Review article

Follicular Lymphoma, a B cell malignancy addicted to epigenetic mutations

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Conflict of interest Jude Fitzgibbon has received research funding from Epizyme. The authors have no other conflicts of interest to declare.

This article to be included in the Special Focus Issue on Epigenetic Drugs

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Abstract

While follicular lymphoma (FL) is exquisitely responsive to immuno-chemotherapy, many patients follow a relapsing remitting clinical course driven in part by a common precursor cell (CPC) population. Advances in next generation sequencing have provided valuable insights into the genetic landscape of FL and its clonal evolution in response to therapy, implicating perturbations of epigenetic regulators as a hallmark of the disease. Recurrent mutations of histone modifiers *KMT2D*, *CREBBP, EP300*, *EZH2, ARIDIA,* and linker histones are likely early events arising in the CPC pool, rendering epigenetic based therapies conceptually attractive for treatment of indolent and transformed FL. This review provides a synopsis of the main epigenetic aberrations and the current efforts in development and testing of epigenetic therapies in this B cell malignancy.

Keywords

follicular lymphoma, epigenetic mutations, epigenetic therapies, EZH2 inhibitors, HDAC inhibitors

Introduction

Follicular lymphoma (FL) is the most common form of indolent non-Hodgkin lymphoma (NHL), accounting for over 20% of all NHL cases. The incorporation of the anti-CD20 monoclonal antibody, rituximab, into treatment regimens has led to considerable improvement in the outlook of FL patients with 5-year overall survival approaching 90%. However, many patients display a relapsing-remitting pattern, with the disease eventually becoming resistant to further therapy, representing a major area of unmet clinical need[1]. These patients include a high-risk subgroup (30% of patients) prone to transformation into a higher-grade lymphoma[2, 3], and a poor-prognostic subgroup (20% of cases) who progresses or relapses within 2 years of receiving first line treatment[1].

At the molecular level, 85%-90% of FL are characterized by the t(14;18) translocation, which places the anti-apoptotic proto-oncogene *BCL2* under the transcriptional control of the *IGH* locus, leading to *BCL2* overexpression[4-7]. Intriguingly, however, t(14;18) alone is not sufficient for malignant transformation as it can be detected in healthy individuals who never develop the disease[8, 9]; therefore, there has been a concerted effort to identify the additional "hits" that cooperate with t(14;18) to induce malignant transformation in GC B cells. Additionally, 10%-15% of FL cases do not harbor the t(14;18) translocation[10], and the pathogenic mechanisms driving the development of this subset of FL remain largely unknown.

The mutational landscape of follicular lymphoma

FL originates from the germinal center (GC) within lymphoid tissues, where B cells undergo clonal expansion and extensive genetic modifications through the processes of somatic hypermutation and class switch recombination[11]. Genome-wide association studies[12], cytogenetic analyses[13-16], gene expression,[17-19] and microRNA profiling[20, 21] have highlighted chromosomal alterations, including 1p36 and 6q losses, components of the tumor microenvironment, and association with *HLA* gene variants, among others[12, 22]. The most significant shift in our

understanding of FL's genetic landscape, however, has occurred following advances in next generation sequencing (NGS). These include mutations in genes involved in immune surveillance, B cell development, BCR-NFkB and JAK/STAT signaling pathways, with, perhaps most strikingly, the identification of a plethora of mutations in series of epigenetic modifiers, such as *CREBBP, EP300*, *EZH2, KMT2D*, *MEF2B*, several members of SWI/SNF nucleosome remodeling complex, including *ARID1A, ARID1B* and *BCL7A,* as well as members of the linker histone H1 and histone H2 gene families[23-32] (Figure 1). Epigenetic deregulation has long been recognized as a cardinal feature of many solid and hematologic cancers and, thus far, four epigenetic drugs have been licensed for the treatment of hematologic malignancies[33]. However, what distinguishes FL is its apparent addiction to epigenetic alterations, with lesions in epigenetic modifiers occurring in nearly all patients[25, 27, 34]. By contrast, in diffuse large B cell lymphoma (DLBCL), a more aggressive form of NHL, mutations in epigenetic modifiers (*CREBBP, EP300, EZH2, and KMT2D)* occur in ~60% of the GC B cell (GCB)-like subtype, which shares a common cell of origin with FL, with fewer epigenetic mutations reported in the activated B cell (ABC)-like subtype[24, 35, 36] and Burkitt lymphoma[37], a rarer form of GC NHL. Intriguingly, epigenetic abnormalities are common in adult-type FL, but are rarely found in pediatric-type nodal follicular lymphoma (PTNFL), a rare variant of t(14;18)-negative FL, where the mutational landscape is genetically light, featuring recurrent mutations in *TNFRSF14* and MAPK signaling pathway genes[38, 39]. The infrequency of epigenetic aberrations and the distinctly more favorable prognosis in PTNFL compared to FL, including t(14;18)-negative FL, further supports the notion that epigenetic deregulation in adult-type FL pathogenesis may support the durability of lymphoma and the resultant clinical course of the disease.

