9, MLL1-AF4 and MLL1-ENL (Ayton & Cleary, 2001). In most cases, the MLL1 fusion proteins retained the N-terminal of MLL1 and lacked the C-terminal SET domain of MLL1 and therefore lost methylation activity (Milne, Martin, Brock, Slany, & Hess, 2005). In addition, MLL fusion proteins interactions with other proteins, i.e.

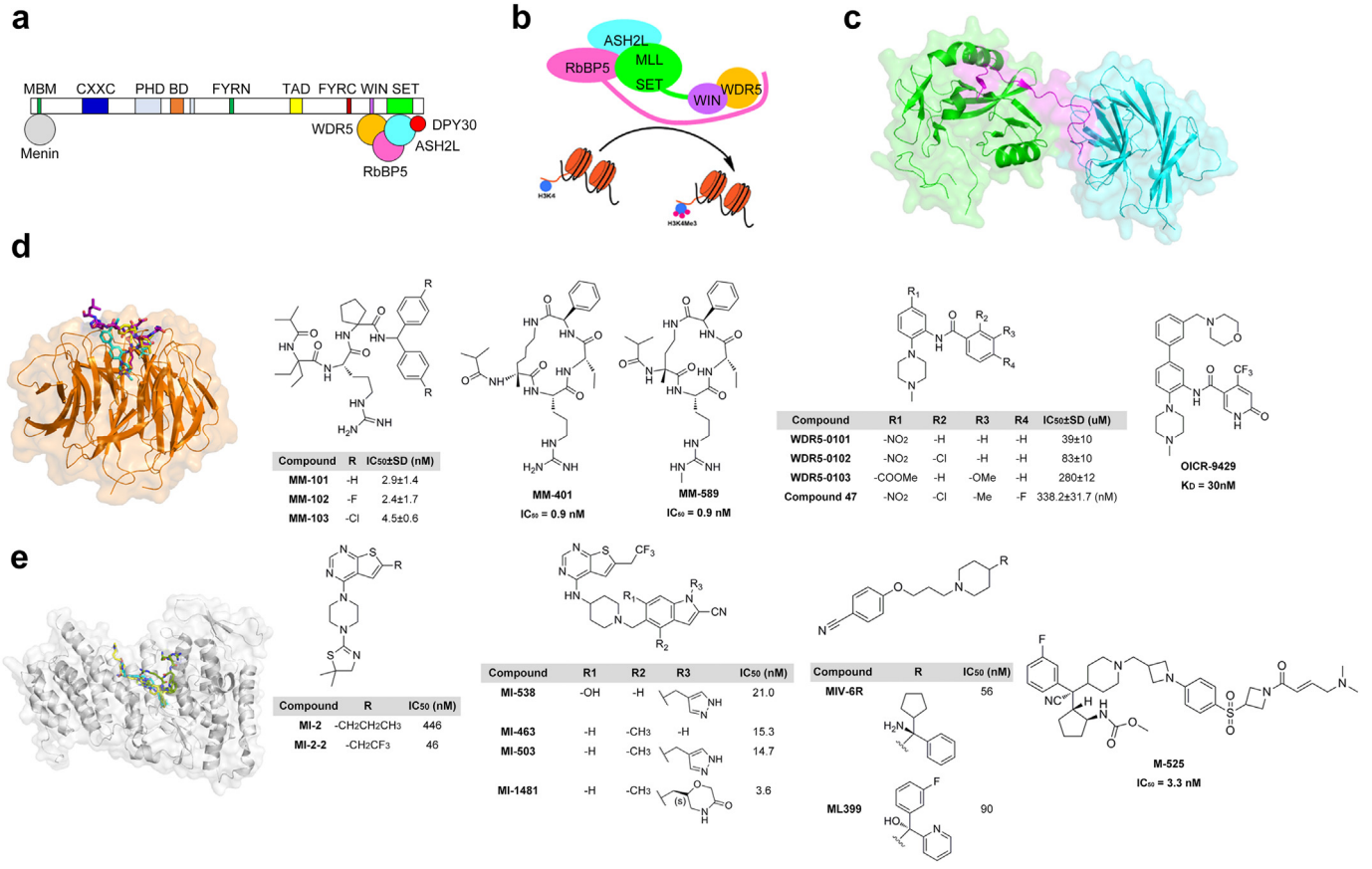

Fig. 3. MLL complex structure, function and potential allosteric sites. (a) The domain structure of MLL1 protein. (b) Cartoon showing the main function of MLL complex in histone medication. (c) The crystal structure of MLL1 (N3861I/Q3867L)-SET/ASH2L/RbBP ternary complex (PDB: 5F6L). (d) (Left) The structure of WDR5 (in orange) in complex with MLL1 WIN peptide (in magenta, PDB: 3EG6) superimposed with structure of WDR5 in complex with OICR-9429 (in cyan, PDB: 4QL1) or MM-589 (in yellow, PDB: 5VFC). (Right) Chemical structures of allosteric inhibitors targeting WDR5-MLL interaction. (e). (Left) Crystal structure of Menin (in grey) in complex with MLL1 MBM peptide (in green, PDB: 3U85) superimposed with structure of menin in complex with MI-1481 (in cyan, PDB: 6BXY) or M-525 (in yellow, PDB: 6B41). (Right) Chemical structures of allosteric inhibitors targeting Menin-MLL interaction.

menin protein, encoded by the MEN1 gene, play pivotal roles in regulating expression of MEIS1 and HOX genes to drive MLL rearranged leukemogenesis (Collins & Hess, 2016). Therefore, developing small molecular inhibitors to block menin-MLL protein-protein interactions is a very attractive therapeutic strategy for MLL leukemia. In addition, the MLL1 fusion proteins needed to cooperate with wild-type MLL1 protein to induce leukemia (Thiel et al., 2010), thus targeting the catalytic activity of MLL1 may be an attractive mechanism for cancer chemotherapy. Interestingly, MLL2 (KMT2B) and MLL1 share an overall similar domain organization and form complex with the same stable set of proteins. However, translocation of MLL2 gene has not been reported in cancer patients. The missenses mutation and overexpression of MLL2 have been correlated with poor prognosis in cancers (Rabello et al., 2018). Deletion of MLL2 in MLL-AF9-transformed cells reduced viability and proliferation and the effect is further amplified with the additional loss of MLL1 gene(Chen et al., 2017), suggesting MLL2 could be an potential target in MLL1 translocated leukemia. In contrary, inactivation of MLL2 due to somatic mutations is associated with the increased risk of the cancers (Augert et al., 2017; Hillman et al., 2018), arguing that MLL2 is a tumor suppressor than an oncogene. Thus, MLL2 could act differently in leukemia and solid tumors. Whether MLL2 inhibition will be an efficacious therapeutic option in MLL rearranged leukemia is waiting for the availability of specific inhibitors of MLL2. Evidence of other MLL family proteins in association with cancer have been summarized in other well written reviews (Vedadi et al., 2017; Yang & Ernst, 2017) and will not be discussed in this paper.

