Epigenetic targets for treatment in acute myeloid leukemia

Myelopoiesis, which involves growth and maturation of granulocytic and monocytic lineages, is highly controlled by lineage-specific hematopoietic transcription factors such as AML1, PU-1, C/EBPα, CBFβ, retinoic acid receptor α (RARα) etc. that cooperatively interact with specific DNA sequences and directly activate or repress the expression of myeloid specific genes. These transcription factors have been found mutated or altered by chromosomal translocations associated to leukemias, indicating their role in the pathogenesis of these malignancies. The post genomic era however, has shown that transcription factors are not the unique key regulators of gene expression. Epigenetic mechanisms such as DNA methylation, post-translational modifications of histone proteins, remodeling of nucleosomes and expression of small regulatory RNAs all contribute to regulation of gene expression and determination of cell and tissue specificity. Deregulation of these epigenetic mechanisms, indeed cooperate with genetic alterations to the establishment and progression of tumors.

In acute myeloid leukemia (AML), which is the clonal expansion of hematopoietic precursors blocked at different stages of myeloid differentiation, the leukemogenetic event is associated with an aberrant silencing of gene transcription. The transforming ability of chromosomal translocation-generated AML fusion proteins (PML/RARα, PLZF/RARα and AML1/ETO), is indeed strictly dependent on their aberrant recruitment of protein complexes containing histone deacetylase (HDAC) and DNA-methyltransferase (DNMT) activities on genes that are relevant to myeloid differentiation and transformation process. By changing nucleosomal packaging of DNA, HDACs remodel chromatin in a gene specific fashion and consequently affect proper cell function, differentiation and proliferation. In particular, deacetylation of histones H3 and H4 by HDACs inhibits transcription, while their acetylation by histone acetyltransferase (HATs) activities results in transcriptional activation.

In acute promyelocytic leukemia (APL), the AML-M3 FAB subtype, the formation of RARα-fusion proteins induces an aberrant recruitment of protein complexes containing HDAC and DNA methyltransferase activities on the all-trans retinoic acid (ATRA) target genes, resulting in their transcriptional silencing. However, the use of ATRA in APL patients represents a paradigmatic example of a highly effective transcriptional/differentiation therapy in leukemias. The clinical efficacy of ATRA in APL is due to its ability at pharmacological doses, to bind the APL-associated PML/RARα fusion protein, to decrease DNA-methyltransferase expression levels, to release the HDAC-repressive complex and to recruit the multisubunit HAT-complex on ATRA-target gene promoters. Interestingly, the ATRA-signaling pathway is constitutively repressed through an HDAC-dependent mechanism in other non-APL AML subtypes, regardless of their underlying genetic lesion. Thus, common pathways of leukemogenesis could lie along the control of specific chromatin structures in terms of transcriptional activation or repression.

By using inhibitors of HDAC activities (Valproic acid and Trichostatin A) in the presence or in the absence of ATRA, we found that all these chromatin remodeling agents are active in restoring or potentiating the effect of ATRA on endogenous target genes resulting in AML blast differentiation.
Thus, therapeutic targeting of aberrant HDAC activities might represent a potentially novel treatment strategy for AML. Recently, a pilot study was carried out in eight refractory or high-risk AML patients not eligible for intensive therapy to assess the biological and therapeutic activities of the HDAC inhibitor Valproic acid (VPA) used to remodel chromatin, followed by the addition of ATRA, to activate gene transcription and differentiation in leukemic cells. Hyper-acetylation of histones H3 and H4 was detectable at therapeutic VPA serum levels (≥50 µg/mL) in blood mononuclear cells from 7/8 patients. This, correlated with myelo-monocytic differentiation of leukemic cells as revealed by morphologic, cytochemical, immunophenotypic and gene expression analyses. Differentiation of the leukemic clone was proven by FISH analysis showing the cytogenetic lesion +8 or 7q- in differentiating cells. Hematological improvement, according to established criteria for myelodysplastic syndromes, was observed in two cases. Stable disease and disease progression were observed in five and one case, respectively. VPA-ATRA is a well tolerated treatment that induces phenotypic maturation of AML blasts through chromatin remodeling. Of note that VPA-ATRA in AML patient blasts induced similar features previously reported in ATRA treated APL patients including the increased WBC number; increased percentage of cells in S phase and terminal differentiation of leukemic blasts with leukemia-specific markers as shown by FISH (10;14). However, in APL, ATRA is per se unable to eradicate the leukemic clone and to cure the disease. Chemotherapy following ATRA treatment strikingly improved the prognosis of APL patients and their cure rate to 70-80% at 5 years.10

Studies performed in normal hematopoietic stem cells revealed an interesting dual action of treatment with either ATRA or inhibitors of HDAC (VPA and TSA) and DNMT activities (5-azacytidine): i) expansion of a primitive hematopoietic stem cell population; and ii) induction of committed myeloid precursors to cell differentiation.15-17 Thus, the chromatin accessibility at specific DNA-binding sites might represent the key event for the activity of cytokines or transcription factors either involved in the maintenance of early hematopoietic stem cell population or in lineage commitment. Some of these factors might be present in the same cellular context and dictate cell fate choice by targeting enzymes with chromatin remodeling activity such as HDACs, HATs or DNA methyltransferases at specific gene loci. ATRA, VPA and 5-azacytidine can therefore initiate a series of events leading to the maintenance of the undifferentiated state in early HSC, or generating a chromatin code coupled to specific differentiation decision in HPC and in AML blasts. We can therefore hypothesize the efficacy of ATRA based regimens in APL due to the epigenetic changes occurring in leukemic progenitors, which render these cells more sensitive to conventional chemotherapy agents. Accordingly, a clinical study performed in 242 non-M3 elderly AML patients recently demonstrated that the adjunction of ATRA to chemotherapy significantly improved their clinical outcome in respect to chemotherapy alone.18 Although we lack a direct proof for an epigenetic priming effect of VPA on gene transcription in AML blasts, VPA augmented the histone acetylation status on chromatin regions surrounding the retinoic acid response element (RARE) of RARα in both the cases tested. RARα is the ATRA receptor gene involved in normal and pathological myelopoiesis.1,19 Histone acetylation levels at ATRA regulatory sites are further increased by ATRA addition to VPA. In agreement with the ligand inducibility of RARα gene, RARα mRNA transcripts were found significantly induced after the addition of ATRA to VPA in all the VPA-ATRA responsive patients. In this view, the VPA-ATRA combination by inducing an epigenetic priming of AML blasts might increase their sensitivity to standard chemotherapy or to other novel therapeutic approaches for AMLs.

References

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