Aberrations of epigenetic modifiers are early and driving events in the pathogenesis of follicular lymphoma

There is emerging evidence that the later age onset of FL is due to accumulation of several oncogenic events within the long-lived B cell progenitors[40-42]. Studies based on temporal profiling of FL and transformed FL (tFL), in addition to rare examples of donor-derived FL occurring several years following allogeneic stem cell transplant and donor lymphocyte infusions where both patients shared the same *IGH* and *IGH-BCL2* rearrangements and somatic mutations, lend credence to the existence of a long-lived pool of common progenitor cells (CPCs) acting as tumor-initiating cells that need to be eliminated in order to improve outcomes[25, 27, 30, 43, 44].

Transformation in FL arises predominantly by divergent evolution (reviewed in [42]), rather than through sequential acquisition of genetic aberrations, with mutations affecting the epigenetic modifiers *KMT2D, EZH2, CREBBP,* and *MEF2B,* being commonly shared between FL and tFL samples. These mutations are typically clonal, supporting a founder role for these events in FL, while mutations in NFkB signaling pathway, B cell development, or cell cycle genes tend to occur as later events presumably leading to the emergence of fitter clones that drive disease progression and transformation. It is worth keeping in mind that individual mutations in epigenetic modifiers are potentially insufficient to give rise to FL, although there has been an intriguing case study of FL occurring in a patient with Rubinstein-Taybi syndrome, a developmental disorder linked to germline *CREBBP* mutations[45]. In a minority of cases, however, evolution through "sparse" CPCs occurs with little clonal resemblance between paired FL and tFL samples and presents different epigenetic mutations acquired independently[25, 27], which is supported by a broad consensus in the literature through the contributions of several groups in the field.

Role of epigenetic regulatory mutations in lymphomagenesis

The potential roles of genetic aberrations affecting epigenetic modifiers in lymphomagenesis have been elucidated in a number of functional studies. Inactivating KMT2D mutations[46, 47] and

gain-of-function EZH2 mutations (a member of Polycomb-group proteins), mainly at tyrosine 641 (Y641) hotspot[23, 28, 48, 49], promote GC proliferation and block differentiation by transcriptional suppression of tumor suppressor genes that regulate B cell development, including KMT2D targets *TNFAIP3*, *SOCS3* and *TNFRSF14* and EZH2 targets *CDKN1A*, *CDKN2A, PRDM1,* and *IRF4*[46, 50-52]. While it has long been assumed that mutations in epigenetic modifiers arise in GC, *in vivo* models have shown that the impact of *KMT2D* mutations is dependent on the stage of B cell development, whereby mutations in early precursor B cells are sufficient to initiate lymphoma while mutations arising at later stages of GC B cell development require additional genetic hits to support malignant transformation[46, 47]. Loss-of-function *CREBBP* mutations in FL have been shown to facilitate immune evasion by downregulating MHC class II expression, associated with reduced T cell infiltration[53]. These inactivating mutations may also contribute to lymphomagenesis by impairing the acetylation of non-histone proteins p53 and BCL6[26], with emerging data showing that the BCL6/HDAC3/SMRT complex maintain the suppression of CREBBP target genes, including MHC class II, in *CREBBP*-mutant cells, rendering HDAC3 inhibition as a potential therapeutic strategy by restoring histone H3 lysine 27 acetylation at target enhancers[54]. More recently, global DNA methylation analysis of follicular lymphoma B cells has revealed hypermethylation of Polycomb-suppressed genes and hypomethylation of heterochromatin regions compared to normal GC B cells. The abnormal DNA methylation programming in FL may potentially cooperate with the underlying somatic mutations in, for example, chromatin modifiers to fixate the normal dynamic transcriptional regulation of B cell differentiation genes[55]. Furthermore, enhancer profiling of FL B cells has identified subsets of abnormally activated or repressed regulatory elements that may also contain somatic mutations that directly impact on transcription factor binding and transcriptional regulation of downstream genes, indicating another mode of aberrant epigenetic contribution to FL pathogenesis[56].