4.3. MLL complex allosteric inhibitors

MLL proteins itself generally exhibit poor H3K4 methyltransferase activity (Dou et al., 2006; Patel, Dharmarajan, Vought, & Cosgrove, 2009). The catalytic domain of MLL proteins resides in large, multi-subunit complexes composed of WDR5, ASH2L, RbBP5, and DPY30 (Fig. 3b–d). MLL2-4, SET1A and SET1B can be fully activated by interacting with RBBP5–ASH2L heterodimer, while the full activation of MLL1 require corporative interaction with RBBP5–ASH2L and WDR5, in which the WDR5 acts as a bridging molecule to facilitate the formation of MLL complexes (Li, Han et al., 2016). In the absence of WDR5, MLL1 interacts with RBBP5–ASH2L with very weak binding affinities and is unable to dimethylate H3K4 *in vitro* (Dou et al., 2006). Knock-down of WDR5 or mutation of key residues on WDR5 led to a dramatic reduction of H3K4 trimethylation *in vivo* (Patel, Vought, Dharmarajan, & Cosgrove, 2008; Wysocka et al., 2005). the histone methyltransferase activity of wild-type MLL1 is required for the full transformative capacity of MLL fusion proteins during leukemogenesis (Klopfenstein et al., 2006; Wang et al., 2009a). This suggested that directly inhibition of the catalytic activity of MLL1 may be a promising approach for cancer chemotherapy. On the other hand, given the importance of WDR5 in MLL1 complex activation, it is also a legitimate idea to targeting WDR5-MLL1 interaction to allosterically inhibit MLL1 functions.

Multiple co-crystal structures of WDR5 in complex with MLL peptide (Alicea-Velazquez et al., 2016; Dharmarajan, Lee, Patel, Skalnik, &

Cosgrove, 2012; Patel, Dharmarajan, & Cosgrove, 2008; Ruthenburg et al., 2006; Song & Kingston, 2008; Zhang, Lee, Brunzelle, & Couture, 2012) and H3 histone (Couture, Collazo, & Trievel, 2006; Han et al., 2006; Ruthenburg et al., 2006; Schuetz et al., 2006) have been determined (Fig. 3d). The binding pocket of WDR5 for both While MLL peptide (so called WIN, WDR5 interaction motif) and histone H3 peptide is suggested to have a good degree of plasticity, a characteristic important for the development of inhibitors. A systematic analysis of MLL1 and H3 sequences led to the identification of two tripeptides, Ac-ARA-NH_2 and Ac-ART-NH2, which bind to WDR5 with *K*i values of 120 nM and 20 nM, respectively (Karatas, Townsend, Bernard, Dou, & Wang, 2010). Subsequently, modifications of Ac-ARA-NH_2 led to several more potent peptidomimetic inhibitors of WDR5-MLL1 interaction with Ki of ≤ 1 nM (MM-101~103, Fig. 3d). Co-crystal structures of MM-101 and MM-102 in complex with WDR5 suggested that the inhibitors mimic MLL WIN peptide for binding to WDR5 (Karatas et al., 2013). However, MM-101 has very modest cellular potency and poor metabolic stability in human microsomes. Structure-based optimization of MM-101 lead to MM-401, a highly selective MLL1 inhibitor with over 3 folds more potency relative to MM-101. MM-401 could also specifically inhibits growth of MLL leukemia (Cao et al., 2014). Further design, synthesis, biological evaluation yielded a new cyclic peptidomimetic, MM-589 (Fig. 3d), which is >40-times more potent than MM-401 in inhibition of cell growth of human leukemia cells harboring MLL translocations (Karatas et al., 2017). Co-crystal structures of these inhibitors in complex with WDR5 provide the structural basis for their high affinity to WDR5.

Besides peptidomimetic inhibitors, several nonpeptide, small-molecule inhibitors have been identified to disrupt WDR5—MLL interaction. Based on high throughput compound libraries screening, three compounds with benzamide scaffold were identified as WDR5-MLL inhibitors (WDR5-0101~0103, Fig. 3d). However, those inhibitors presented weak MLL KMT inhibitory activity with the most potent inhibitor WDR5-0103 showing an IC_{50} of ~40 μM (Senisterra et al., 2013). Based on the crystal structure of WDR5 in complex with WDR5-0102, a systematic optimization led to compound 47 with more than 25-fold increased activity in displacing WIN peptide (Bolshan et al., 2013). However, no cell-based studies have been reported for these inhibitors, assuming either lack of cell permeability or not potent enough. Further modification of compound 47 resulted in OICR-9429, a potent small-molecule inhibitor of the WDR5-MLL1 interaction (Kd (SPR) = 30 nM) (Getlik et al., 2016) (Fig. 3d). This compound showed significant biological activity in several cancer models that are related to WDR5-MLL1 interactions. The mutations of CCAAT/enhancer binding protein α (C/EBPα), a master regulator myeloid gene expression programs in the hematopoietic system, are present in 9% of patients with AML. C/EBPα p30, the most common type of *CEBPA* mutations in AML, preferentially interacts with WDR5. Significantly, OICR-9429 selectively inhibited proliferation and induced differentiation of human AML cells that express p30 (Grebien et al., 2015). Another study showed that OICR-9429 could inhibit the proliferation of tumor cells with gain of-function (GOF) p53 mutants, which were found to upregulate the transcription of MLL1 and MLL2 leading to a global increase of histone methylation (Zhu et al., 2015). Furthermore, OICR-9429 was shown to block the N-Myc/WDR5 interaction, thereby reducing neuroblastoma cell proliferation (Sun et al., 2015). Afterwards, further optimizations of the benzamide scaffold have been reported (Chen, Li et al., 2018; Li, Chen, Wang et al., 2016; Li, Chen, Xu et al., 2016; Li, Wang et al., 2016). More recently, by using fragment-based design approach, Wang et al identified several chemically distinct hit compounds, which were expanded from 6,7-dihydro-5H-pyrrolo[1,2-a] imidazole fragment, as novel and potent WDR5-MLL inhibitors. However, most of the inhibitors showed modest activities in cells, preventing the further applications of the compounds in animal tumor models.

Besides the core components, menin encoded by the multiple endocrine neoplasia 1 (Men1) gene is also a highly specific partner of wild-type MLL1/2 and all MLL1 fusion proteins (Shukla et al., 2010). Association of menin with MLL fusion proteins upregulates expression of target genes, such as *HOXA9* or *MEIS1*, and is essential for leukemogenic transformation (Robert et al., 2003). In addition, the interaction of menin with the wild-type MLL plays an important role in various solid tumors, including pediatric gliomas (Li, Zhang et al., 2018), prostate cancer (Malik et al., 2015), and hepatocellular carcinoma (Holoch & Margueron, 2017). Therefore, disrupting menin-MLL interaction is also an effective therapeutic strategy (Robert et al., 2003). A series of biochemical and structural studies were carried out to identify the menin-MLL interaction motif, suggesting that two short motifs, MBM1 and MBM2, in the N-terminus of MLL interact with Menin with high binding affinity (Grembecka et al., 2012; Grembecka, Belcher, Hartley, & Cierpicki, 2010; Huang et al., 2012; Meyer et al., 2009; Robert et al., 2003; Shi et al., 2012). Based on the crystal structure of menin-MLL, multiple inhibitors of menin-MLL were developed (Fig. 3e).

