Editorial

ENL YEATS domain: targeting the acute myeloid leukemia epigenome

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Provenance: This is an invited Editorial commissioned by the Executive Editor-in-Chief Dr. Hualin Sun (Jiangsu Key Laboratory of Neuroregeneration, Nantong University, Nantong, China).

Comment on: Wan L, Wen H, Li Y, *et al.* ENL links histone acetylation to oncogenic gene expression in acute myeloid leukaemia. Nature 2017;543:265-9.

Received: 04 August 2018; Accepted: 07 August 2018; Published: 23 August 2018.

doi: 10.21037/biotarget.2018.08.01

View this article at: http://dx.doi.org/10.21037/biotarget.2018.08.01

Acute myeloid leukemia (AML) is a hematological cancer characterized by the quick proliferation and accumulation of immature myeloid cells in the bone marrow, with an impaired differentiation program. AML is developmentally related to normal hematopoiesis and, in the same way, sprouts from a population of immature progenitors, namely leukemic stem cells (LSCs) (1). Despite significant advancements in AML therapy and high rates of complete remission (CR) after induction chemotherapy, many patients relapse and die from the disease (2). In the effort to improve the clinical outcome of AML patients, the identification of disease-specific alleles harbored by the malignant clone, in recent years, has triggered the development of molecular-based targeted therapies (3). However, these approaches failed to significantly increase the cure rate for AML patients, and produced disappointing results when employed as single therapeutic agent. On the one hand, the low rate of success of molecular-targeted therapy could be related to the high AML heterogeneity in terms of it underlying genetics. On the other hand, the presence and persistence of mutations in epigenetic regulators, which play a major role in hematopoietic (HSCs) and leukemia stem cells (LSCs) self-renewal, survival or differentiation (4), could be a relevant contributor to the failure of therapies targeting genetic aberrations.

Epigenetics is considered as the sum of hereditable modifications in the pattern of post-translational changes, leading to the development of a new phenotype, not encoded in the DNA sequence (5). The epigenome is an ensemble of chemical compounds contiguous to the DNA, responsible for the modification of the genome without altering the DNA sequences; it is dynamically regulated by chemical changes of DNA, RNA, and histones, around which DNA is packaged. Last but not least, the epigenome has a determinant role in controlling which genes have to and which haven't to be active in a particular cell. Interestingly, the characterization of the genomic landscape of AML (6) has demonstrated the presence of mutations in genes encoding epigenetic regulators. Accordingly, epigenomic dysregulation plays a strong role in AML pathogenesis and, as we will discuss later on, in the maintenance of AML clones.

In AML, there are 3 types of epigenetic regulators that can be affected by mutations. The first is represented by epigenetic "writers," such as DNA methyltransferases, histone methyltransferase (HMT), and histone acetyltransferases (HAT), which place acetylation and/or methylation on DNA or histones. The second consists of the epigenetic "erasers", including histone demethylase (HDM) and histone deacetylase complexes (HDACs), which may remove the epigenetic marks deposited by "writers". The third regulators are the epigenetic "readers", capable to specifically bind to distinct epigenetic marks, in order to transfer a specific information to the downstream effectors (7).

Interestingly, founder AML clones may have recurrent genomic mutations in epigenetic writer, reader, and eraser

proteins, such as DNA methyltransferase 3A (DNMT3A), TET1/2, IDH1/IDH2, EZH2, mixed-lineage leukemia (MLL), NSD1/3, AF10, ENL, or in other regulators of the epigenome. Moreover, these recurrent alterations persist after chemotherapy (7). Accordingly, mutations in epigenetic regulators may contribute to a premalignant state; the subsequent acquisition of cooperating mutations by the premalignant cell will result in the development of cancer.

The importance of chromatin modulators in AML pathogenesis, was discovered few years ago, when chromosomal translocations and fusion oncogenes were noticed to have a causative role in AML.

In particular, MLL/KMT2A, an epigenetic "writer", is responsible for the expression of a MLL-fusion protein, which triggers a cascade of downstream transcriptional programs (e.g., the HOX/MEIS genes) responsible for maintaining the self-renewing state in transformed hematopoietic progenitors (4).