Taken together, these lines of evidence demonstrate a pivotal role for epigenetic abnormalities, not only as initiating events but also as potential drivers of progression, transformation, and modulators of the tumor microenvironment. The particular enrichment of epigenetic lesions in the CPC population also underscores the potential of epigenetic therapies as means of eradicating the founder clone in FL. These experiments equally highlight the challenges faced in predicting the mechanisms by which these mutations exert their effects and whether each genetic lesion is acting alone or there is some concerted effort in shifting a B cell towards malignancy.

Mutations in epigenetic modifiers can predict patient outcomes in FL

FL patients who relapse within 2 years of receiving R-CHOP treatment present a significant clinical challenge. The follicular lymphoma international prognostic index (FLIPI)[57] is useful to predict prognosis based on clinical and basic laboratory parameters; however, FLIPI tends to overestimate high-risk patients at diagnosis[58] and cannot be used effectively to inform clinical decisions. Therefore, in recent years, there have been efforts to develop new experimental models, the first of which, m7-FLIPI, incorporates the mutational status of seven genes, including the five epigenetic modifiers *EZH2*, *CREBBP*, *EP300*, *MEF2B,* and *ARID1A*[34]. m7-FLIPI has been shown to be more effective at distinguishing between high- and low-risk FL patients measured by 5-year failure-free survival following first line R-CHOP, with almost half of the patients classified as high-risk by FLIPI re-classified as low-risk by m7-FLIPI. This new prognostic tool is also more accurate in predicting disease progression within 24 months, a surrogate endpoint for overall survival, and in identifying the small subset of patients who are at the highest risk of early treatment failure following first line R-CHOP. Critically, high risk patients tend to carry *CREBBP* or *EP300* mutations, while *EZH2*- or *MEF2B*-mutant cases are associated with low risk disease and favorable outcome[34] and may benefit from less intensive R-CHOP regimens. However, it is still unclear how individual genetic lesions affect sensitivity to treatment (outcome predictors) or aggressiveness of the disease

(prognostic marker). Overall, the inclusion of epigenetic modifier mutations in patient risk stratification holds promise for more accurate prediction of patient outcomes following first line treatment with R-CHOP, although this needs further improvements in prospective studies before they can be translated into routine clinical practice.

Epigenetic inhibitors in follicular lymphoma therapy

Current treatment strategies for FL are based on a "one size fits all" approach that fails to take into account the specific genetic and epigenetic aberrations in different patients. Going forward, a more considered approach may be most beneficial for high risk patients who relapse within the first 2 years and the patients who transform to more aggressive lymphoma. The abundance and the co-founding role of epigenetic modifier mutations in indolent FL and tFL, together with the reversibility of epigenetic abnormalities, offer a platform to apply small molecule inhibitors that target these epigenetic aberrations in a precision medicine approach.

Targeting gain-of-function EZH2 mutations as emerging epigenetic therapies

EZH2 gain-of-function hotspot mutations are oncogenic drivers in 25% of FL patients that remain stable during disease relapse and/or transformation[23, 25, 28], therefore providing a *bona fide* drug target for FL therapy. Several S-adenosylmethionine-competitive EZH2-selective inhibitors (EZH2i) have been developed in recent years, including GSK-126, EPZ-6438, and CPI-1205, with superior EZH2 selectivity over EZH1 and other histone methyltransferases. These EZH2i have shown significant inhibitory effects against *EZH2*-mutant lymphoma cell growth and survival in pre-clinical studies[59-63] and are currently being tested in phase I/II clinical trials (Table 1). Preliminary trial results of EPZ-6438 [NCT01897571] in a small subset of B-NHL patients indicate 60% objective response (partial or complete response)[64]. While encouraging, the clinical efficacy of EZH2i awaits data in large series of patients accounted for the mutational status of *EZH2* and cell of origin. The intriguing responses in *EZH2* wild type patients may be caused by underlying genetic abnormalities such as inactivating mutations in H3K27 demethylase machinery[60] or by targeting the EZH2's non-

enzymatic role consistent with findings in SWI/SNF-mutant cancers[65], suggesting that epigenetic modifiers may cooperate in driving FL. Critically, however, identifying reliable diagnostic biomarkers for patients who will benefit from EZH2i therapy remains challenging.