By applying a competition-based fluoresce polarization assay, the first small-molecule inhibitors of meinn/MLL interaction with thienopyrimidines scaffold were identified. The most potent compound, MI-2, inhibits the menin-MBM1 interaction with IC_{50} = 446 nM (Grembecka et al., 2012). Based on the co-crystal structure of menin-MI-2 complex, MI-2 was optimized to an inhibitor MI-2-2 with 10-fold more potency (IC_{50} = 46 nM), they all bind to the same central cavity on menin that is mimicking the binding mode of MBM1 (Shi et al., 2012). However, due to the modest cellular activity and poor metabolic stability of MI-2-2, this compound is not suitable for *in vivo* studies. Based on the crystal structure of the menin-MI-2-2 complex, chemical modifications for optimization of both activity and drug-like properties have been carried out (Pollock et al., 2015), leading to discovery of several benzylpiperidine menin-MLL inhibitors with high potency and oral bioavailability. MI-538 (IC_{50} = 21 nM) demonstrated potent activity, selectivity, polarity, pharmacokinetic profile, as well as pronounced effect in a mouse model of MLL leukemia (Borkin et al., 2016). MI-463 (IC_{50} = 15.3 nM) and MI-503 (IC_{50} = 14.7 nM) showed profound activity in MLL leukemia cells, mouse models of MLL leukemia and MLL leukemia patients (Borkin et al., 2015). Recently, compound MI-1481 with very potent activity (IC_{50} = 3.6 nM) (Borkin et al., 2018) was identified, which is also effective in MLL leukemia cells and *in vivo* in MLL leukemia models (Borkin et al., 2018).

The second class of menin-MLL inhibitors are based on the methylpiperidine scaffold (Fig. 3e). High throughput screening combined with medicinal chemistry optimization led to the identification of MIV-6R (IC_{50} = 56 nM), which demonstrated strong and selective effects in MLL leukemia cells (He et al., 2014). Subsequently, A similar compound of hydroxylmethylpiperidine structure, ML399, was identified, which inhibited the menin-MLL interaction with IC_{50} = 90 nM (Senter et al., 2015). By using MIV-6R as the starting point for structure-based optimization, M-525 was discovered as the first-in class, irreversible inhibitor targeting the menin-MLL interface (IC_{50} = 3.3 nM). The co-crystal structure of menin in complex with M-525 showed that its acrylamide group forms a covalent bond with the sulfur atom of Cys329, leading to the irreversibility of M-525. M-525 demonstrates more potent cellular activity in human leukemia cell lines carrying MLL fusions than its corresponding reversible inhibitors, suggesting that irreversible inhibition of menin may be a promising therapeutic strategy for MLL leukemia (Xu, Aguilar et al., 2018). Afterwards, the same group identified another highly potent, noncovalent menin inhibitors M-89 (IC_{50} = 5.0 nM), which is effective both *in vitro* and *in vivo* (Aguilar et al., 2019). Recently, some other small-molecule menin-MLL inhibitors were discovered (Li et al., 2014; Xu et al., 2016; Yue et al., 2016; Zhong et al., 2016). In addition to small-molecule inhibitors, peptidomimetics MCP-1 mimicking MBM1 strongly binds to menin with a IC_{50} value of 18.5 nM, but efforts to further optimize the cell-permeable property remains to be made (Zhou et al., 2013). The most notable advancement is the recent FDA approval of another novel Menin-MLL inhibitor KO-539 for clinical trials in relapsed or refractory

AML (Francis Burrows et al., 2018). The starting point of KO-539 was from a biochemical high throughput screening and was optimized by structure based rational design. KO-539 is a potent, and selective inhibitor of menin-MLL interaction. It potently inhibits the growth of MLL-rearranged cell lines and is remarkably effective in treating MLL leukemias in various *in vivo* models. Recently, another potent and selective Menin-MLL inhibitor SNDX-5613 (VTP-50469) has entered into phase I/II clinical trials for elapsed/refractory (R/R) acute leukemias (Table 2; NCT04065399). It will be interesting to see if these inhibitors can be proved to be an efficacious strategy targeting Menin-MLL for AML treatment.

5. SWI/SNF complex machinery

5.1. SWI/SNF function

The mammalian SWI/SNF complexes which are ATP-dependent chromatin remodeler usually contain 9 ~12 subunits including highly conserved core and variant subunits (Fig. 4). Based on distinct composition of variant subunits, SWI/SWF complexes can be divided in two major subclasses, SWI/SNF-A (also known as BRG1-associated factor, BAF) and SWI/SWF-B (also known as polybromo BRG1-associated factor, PBAF). BRG1 or BRM, BAF155, BAF170, SNF5 are found as the evolutionarily conserved components. The ATPase domain of BRG1 and BRM is responsible for ATP hydrolysis, and the bromodomain specially recognizes ε-N-lysine acetylation motifs (Phelan, Sif, Narlikar, & Kingston, 1999). ARID1A and ARID1B only exist in SWI/SNF-A while BAF200, BAF180 and BRD7 are particularly found in SWI/SWF-B. Also, it has been found that different cell types may have distinct subunits configuration that resulting in lineage-specific biological roles (Wang et al., 1996). Although the diversity and heterogeneity are the characters of SWI/SNF complex, the fundamental role of the chromatin remodelers is to convert the energy generated from ATP hydrolysis into the ability of ATP-dependent DNA translocation. The remodeling ATPase subunit is generally separated into torsion and tracking subdomains which play vital roles in nucleosome sliding and translocating, thereby changing the activity state of chromatin domains. The remodeling process can be divided into three steps. SWI/SNF complexes first bind to nucleosome DNA via its ATPase subunit which leads to the disruption of histone-DNA interaction. The translocation domain then undergoes an