This gene was one of the first epigenetic regulators known to be involved in leukemia pathogenesis through the expression of a MLL-fusion protein. Furthermore, the oncogenesis driven by MLL-fusion protein, necessitates the synchronized action of several chromatin modulators, needed for activation of HOX/MEIS genes, such as the histone methyltransferase DOT1L (8). The DOT1L protein biochemically works together with most of the usual MLL-fusion partners, such as AF4, AF9, ENL, AF10, and AF17 (9-12) epigenetic regulators in AML leading to oncogenic transcriptional activation. Apart from pathogenesis, it is becoming increasingly relevant the possible role of mutation in epigenetic regulators for AML maintenance and, thus, disease resistance or relapse.

Chromatin modifications are relevant for several biological processes; they are often established and recognized by conserved protein domains of multisubunit protein complexes. The most well-known chromatin-modifying complexes are: (I) bromodomains, recognizing acetyl-lysines; (II) chromodomains, recognizing methylated lysines; (III) YEATS domains. Proteins, that contains these domains (YEATS, bromo and chromo), act as effectors. They let chromatin be more accessible to RNA polymerase and transcriptional factors, recruit complexes able to remodelling the nucleosomes, or make additional chemical changes.

The YEATS are a new family of histone acetyllysine readers, different from other well-known readers in regard to protein fold, pocket generation and acetyllysine recognition (9), strictly linked to chromatin modifications and transcription. The YEATS domain family of proteins has more than 100 members in approximately 70 different eukaryotic species. The first five proteins discovered to contain this domain (Yaf9, ENL, AF9, Taf14 and Sas5) gave the name to the YEATS domain, which was conserved during evolution, from yeast to human (10). In humans, the best characterized proteins containing YEATS domain are AF9, ENL and GAS41, and all of them are strongly linked to cancer. GAS41 is amplified in glioblastomas and astrocytomas; AF9 and ENL are frequently fused with MLL protein, resulting in the development of fusion proteins, that are oncogenic drivers in AML and ALL.

Recently, it has been shown that ENL protein links histone acetylation to oncogenic gene expression in AML (11). It was discovered that ENL YEATS domain binds, *in vitro*, to histone H3 and H1, suggesting an overall function of YEATS domains in histone binding and chromatin modification. Wan *et al.* (11) identified ENL YEATS domain as a histone acetylation reader, regulating AML oncogenic transcriptional programs. They found ENL protein able to bind to acetylated histone H3, and then colocalize with H3K27 and H3K9ac on the promoters of genes essential for leukemia, which are actively transcribed. Interestingly, the authors showed that YEATS domain containing protein ENL, but not its paralog AF9, is required for AML maintenance (11). The disruption of the interaction between histone acetylation and the YEATS domain through structure-based mutagenesis, significantly decreased RNA polymerase II recruitment to ENL target genes, resulting into the suppression of oncogenic gene expression patterns. The requirement of YEATS domain for the ENL-dependent maintenance of oncogenic gene expression in AML, was demonstrated by rescue experiments with ectopically expressed murine WT or mutant ENL proteins in cells expressing a human ENL sgRNA (11). Transcriptional profiling analyses showed that WT WNL, but not the F59A or Y78A mutant, restored the transcriptional changes caused by the ENL sgRNA (11). As a whole, all the finding of the paper by Wan *et al.* (11) establish a central role for the interaction between ENL YEATS and histone acetylation in the regulation of oncogenic gene expression and AML maintenance. Moreover, the Authors demonstrated that the disruption of ENL's functionally additionally sensitized AML cells to BET (bromodomain and extra-terminal) inhibitors (11). Accordingly, pharmacologic targeting of chromatin through the displacement of ENL could represent a possible future therapeutic option for AML, with or

without BET inhibitors (11).