Based on recent studies, there is also the prospect of EZH2i as a means of eradicating cancer stem cells. This has been exemplified in chronic myeloid leukemia where leukemic stem cells, which are typically resistant to first-line tyrosine kinase inhibitors, are sensitive to EZH2i due to their abnormal EZH2 gain-of-function status[66, 67]. Therefore, it is worth testing the efficacy of EZH2i in targeting the subset of *EZH2*-mutant FL CPCs to prevent them repopulating subsequent disease relapses or transformation.

HDAC inhibitors may benefit high-risk follicular lymphoma patients

Loss-of-function mutations of *CREBBP/EP300* leading to acetylation imbalance are associated with high-risk FL patients[34], therefore providing a rationale for histone deacetylase inhibitors (HDACi) therapy for this patient category. A number of HDACi have been tested in cancer trials in recent years, with vorinostat, which is licensed for the treatment of advanced cutaneous T cell lymphoma[68], being the most exhaustively tested orally bioavailable HDACi against lymphoid malignancies (Table 2). The efficacy of vorinostat against relapsed/refractory FL has been shown in phase I/II trials with an objective response rate of ~50%[69, 70] and median progression free survival (PFS) of 30.5 months[70]. Interestingly, vorinostat has shown inferior efficacies against non-FL type B-NHLs, including mantle cell and marginal zone lymphomas that are deplete of acetylation-compromising genetic lesions. There may be value, therefore, in correlating *CREBBP/EP300* mutation status with response to HDACi in order to identify patients most likely to derive benefit from these therapies. One case in point is the association of *MEF2B* mutations in DLBCL with response to the HDACi panobinostat, in a phase II trial[71]. Furthermore, a recent study in Kabuki syndrome, a congenital genetic disorder, has linked loss-of-function *KMT2D* mutations to sensitivity to HDACi[72], a biomarker that can be investigated in future HDACi trials. There are also a number of

caveats limiting widespread application of epigenetic therapies. In the example of HDACi, the exact mechanism of action in patients is poorly understood, as they can exert their function by acetylating both histone and non-histone proteins that are implicated in several biological processes, including cell cycle[73], DNA damage response, apoptosis[74, 75], angiogenesis[76], and regulation of tumor immunology[77]. It is important, therefore, that we include routine testing of mutations as part of existing clinical trial programs in FL and that we equally increase our efforts in understanding the functional roles of these inhibitors within the context of complex tumor genetics.

Lysine specific demethylase inhibitors: a new area of epigenetic therapy

Despite >80% of FL cases carry *KMT2D* mutations[24, 25, 36], no attempt has been made to target the resulting epigenetic abnormalities.[24, 26]. One therapeutic strategy may be to inhibit lysine demethylases (KDMs) that regulate H3K4 methylation, in order to counteract the H3K4 methylation imbalance in loss-of-function *KMT2D* mutants. The KDM family consists of seven subfamilies, with the KDM1 and KDM5 families both able to regulate H3K4 methylation. A number of inhibitors of these two families have been reported, although neither family has been investigated in lymphoma. Several inhibitors of the KDM1 family member LSD1 are currently undergoing clinical trials for other malignancies, such as acute myeloid leukemia (TCP[78], ORY-1001,[79] and GSK2879552[80]). More recently, three potent KDM5 inhibitors (EPT-103182[81, 82], CPI-455,[83] and KDM5-C70[84]) have been reported in pre-clinical studies, all of which demonstrate selectivity over other KDM families, increase H3K4me3 levels and cause significant inhibition of cell proliferation in myeloma[84] and drug-resistant small cell lung cancer[83]. There is value, therefore, in exploring LSD1 and KDM5 inhibitors as a means of potentiating a new area of epigenetic therapeutics for *KMT2D*-mutant FL.

Conclusion

The landscape of FL demonstrates a complex pattern of frequent mutations in epigenetic modifiers that are co-founding events arising in the CPC and remain stable during disease progression and transformation, providing a compelling rationale for using epigenetic therapies.

These aberrant epigenetic mechanisms have also been implicated in predicting patient outcomes as demonstrated by the m7-FLIPI model, which links 7 mutations, 5 of which with epigenetic function, to FL prognosis. While promising, it is yet to be determined whether these mutations can be used to robustly stratify patients at diagnosis to inform clinical decision making, and whether they have any predictive value in determining response to epigenetic therapy in particular.