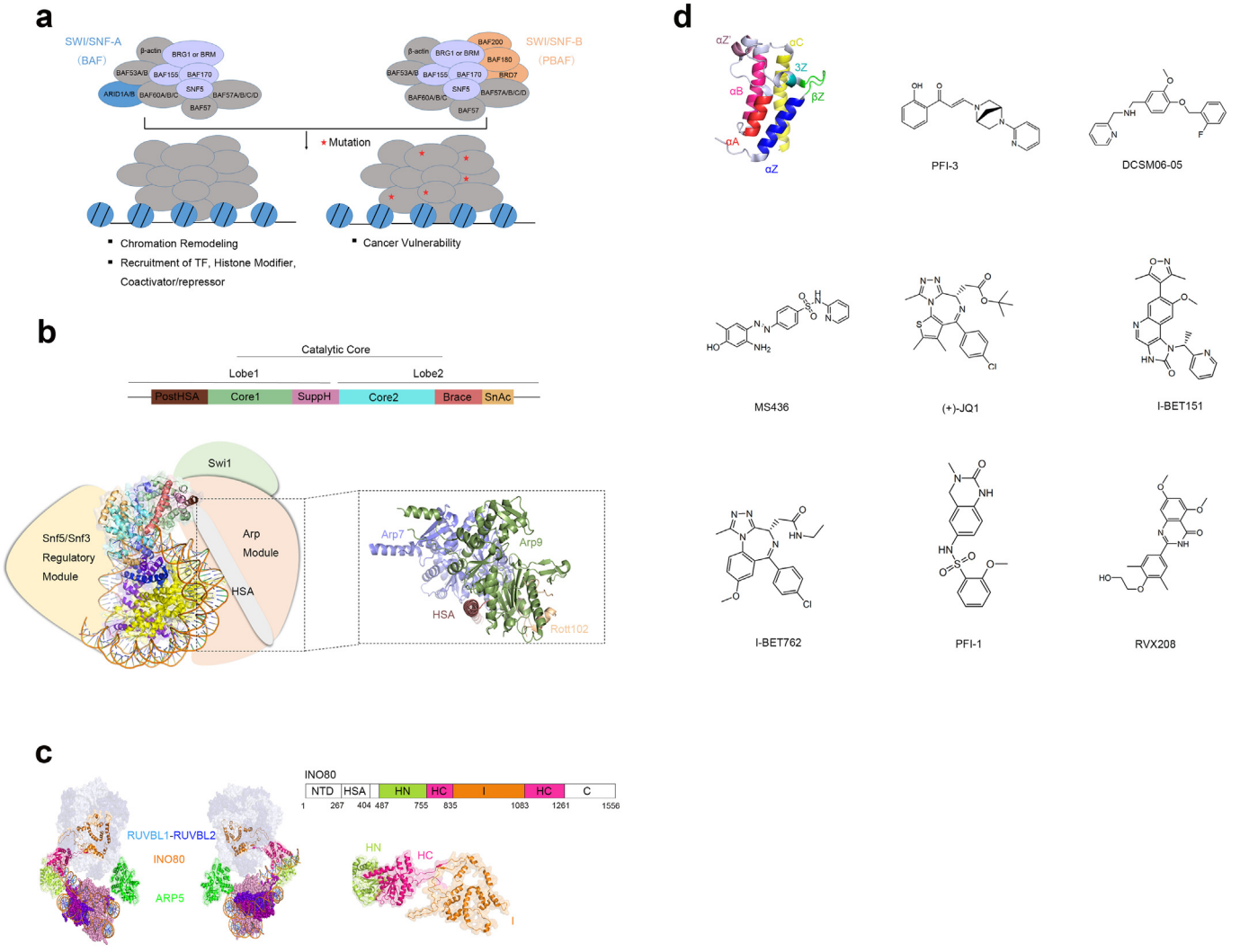

Fig. 4. SWI/SNF structure, function and potential allosteric sites. (a) Schematic diagram of two families of Human SWI/SNF complexes. (b) Structure of *S. cerevisiae* SNF5 in complex with nucleosome. Snf2-nucleosome core particle (NCP) is PDB: 5X0Y; Arp module is PDB: 4I6M. (c) Structure of human INO80 chromatin remodeling complex (PDB: 6HTS). (Left) INO80-nucleosome structure reconstructed with RUVBL1, RUVBL2, ARP5. N-terminal helicase domain (HN), C-terminal helicase domain (HC), INO80 insert domain (I) are shown in green, hot pink, orange. Histone is shown in spheres. NTD, N-terminal domain; HSA, helicase-SANT-associated domain, C, C-terminal domain are not shown. (Right) Detailed structure of chromatin-remodeling ATPase INO80. (d). BRG1 bromodomain structure (PDB: 2GRC) and small molecular inhibitors (PFI-3; DCSM06-05) targeting BRG1/BRM-bromodomain as well as some representative BET bromodomain inhibitors.

ATP-dependent conformational change causing the torsion subdomain to drag DNA from the linker region into the nucleosome and thereby DNA loop is formed. Subsequently, the DNA loop will propagate in a 3′→5′ direction directed by the tracking subdomain, which emerges DNA wave along the nucleosome. These loose structures allow epigenetic enzymes binding and thus enable gene repression or activation. For example, in terms of repression execution, SWI/SNF can recruit HDACs which erase acetyl groups from an ε-N-acetyl lysine amino acid on a histone, promoting chromatin packaging and compaction (Wilson & Roberts, 2011). Alternatively, DNA restructuring can also be achieved by histone-octamer ejection or insertion. Although the mechanism of which is obscure, emerging evidence has indicated that histone chaperons, such as ASF1 and Nap1, are capable of acting coordinately with SWF/SNF complexes to remove histone octamers from DNA (Andrews, Downing, Brown, Park, & Luger, 2008; Gkikopoulos, Havas, Dewar, & Owen-Hughes, 2009).

5.2. Dysfunction of SWI/SNF complex in cancer

Increasing reports have linked SWI/SNF complexes' multifunction to cancer. Loss-of-function genetic mutations are often found in human cancer, for example, more than 20% of various human tumors carry mutations of genes in SWI/SNF complexes (Kadoch & Crabtree, 2015), and therefore are considered as critical tumor suppressors. However, in different malignant cancers context, whether SWI/SNF serves as tumor suppressor or oncogenes should be evaluated on a case by case basis. SNF5, one of the highly conserved subunits has been found to be able to deter tumor progression in malignant rhabdoid tumors (MRT) (Biegel et al., 1999; Versteege et al., 1998), and loss-of-function mutations of SNF5 accelerates oncogenesis. Wilson et al. found that imbalanced epigenetic antagonistic mechanisms between SWI/SNF complexes and PRC2 may be responsible for the tumor initiation with the deficiency of SNF5 (Wilson et al., 2010). SWI/SNF and PRC2 are mutually exclusive when binding to chromatin, and thereby EZH2 activity is significantly upregulated due to lacking competitive suppression derived from SNF5, resulting in abnormal epigenetic silencing of Polycomb target genes and hyperactivated stem cell signatures to enable tumor growth (Wilson et al., 2010). On the other hand, although genetic mutation of ATPase of SWI/SNF has been identified as tumor suppressor, several reports have found that BRG1 may exhibit oncogenic properties in several tumors including acute leukemia (Buscarlet et al., 2014; Shi et al., 2013), pancreatic cancer cells (Liu et al., 2014) and poor colorectal cancer (Liu et al., 2014). Interestingly, it was indicated BRG1 has total opposite functions at different course of Kras-driven pancreatic ductal adenocarcinoma (PDA) development. During the stage of precancerous lesions, BRG1 prevents pancreatic duct cells from dedifferentiating owing to decreasing the maturity of duct cells. Nevertheless, it is unexpected that BRG1 converts into an oncogene in the following stages of neoplasia as evidenced by accelerating EMT process which probably through activation of *Hmga2* (Roy et al., 2015).

