Epigenetic changes in myelodysplastic syndrome and acute myeloid leukemia: novel targets for therapy

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Abstract

Epigenetic modifications, defined as DNA changes other than changed nucleotide sequence consist mainly of hypermethylation of gene promoters and histone deacetylation. Through these mechanisms, genes, including tumor suppressor genes can have a functionally altered expression and therefore be "silenced". Epigenetically silenced tumor suppressor genes are common events in myelodysplastic syndromes (MDS) and acute myeloid leukemia (AML), usually correlating with poor prognosis. Epigenetic modifications may be useful targets for therapy in MDS and AML. Two groups of drugs, demethylating agents (azacytidine and decitabine) and histone deacetylase inhibitors (phenyl-butyrate, valproic acid and suberoylanilide hydroxamic acid) have recently been proven effective, either alone or in combinations, by several clinical trials in high-risk MDS and elderly high-risk AML. These agents were able to induce responses, even complete responses in patients known to have an extremely poor prognosis. In conclusion, epigenetic changes are important events in MDS and AML pathogenesis and progression; targeting these mechanisms is one of the strategies that have lately been incorporated in the therapeutic armamentarium of high-risk MDS and AML patients.

Rezumat

Modificările epigenetice, definite ca modificările ADN-ului altele decât schimbarea secvenței nucleotidice constau în principal în hipermetilarea regiunilor promoter ale genelor şi în de-acetilarea histonelor. Prin aceste mecanisme, genele, inclusiv genele supresoare tumorale suferă alterări funcționale, expresia lor fiind practic "cenzurată". Cenzurarea epigenetică a genelor supresoare tumorale este relativ frecventă în sindroamele mielodisplazice (SMD) şi leucemiile acute mieloide (LAM), aceste evenimente corelându-se deseori cu un prognostic infaust. Modificările epigenetice constituie însă ținte utile pentru tratamentul acestor afecțiuni. Două grupe de molecule, agenții demetilanți (azacitidina şi decitabina) şi inhibitorii histon-deacetilazelor (fenil-butiratul, acidul valproic şi acidul hidroxamic suberoilanilidic) s-au dovedit eficienți, atât ca monoterapie cât şi în combinații, în tratamentul SMD şi LAM la batrâni, în cazurile cu risc crescut. Aceşti noi agenți terapeutici au fost capabili să inducă răspunsuri obiective, chiar remisiuni complete la aceşti pacienți, cunoscuți ca având un prognostic deosebit de grav. In concluzie, modificările epigenetice sunt evenimente importante în patogeneza şi progresia SMD şi LAM; tratamentul țintit pe aceste modificări a fost încorporat recent în arsenalul terapeutic al pacienților cu SMD şi LAM cu risc crescut.

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Epigenetic modifications define potentially reversible DNA and chromatin changes, transmitted from a cell to its progeny, able to induce altered gene expression without changing DNA sequence and without adding or deleting any genetic information.

DNA normally exists in a complex configuration with proteins such as histones. Histones organize DNA into units named nucleosomes, which basically consist of DNA wrapped around a histone core. A histone core consists of two copies of each of four different core proteins (H2A, H2B, H3, H4). Histones can be acetylated and methylated at N-terminal lysine residues. The protein-DNA interactions mediate packaging of DNA from ultra compact (the visible chromosomes during mitosis) to most relaxed (the fine chromatin observed under the microscope in immature cells). This chromatin remodeling process is determined largely by the balance between histone acetylation and DNA methylation. When histones, especially H3 and H4 are hyper acetylated and DNA is not methylated the chromatin is "active", the corresponding genes being ready for transcription, whereas when H3 and H4 are not acetylated and DNA is methylated, the chromatin is "inactive" leading to *epigenetic silencing* of the corresponding genes, as shown in *Figure 1* [3,15].

Epigenetic silencing is essential for the normal development of mammalian cells, as only a certain genetic repertoire gets expressed in each cell type despite the common DNA blueprint[9]. However, over the past decades, it has become apparent that cancer cells use the process of epigenetic silencing to their advantage by silencing the expression and function of genes that counteract the malignant phenotype, the so-called tumor-suppressor genes[7]. Regardless of the underlying DNA defects, epigenetic silencing has been found to be one of the common downstream events in myelodysplastic syndrome (MDS) and acute myeloid leukemia