Collectively, our understanding of abnormal epigenetic mechanisms in FL is still at its infancy and there are a number of hurdles to overcome if the epigenetic therapies are to realize their full potential clinically. From a biological perspective, we need a better understanding on whether the aberrant epigenetic modifiers act alone or cooperate in driving the B cell malignancy and what functional impact they have on downstream pathways. From the therapeutics perspective, future research impetus should focus on determining how these epigenetic therapies exert their effects in patients and rationalize treatment combinations in order to maximize their efficacy. Despite the challenges, we are in an exciting era of precision medicine where advanced technologies can help us routinely characterize the genetic and epigenetic landscape of individual patients for hypothesis-driven therapeutic strategies whose impact on patient outcomes are being revealed from ongoing clinical trials.

Disclosure of potential conflicts of interest

Jude Fitzgibbon has received research funding from Epizyme. The authors have no other conflicts of interest to declare.

Funding

K Korfi, S Ali, J Heward and J Fitzgibbon are supported by Cancer Research UK Programme Grant [C15966/A15968] and Bloodwise Programme Grant [15002]. S Ali is also a recipient of Cancer Research UK Clinical Careers Committee research bursary [C56515/A21397].

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Table 1. Current clinical trials with investigational EZH2i in B cell malignancies including FL.

Novel Agent	Clinical Trial No	Clinical Trial Phase	Estimated Enrollment	Eligibility Criteria	Trial Arms	Additional Remarks
EPZ-6438 (Tazemetostat)	NCT01897571	Phase I (closed) Phase II (recruiting)	350	Phase I: B-cell lymphomas, advanced solid tumors Phase II: DLBCL, FL, tFL, PMLBCL	Phase I: single arm (safety study); Phase II: 5 cohorts based on histology, cell of origin, and *EZH2* mutation status (efficacy study)	Phase I preliminary results: 9/15 OR[64]
GSK2816126	NCT02082977	Phase I (recruiting)	169	Relapsed/refractory DLBCL, tFL, other NHL, MM, solid tumors	Single arm (safety study)	After RP2D is established, in part 2, GCB-DLBCL, tFL

						and MM patients will be assigned to two cohorts based on *EZH2* mutation status. Outcome data is not available.
CPI-1205	NCT02395601	Phase I (recruiting)	41	B-cell lymphoma	Single arm (safety study)	Outcome data not available.

DLBCL; diffuse large B cell lymphoma; FL, follicular lymphoma; MM, multiple myeloma; NHL, non-Hodgkin lymphoma; OR, objective response; PMLBCL, primary mediastinal large B-cell lymphoma; RP2D, recommended phase 2 dose; tFL, transformed FL.

Table 2. Selected HDACi in phase I/II clinical trials for NHL.

Agent(s)	Clinical Trial No	Clinical Trial Phase	Enrollment	Eligibility Criteria	Trial Arms	Additional Remarks
Vorinostat	NCT00253630	Phase II (completed)	37	relapsed/refractory indolent NHL	Single arm (safety/efficacy study)	ORR: 47% FL, 22% MZL, 0% MCL[70]
Vorinostat	NCT00875056	Phase II (active, not recruiting)	54	relapsed/refractory indolent NHL	Single arm (safety/efficacy study)	ORR: 49% FL, 43% non-FL indolent NHL, 0% MCL[69]
Vorinostat + Rituximab	NCT00720876	Phase II (active, not recruiting)	33	Indolent NHL	Single arm (safety/efficacy study)	ORR: 50% FL and MZL, 33% MCL[85]
PCI-24781 (Abexinostat)	NCT00724984	Phase I/II (completed)	55	Phase I: relapsed/refractory lymphoma Phase II: Relapsed/refractory FL, MCL	Single arm (safety/efficacy study)	ORR: 64% FL, MCL: 27%[86]
Panobinostat	NCT01261247	Phase II (active, not recruiting)	41	Relapsed/refractory NHL	Single arm (efficacy study)	Outcome data not available.