5.3. Potential SWI/SNF allosteric site

The structure of SWI/SNF complex is important for understanding the molecular basis of the complex machinery. Progress has been made recently due to the advancement of EM technology. In 2017, Liu et al. reported a EM structures of Snf2 in complex with a mononucleosome from *Saccharomyces cerevisiae,* showing how Snf2, the ATPase domain within the complex interact with nucleosome substrate (Liu, Li, Xia, Li, & Chen, 2017). The same group recently determined the structures of *Saccharomyces cerevisiae* Snf2 bound to the nucleosome in the presence of ADP and ADP-BeFx (Fig. 4b), further revealing a fundamental mechanism for the DNA translocation that underlies chromatin remodeling (Li et al., 2019). Recently, structures of human INO80 ATPase in complex with nucleosome have been reported (Aramayo et al., 2018; Ayala et al., 2018)(Fig. 4c), with extended resolution to atomic level from the earlier report by the same lab (Saravanan et al., 2012), providing great details of potential mechanism and the dynamics of INO80 in nucleosome sliding. However, those structures are far from complete, as the remodelers contain more than 15 subunits. Zhang et al. recently published an improved 3D EM reconstruction of intact SWI/SNF complex, which provided us some new visions on functional regulation of this large ATP-dependent chromatin remodeler (Zhang, Wang et al., 2018). Combined with previous structural information and their EM studies, the functional significance of Snf5 subunit and Arp module is emphasized. Arp module (Arp7, Arp9, Rott42) and Snf5 subunit directly interact with lobe1 and lobe2 respectively (Schubert et al., 2013; Sen et al., 2017; Szerlong et al., 2008; Xia, Liu, Li, Fang, & Chen, 2016). Besides the critical roles of Snf5 in recruitment Snf2 ATPase domain to DNA, it is worth to highlight vital roles of the mobile Arp module in modulating the activity of Snf2 ATPase domain. Arp module interacts directly with helicase-SANT–associated (HSA) domain from Snf2 (Szerlong et al., 2008). With the observation of the substantial conformational alternations of Arp module in three intermediate states of SWI/SNF binding to a nucleosome, it is tempting to propose that allosteric sites may exist within the binding surface between Arp module and HSA domain, which may engage with SWI/SNF activity modulation. Nevertheless, a few allosteric sites have already been extensively studied and will be highlighted in this review.

BRG1 is a core subunit in human SWI/SNF chromatin remodeling complex. BRG1 possesses an evolutionarily conserved ATPase domain as well as a bromodomain. The ATPase domain is the major catalytic module, while the bromodomain is responsible for anchoring the whole complex to nucleosomes where histones are specifically acetylated (Sif, 2004). BRG1 mutations occurred in many tumors are missense mutations mainly around its catalytic ATPase domain. Thus, although directly targeting to ATPase site of BRG1 is an attractive strategy, so far, no small molecular inhibitor has been reported. We speculate that allosteric sites may exist in the interaction interface between BRG1 and associated proteins, which may play critical roles in chromatin remodeling and development. Indeed, allosteric molecules that inhibit both BRG1 and BRM1 ATPase activities have been identified recently by high throughout screen using a biochemical ATPase assay. Further optimization of this urea moiety containing compound led to improved ATPase inhibition activity with the most potent compounds reached to an IC_{50} of greater than 5 nM to both BRG1 and BRM1 (Papillon et al., 2018). The binding mode of the inhibitors were confirmed to be in the N-RecA lobe of the BRM, an allosteric site proximate to the ATP binding site. Importantly, those compounds showed selective cellular activities as they inhibit BRM-dependent KRT80 gene expression in cancer cell with increased proliferation activity in BRM-dependent BRG1-mutant cutaneous melanoma cell line. In a BRG1-mutant human lung cancer mouse xenograft mice model, one of the compounds (compound 14) showed modest tumor growth inhibition correlated with reduced PD readout of KTR80 gene expression, suggesting this series of compound can be further modified to achieve better pharmacological efficacy.

While this discovery is encouraging, great efforts have been taken in developing inhibitors against the bromodomain of BRG1, which is crucial for SWI/SNF complex to exert proper functions. Similar to other bromodomains, the structure of BRG1 bromodomain is bundled with five α-helices: αZ, αZ', αA, αB, αC, and 3Z (Fig. 4d). The acetyl-lysine binding groove locates at the ZA loop of αZ and αA helices and the BC loop of αB and αC helices, in where inhibitors also directly bind in competition with acetyl-lysine. A small βZ unique to BRG1 is found in the ZA loop, which may help to design BRG1 selective inhibitor (Singh, Popowicz, Krajewski, & Holak, 2007).

The importance of epigenetic modifications in diseases have been fully recognized in recent years. Bromodomains are the specialized readers of ε-N-acetyl lysine marks on histone tails and have been found to be dysregulated in oncology and inflammation (Ho et al., 2013; Pavlopoulou & Kossida, 2009; Wang et al., 2013; Xia et al., 2014;

Yu et al., 2015). Inhibitors selectively targeting BET (bromodomain and extra-terminal) family of bromodomains have emerged as a druggable family supported by strong pre-clinical results in various *in vivo* animal tumor models. Quite few BET bromodomain inhibitors are in phase I or phase II clinical trials for hematopoietic malignancy or solid tumors (Pervaiz, Mishra, & Gunther, 2018; Sermer, Pasqualucci, Wendel, Melnick, & Younes, 2019) (Fig. 4d). Meanwhile, inhibitors targeting non-BET bromodomains have also gained interest from research institutes and pharmaceuticals companies (Brand et al., 2015; Filippakopoulos & Knapp, 2014), although no inhibitors have reached to clinical stage.

In 2016, PFI-3 which derived from fragment-based screening was reported to be the first small molecule targeting BRG1/BRM-bromodomain (Fig. 4d) (Gerstenberger et al., 2016). The feature of PFI-3 is the bridged piperazine which is supposed to be related to the selectivity of family VIII bromodomains and thus PFI-3 which is a potent chemical inhibitor targeting BRG1/BRM-bromodomain can be warranted to probe SWI/SNF complex dysfunctions in context with diseased models. In 2018, another hit DCSM06-05 with an IC_{50} value of nearly 9.0 μM was published (Fig. 4d) (Lu et al., 2018). Docking simulations implied that DCSM06-05 exhibits similar binding mechanism of PFI-3 and its 2-pyridine moiety is essential for the inhibition activity because of the hydrogen bond formed between the 2-pyridine and residue F1409 of BRM, a key interaction in driving the binding affinity. Overall, the BGR1/BRM bromodomain inhibitor still at its infant time. Significant efforts are necessary in order to assess whether targeting bromodomain can be a meaningful strategy in allosterically interrogating SWI/SNF chromatin remodeling activities for the treatment of the complex driven cancers. Aside from the aforementioned progress, no other allosteric sites within SWI/SNF complex have been reported and the scientific community is waiting for further exploration through phenotypic screenings or structure-based *de novo* design, based on recent advancements. Such investigation will thereby accelerate the discovery of additional allosteric modulators targeting SWI/SNF complex.