The last few years have seen extraordinary advancements in the development of new drugs and treatment strategies for AML. New monoclonal antibodies, tyrosine kinases, nuclear export, small-molecule inhibitors of signaling pathway mutations were developed and clinically tested. The vast majority of these therapeutic strategies are driven by specific mutations recurrently found in AML patients, but these molecularly-targeted approaches often failed, probably because the lack of efficacy on somatic mutations of DNA/chromatin modifiers, such as DNMT3A and IDH1/2, for example. Thus, targeting the epigenome of genetically defined AMLs with inhibitors specific to chromatin modulators is probably the newest frontier of precision medicine in AML. This is mainly because deep sequencing of samples from AML patients but also from pre-leukemia disorders, such as myelodysplastic syndromes or CHIPs, showed numerous somatic mutations of genes implicated in epigenetic modulation and RNA splicing (6,12,13). Moreover, epigenetic modifiers mutations involving DNMT3A, IDH1/2, ASLX1, and other epigenetic regulators, were frequently detected in patients with CHIPs, or in AML patients in CR. As a consequence epigenetic deregulation is supposed to have an essential role in AML initiation, clonal evolution and relapse, thus the therapeutic targeting of the AML epigenome has clearly become more and more fascinating in recent years. Last but not least, epigenetic alterations present in AML cells can be reverted using specific drugs, whereas genomic abnormalities are difficult to overturn. This is because several epigenetic regulators have enzymatic activity, which may be more responsive to therapeutic targeting using small-molecule inhibitors than other classes of proteins such as transcription factors (4). Finally, targeting the epigenome may also contribute to eliminate LSCs, as chromatin modulators mutations are frequently seen in founding AML clones.

The relevance of the paper by Wan *et al.* stands in the opportunity of targeting different categories of the epigenetic regulation. As previously mentioned, the modification of DNA or histones is dynamically regulated by three different epigenetic modulators. Epigenetic writers, such as MLL protein complex, G9A, EZH2, DOT1L and PRMT1, are enzymes catalyzing chromatin modification. Epigenetic erasers, such as histone deacetylases, LSD1 and KIDM4C, remove the modification. Epigenetic readers or effectors, such as bromodomain proteins (e.g., BET), NUP98-PHF23 or NUP98-JARID1A, identify the change to produce biological consequences. Remarkably, an increasing amount of epigenetic writers and erasers acquire either activating or loss-of-function mutations in AML, thus supporting the suggestion that epigenetic dysfunction is fundamental in AML pathogenesis (8).

A series of preclinical and clinical studies testing specific inhibition of histone-modifying enzymes and regulatory proteins as potential AML therapeutics are ongoing. Interestingly, there are representative compounds sub-grouped into the categories targeting either the writing, reading or erasing function of epigenetic modulators. An exhaustive discussion of these compound is not the aim of this paper. However, it is important for us to underline some relevant aspects.

First of all, targeting the epigenome might probably be more relevant in the pre-leukemia disorders, such as MDS or, eventually, CHIPs. Even if most mutation in epigenetic regulators may be relevant for AML pathogenesis, cancer cells have multiple dependencies, and molecular-targeting, with the exception of acute promyelocytic leukemia, has not proven to be curative for any AML subtype. Secondly, the immune system has a major role in AML initiation, development and relapse. A dysfunction in the immunological synapse with the induction of tolerogenic mechanisms favoring the immune-escape of AML was demonstrated by several groups comprising our (14), and the only curative option in 2018 is still allogeneic stem cell transplant. In this view, it will be interesting to evaluate possible chromatin modifications in the cell of the immune system, in order to see if mutation in epigenetic regulators influences the fate also of B and T lymphocytes and NK cells.

In conclusion, it is very important to identify novel target to be hit by new pharmacological therapy, possibly by combining different therapies, in order to knockout a large number of different target, and thus reducing the probabilities of AML relapse. In this view, the ability of targeting multiple sites of the epigenome, by combining different small-molecule inhibitors of specific chromatin factors, clearly represent a novel and promising therapeutic strategy in the treatment landscape of AML.

Acknowledgements

The study was supported in part by AIL Pesaro Onlus.

Footnote

Conflicts of Interest: The authors have no conflicts of interest to declare.

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doi: 10.21037/biotarget.2018.08.01
Cite this article as: Loscocco F, Visani G, Isidori A. ENL YEATS domain: targeting the acute myeloid leukemia epigenome. Biotarget 2018;2:12.