CUDC-907 (Dual HDAC/PI3K inhibitor)	NCT01742988	Phase I (recruiting)	138 (estimated)	Relapsed/refractory lymphoma or MM	CUDC-907 vs CUDC-907 + Rituximab (dose escalating arms)	ORR: 55% DLBCL, 0% MM and other lymphoma [87]
Mocetinostat	NCT02282358	Phase I/II (recruiting)	56 (estimated)	Relapsed/refractory DLBCL and FL (*CREBBP/EP300*-mutant)	Single arm (safety/efficacy study)	Outcome data not available.

CTCL, cutaneous T cell lymphoma; DLBCL; diffuse large B cell lymphoma; FL, follicular lymphoma; MCL, mantle cell lymphoma; MM, multiple myeloma; MZL, marginal zone lymphoma; ORR, objective response rate; NHL, non-Hodgkin lymphoma.

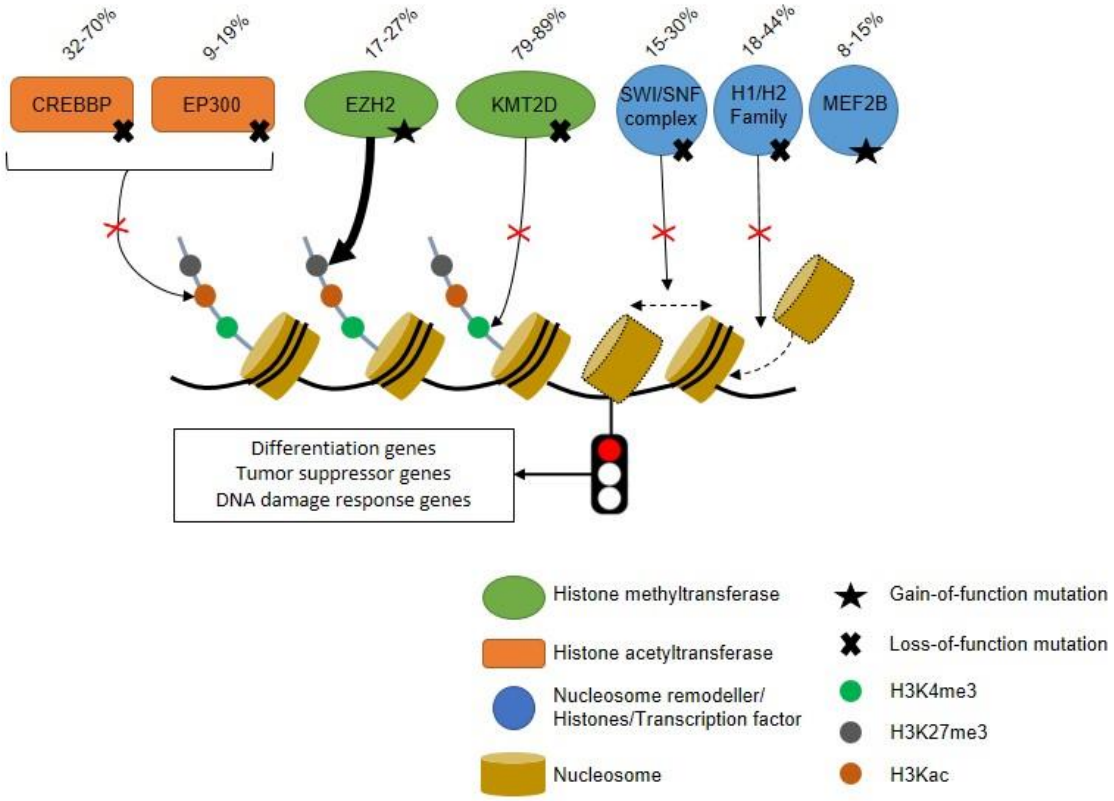

Figure 1. Frequently mutated epigenetic modifiers in FL. Schematic model of recurrent mutations affecting epigenetic modifiers in FL with reported frequencies for KMT2D[24, 25, 27, 34], EZH2[25, 28, 32, 34], CREBBP[25-27, 34], EP300[26, 27, 32, 34], linker histone H1 and histone H2 family[25, 27, 32], SWI/SNF complex members (ARID1A, ARID1B, BCL7A)[27, 32, 34], and MEF2B[23, 25, 32, 34] depicted above. Mutations in epigenetic modifiers occur concurrently in majority of FL patients and mechanistically it is not clear whether they act alone or cooperatively in driving B cell malignancy. H3Kac, histone H3 lysine acetylation; H3K4me3, histone H3 lysine 4 trimethylation; H3K27me3, histone H3 lysine 27 trimethylation.