6. DNMT1 complex machinery

6.1. DNMT1 function

DNA methylation, which is a major epigenetic modification, plays an important role in regulation of transcription, genome stability, genomic imprinting, X-chromosome inactivation and retrotransposon silencing (Bird, 2002; Li, 2002; Reik & Lewis, 2005). Mammalian genomes encode three active DNMTs: the *de novo* methyltransferases DNMT3A and DNMT3B as well as the maintenance methyltransferase DNMT1 (Aoki et al., 2001; Li, Bestor, & Jaenisch, 1992; Okano, Xie, & Li, 1998). These enzymes catalyze the transfer of a methyl group from SAM to the C5 position of cytosines within CpG dinucleotides (Cheng & Blumenthal, 2008; Law & Jacobsen, 2010). As a maintenance methyltransferase, DNMT1 shows a preference for hemimethylated DNA and is thus responsible for DNA methylation maintenance during cell replication. Mouse knockout studies have indicated that DNMT1 is essential for embryonic development (Li et al., 1992), suggesting the critical role of DNMT1. DNMT1 is necessary to maintain global methylation and aberrant CpG island methylation in human cells, through regulation by multiple proteins. For instance, LSD1 demethylates and stabilizes DNMT1, thus is required for maintenance of global DNA methylation (Wang, et al., 2009a). Breast cancer-associated gene 1 (BRCA1), a breast tumor suppressor gene, prevents global DNA hypomethylation *via* positively regulating DNMT1 expression (Shukla et al., 2010). Besides, ubiquitin-like with PHD and ring finger domains 1 (UHRF1) is essential to maintain global and local DNA methylation by directly interacting with DNMT1 (will be discussed in more detail below) (Robert et al., 2003). Recently, Stella, a factor essential for female fertility, is found to safeguards the unique oocyte methylome by preventing *de novo* methylation mediated by DNMT1 (Li, Zhang et al., 2018). DNMT1 also plays an important role in regulating pathways that are sufficient for achieving targeted generation of β cells from adult pancreatic α cells (Chakravarthy et al., 2017), confirming the importance of DNMT1.

6.2. DNMT1 dysfunction in cancer

Currently, multiple researches have suggested that DNMT1 is involved in several disease pathways. Aberrant expression of DNMT1 has been demonstrated to be highly associated with various cancers, including gastric (Etoh et al., 2004), pancreatic (Peng et al., 2005; Peng et al., 2006), colon (Chen et al., 2007; Robert et al., 2003), and bladder cancers (Fournel, Sapieha, Beaulieu, Besterman, & MacLeod, 1999). Mutations of DNMT1 have been reported to cause hereditary sensory and autonomic neuropathy type IE (HSAN IE) and autosomal dominant cerebellar ataxia, deafness and narcolepsy (ADCA-DN), two discrete autosomal dominant neurodegenerative syndromes which belong to the same disease spectrum, with variable degrees of overlap (Hamidi, Singh, & Chen, 2015; Maresca, Zaffagnini, Caporali, Carelli, & Zanna, 2015). To date, 18 different nonsynonymous mutations of DNMT1 have been identified, all of them occur in exon 20 or 21, which encode the replication foci targeting sequence domain (RFTS) domain. Multiple evidences demonstrated that RFTS domain regulates DNMT1 binding to heterochromatin during the late S phase and persistent chromatin association during the G2 and M phase (Easwaran, Schermelleh, Leonhardt, & Cardoso, 2004). This domain also plays an important role in function, enzymatic activity, and subcellular localization of DNMT1 (Takeshita et al., 2011). Therefore, some mutations, i.e., Y495C and D490E & P491Y identified in HSANIE patients, caused premature degradation of the protein, educed methyltransferase activity and impaired heterochromatin binding in G2 phase, resulting in global DNA hypomethylation and site-specific hypermethylation (Klein et al., 2011). Three missense mutations identified in ADCA-DN patients, A554V, V590F and G589A, were speculated to affect the interaction with other proteins, i.e., HDAC2, or the DNA- binding (Winkelmann et al., 2012). Particularly, DNMT1 mutations P491Y and Y495C identified in HSAN IE patients not only impair DNMT1 heterochromatin association, but also UHRF1 interaction. Interaction of UHRF1 with DNMT1 releases the TS domain and enables catalytic activity of the CTD (Bashtrykov, Jankevicius, Jurkowska, Ragozin, & Jeltsch, 2014).

6.3. DNMT1 complex potential allosteric sites

To date, several crystal structures of DNMT1 have been determined, revealing that DNMT1 is a large, multi-modular protein (Fig. 5a) (Kanada, Takeshita, Suetake, Tajima, & Nakagawa, 2017; Song, Rechkoblit, Bestor, & Patel, 2011; Song, Teplova, Ishibe-Murakami, & Patel, 2012; Takeshita et al., 2011; Zhang et al., 2015). DNMT1 is composed of several domains that are listed here in the order from the N- to the C-terminus. The RFTS domain, directly interacts with the DNA-binding pocket of the catalytic domain, thereby exerting an autoinhibitory effect on DNMT1 activity (Takeshita et al., 2011). The following domain is the CXXC domain, which comprises a zinc finger and binds unmethylated DNA (Song et al., 2011). Next, the two bromo-adjacent homology (BAH1, BAH2) domains are required for the folding of DNMT1 enzyme. The N- terminal and C- terminal part of DNMT1 is joined by a flexible linker composed of lysine-glycine (KG) repeats. The C-terminal methyltransferase domain (CD) is further divided into two subdomains, the catalytic core and the target recognition domain (TRD). The catalytic pocket is composed of a cofactor SAM and a substrate cytidine binding site. As we mentioned earlier, two approved drugs, Azacitidine and decitabine, are known DNMT methyltransferases inhibitors. Both compounds are converted to the activated triphosphate 5-Aza-dCTP and subsequently incorporated into DNA, then trap the DNMT enzyme by forming irreversible covalent complex, leading to proteasome-mediated degradation of the complex. Although those compounds are quite effective in treating hemopoietic malignancies

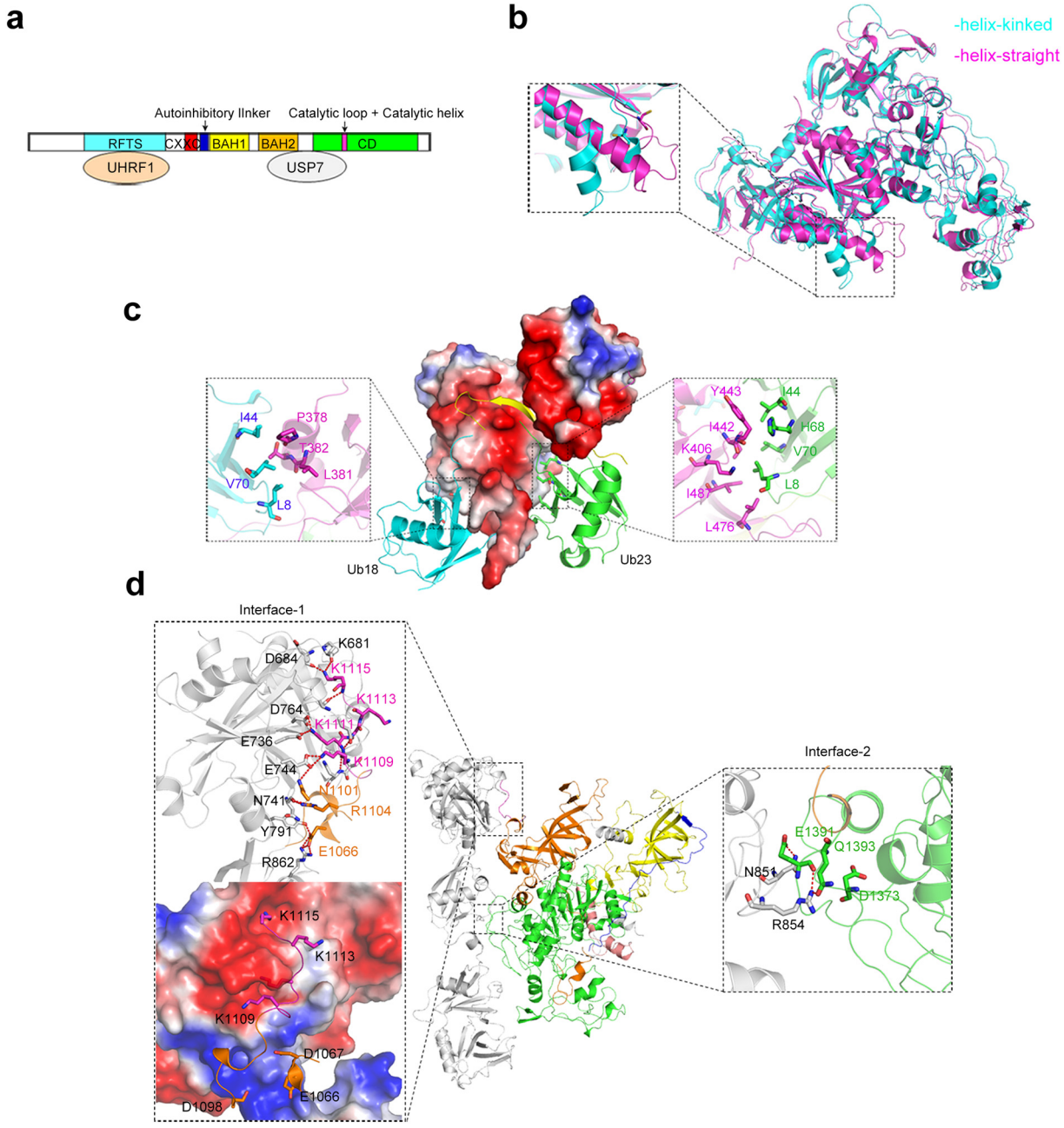

Fig. 5. DNMT1 structure, function and potential allosteric sites. (a) Schematic diagram of DNMT1 domain structure and regions for interacting with UHRF1 and USP7. (b) Alignment of two states of DNMT1. The helix-kinked (PDB: 3PT9) and -straight DNMT1 (PDB: 4DA4) are shown in cyan and magenta, respectively. (c) Interface between DNMT1 and H3UB2 (PDB: 5WVO). (d) Interface between DNMT1 and USP7 (PDB: 4YOC) that may be important for allosteric regulation of DMT1 function.

such as AML and myelodysplastic syndrome (MDS), severe side effects are quite common in patients due to DNA incorporation induced genome instability related toxicities. Therefore, reversible and selective DNMT inhibitors could overcome the liabilities of irreversible inhibitors to broaden clinical applications with improved safety profiles. To this end, current DNMT inhibitors are mainly developed targeting different parts of the catalytic pocket (the DNA, the SAM, or both) (Lopez, Halby, & Arimondo, 2016). However, most of the identified DNMT1 small-molecular inhibitors suffer from weak activity, poor selectivity and toxic side-effects. Therefore, targeting allosteric sites might be a potential strategy that can be explored for next generation inhibitors.

In combination of *in vitro* enzymatic and cellular assays, x-ray crystallography, and computational simulations, we previously identified a conformational transition site which may serve as a potential allosteric site to develop DNMT1 inhibitors with novel mode of action (MOA). This site is composed of catalytic loop and N-terminal portion of the catalytic helix (residues C1227-S1249) which underwent dramatic conformational changes between different states of DNMT1 (Fig. 5b). Moreover, these dynamic structural properties are conserved in different DNMTs and are involved in the process of enzyme catalysis. In supporting of this, mutations of the key residues involved in the conformational transition, N1248 and R1279 didn't affect the binding affinities of substrates or cofactors to DNMT1, however, can reduce the enzymatic activity both *in vitro* and in cells, suggesting structural transition of DNMT1 catalysis could create intermediate states. Oppositing to SAM cofactor binding site, the sequence of this potential allosteric site is not conserved in different DNMTs, thus providing the advantage of finding selective inhibitors (Ye et al., 2018).

Multiple studies have demonstrated that DNMT1 alone cannot maintain global DNA methylation during somatic cell divisions (Spada et al., 2007). The E3 ubiquitin ligase UHRF1 is now very well established as a key regulator of DNMT1-directed DNA methylation maintenance (Bostick et al., 2007), which directly stimulates the catalytic activity of DNMT1 by interacting with the RFTS domain and relieving the autoinhibition effect (Arita, Ariyoshi, Tochio, Nakamura, & Shirakawa, 2008; Bostick et al., 2007; Cooper et al., 2016). Disruption of DNMT1/UHRF1 interactions acts as an oncogenic event, which promotes tumorigenesis of human and mice glial cells (Hervouet et al., 2010). UHRF1 specifically recognizes hemimethylated DNA via its SET- and RING-associated (SRA) domain (Arita et al., 2008; Avvakumov et al., 2008; Bostick et al., 2007; Hashimoto et al., 2008; Qian et al., 2008; Sharif et al., 2007), which helps recruit DNMT1 to DNA replication sites. The C-terminal RING domain of UHRF1 acts as an E3 ubiquitin ligase to target histone H3 at Lys18 and/or Lys23 (Harrison et al., 2016; Karagianni, Amazit, Qin, & Wong, 2008), the ubiquitinated H3 (H3Ub) is subsequently recognized by RFTS domain of DNMT1 (Nishiyama et al., 2013; Qin et al., 2015). H3Ub functions as a unique chromatin mark, and DNMT1 targets it for its recruitment to and activation at hemimethylated DNA sites, which is critical for the proper subnuclear localization of DNMT1 and maintenance of DNA methylation (Ishiyama et al., 2017; Li, Wang et al., 2018). Recently, the crystal structure of the RFTS domain in complex with H3-K18Ub/23Ub was determined, revealing structural and mechanistic insights into UHRF1-mediated DNMT1 activation in the DNA methylation (Ishiyama et al., 2017). As shown in Fig. 5c, the two ubiquitins are simultaneously bound to the RFTS with a combination of canonical hydrophobic and atypical hydrophilic interactions, providing potential binding site for allosteric regulation. Although the complex structure of DNMT1/UHRF1 complex has not been reported, recent study showed that UHRF1 N-terminal ubiquitin-like domain (UBL) directly interacts with DNMT1, and the binding could stimulate DNMT1 enzymatic activity (Li, Wang et al., 2018). Therefore, the interactions between UBL and DNMT1 could be important in guiding the development of such allosteric inhibitors in the future.

Besides UHRF1 dependent ubiquitination, the stability, activity and abundance of DNMT1 during the cell cycle is also governed by deubiquitination via the ubiquitin specific peptidase 7 (USP7, also known as herpes virus associated ubiquitin specific protease (HAUSP)) which protects DNMT1 against proteasomal degradation (Du et al., 2010; Felle et al., 2011; Qin, Leonhardt, & Spada, 2011). USP7 could also prevent autoubiquitinylation of UHRF1, thus plays an important general role in epigenetic regulation (Nicholson & Suresh Kumar, 2011). The stable, soluble DNMT1/USP7 complex associates with UHRF1 to form a trimeric complex on chromatin (Felle et al., 2011). The crystal structure of DNMT1/USP7 complex showed that there are two intermolecular interfaces (Fig. 5d). The interface-1, which involves the Lysine residues within DNMT1's KG linker and an acidic groove of USP7, primarily mediates the interaction between the two proteins (Cheng et al., 2015), whereas residues on the Interface-2 are less important. Disruption of interface-1 promotes the degradation of DNMT1 (Cheng et al., 2015), indicating that this interface is a potential allosteric site for the design of DNMT1 inhibitors. Many inhibitors directly binding to the catalytic site of USP7 have been developed (Zhou et al., 2018). A highly potent, selective and substrate ubiquitin non-competitive inhibitor that binding to an novel allosteric site of USP7 was also reported recently (Gavory et al., 2018).

7. The future of Epigenetic allosteric modulator

Precision medicine, or personalized medicine is a revolutionary medical model for disease prevention and treatment that considers individual differences in lifestyle, environment, and biology. Systemic analysis of genetic content, molecular pathway and cellular activities of a patient is a must have step to diagnosis for optimal treatment of diseases such as cancer. The inherited genome does not determine the overall risk profile of an individual's risk. It is now well established that epigenetic alterations are an important driver of phenotype that broadly exist in human diseases. DNA methylation, chromatin remodeling, and histone modification are the epigenetic mechanisms implicated in cancer prevention and treatment. Thus epigenetic signature has become valuable biomarkers, take for example, abnormal DNA methylation patterns have been successfully utilized in clinical testing for diagnosis and treatment of cancer patient (Koch et al., 2018), and H3K27me3 is the hall mark of gene repression that is often associated with poor prognosis and progression in many cancers (Sawan & Herceg, 2010). However, it is also quite common that heterogeneity in the chromatin landscape of cancers could be widespread exist (Rendeiro et al., 2016). Therefore, accurate and robust epigenetic tests are important in order to precisely apply epigenetic medicines to patients in maximizing effects while minimizing the potential side effects.

As summarized in Table 2, there are quite few epigenetic drugs currently are in clinical studies against hematologic malignancies or solid tumors. Among them, MAK683 of EED binder (Table 1), PTC-596 of BMI-1 binder, and KO-539 of menin-MLL interaction, are most notable drug candidates binding to an allosteric site to modulate the activities of the corresponding complex machinery. While it is still very early for those compounds to be successful in their clinical trials, it is still exciting to see the concept of allosteric modulation targeting disease-relevant complex machinery, becoming more and more attractive strategy in drug discovery. Given the complexity of tumorigenesis and heterogeneity of cancers, carefully select appropriate patients based on conceivable biomarkers would be critical to assure the likelihood of positive treatment response for such drugs in precision medicine.

Benefits of allosteric modulators have been well established around the world and which has attracted more and more researchers to devote themselves into the discovery of epigenetic allosteric modulators. Epigenetic machinery in cancer have been emphasized over the past years, yet the limited applications of epigenetic therapies in clinical and fewer available selective and applealing probes demands better and more effective novel epigenetic inhibitors. Many epigenetic enzyme families possess conserved catalytic domains which share similar structures. This could be attributed to non-selective properties for many of the epi-drugs and epi-probes which could cause non-specific epigenetic landscape alternations and unexpected off target adverse effects. Allosteric modulators target sites are often unique, not directly participating in catalytic process, and are indispensable and pivotal in regulating the activity and biological functions of a complex. Therefore, developing allosteric modulators targeting epigenetic complexes not only may provide a new path for improving the poor selectivity of epi-drugs and epi-probes, but may expanding the target space making undruggable proteins to be druggable. Moreover, comparing to orthosteric sites, there are more than one allosteric site within one target, and thus allosteric modulators can exert their pharmacological effects when endogenous ligand exists in a saturable manner. Such characteristics can attenuate normal fluctuations in proteins activity and extend disease applications by mitigating potential safety liability. However, with the circumstance that epigenetic allosteric modulators reported were serendipitously discovered through HTS, developing epigenetic allosteric modulators are still facing many challenges to overcome. Extensive research needs to be explored in charting much clearer path forward in the journey of epigenetic allosteric modulator discovery. To solve these problems, it is urgent to develop reliable and standardized principles in order to identify allosteric sites and corresponding drug candidates. While traditional high throughput screening against a large size compound library using biochemical or cellular phenotypical assays is still powerful approach, it is far more from efficient due to difficulties including limitation of compound diversity, hit deconvolution and confirmation (Eder, Sedrani, & Wiesmann, 2014). It is exciting that emerging technologies could pave new directions in allosteric modulator discovery. Among them, DNA-encoded chemical library (DEL) technologies

which can deeply extend the chemical spaces to millions even billions are increasingly appreciated in drug discovery (Goodnow Jr., Dumelin, & Keefe, 2017). In addition, with the rapid advancement of deep learning technologies, artificial intelligence is rising as a potential powerful and revolutionary application in *de novo* design and generation of meaningful new biologically active molecules with desired druggable properties (Hessler & Baringhaus, 2018; Sharma & Sharma, 2018). Furthermore, current computational technologies can predict 3D structure of a large size protein (Li, Fooksa, Heinze, & Meiler, 2018), can create proteins with novel sequence and unique structure yet present similar physiological functions with naturally existing protein (Shen et al., 2018; Silva et al., 2019), and can more accurately predict the protein-protein interaction interfaces (Gao, Zhou, & Skolnick, 2019). Importantly, technologies can search, define novel druggable site/pocket through de novo simulation (Bai, Morcos, Cheng, Jiang, & Onuchic, 2016; Xu, Wang et al., 2018). Even more, artificial intelligence can screen compound in high speed to identify hit, optimize the potency and pharmacological properties (Xu et al., 2019). With the promising of technologies like DEL and artificial intelligence, the discovery of more biologic active allosteric site binding inhibitors/activators will be accelerated in targeting epigenetic complex machinery. This for sure will exhibit great promise to facilitate the development of novel MOA driven chemical tools towards the investigation of mechanisms of critical nodes in epigenetic network, more importantly, to foster a new paradigm applications of epigenetics therapeutics for the treatment of human diseases.

Declaration of Competing Interest

All authors declare no competing interest.

Acknowledgements

We gratefully acknowledge financial support from the sponsor-id="http://dx.doi.org/10.13039/501100001809">National Natural Science Foundation of China (21877095 to K.Z., 91853205, 81625022 and 81821005 to C.L., 81728020 to H.W.), Key Research Project of Shandong Province (2017GSF18177 to K.Z.& H.W.), Natural Science Foundation of Shangdong Province (ZR2018LH025 to H.W.), Key Research Project of Yantai (2019XDHZ102 to H.W.), the National Science and Technology Major Project (2018ZX09711002 to C.L.), the sponsor-id="http://dx.doi.org/10.13039/501100003399">Science and Technology Commission of Shanghai Municipality (9XD1404700 to C.L.), K. C. Wong Education Foundation to C.L. and Taishan Scholarship to K. Z.

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