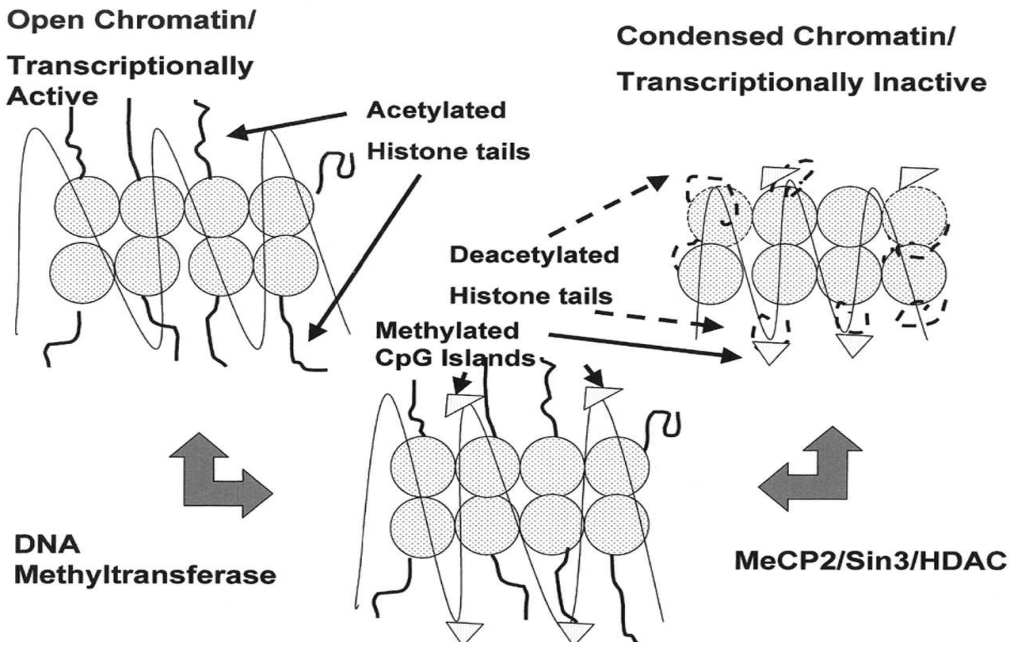

Figure1. Impact of chromatin remodeling on gene transcription. Methylated cytosine residues in CpG (cytosine-guanosine) dinucleotides in promoter regions of genes recruit transcriptional repression complexes including histone deacetylases (HDAC), methyl CpG binding protein 2 (MeCP2) and Sin3. The deacetylated lysine tails of the histones interact tightly with DNA, rendering the chromatin transcriptionally inactive (15).

(AML) pathogenesis. Two interconnected molecular mechanisms are key to the process of epigenetic silencing: **DNA methylation** of the promoter regions of genes and **histone modifications**.

DNA methylation occurs by covalent addition of a methyl group at the 5' carbon of the cytosine ring, resulting in 5-methylcytosine (*Figure 2*). These methyl groups project into the major groove of DNA and effectively inhibit transcription. In mammalian DNA, 5-methylcytosine is found in approximately 4% of genomic DNA, primarily at cytosine-guanosine dinucleotides (CpG). CpG concentrations (islands) are typically found in or near promoter regions of genes. It was found that if such regions are heavily methylated the corresponding genes do not get expressed. Hematologic malignancies demonstrate a link between methylation and the neoplastic phenotype[8]. AML and MDS are characterized by the hypermethylation and silencing of multiple genes. This process can occur early and may be detected in cases of low-risk MDS but, in general, it is associated with disease progression. In MDS, for example, the cyclin-dependent kinase inhibitor P15 is a frequent target of aberrant methylation, and its inactivation is associated with an increased risk of progression to AML[12]. A recent study showed that the gene encoding alpha-catenin (CTNNA1) is expressed at a much lower level in leukemia-initiating stem cells from individuals with AML or MDS with a 5q deletion than in individuals with MDS or AML lacking a 5q deletion or in normal haematopoietic stem cells and that this is due to suppression by both methylation and histone deacetylation of the CTNNA1 promoter[13]. A number of other genes are similarly affected, including DAP kinase, regulators of apoptosis, SOCS1, involved in cytokine regulation, E-cadherin, involved in cell adhesion and others. Methylation of these genes occurs particularly in "high-risk" MDS and an increased degree of methylation is associated with progression towards AML[3].

Histone modifications include acetylation, methylation, phosphorylation and ubiquitination. Acetylation seems to be the most important histone-modulation process. In general, acetylation of lysine residues on histone tails is associated with transcriptionally active chromatin (euchromatin), whereas deacetylated histones are associated with transcriptionally inactive chromatin (heterochromatin). Histones are acetylated by enzymes which contain histone acetyltransferase activity; in contrast, deacetylation is mediated by histone deacetylases (HDAC). Several human HDACs have been identified to date, classified in 2 major classes: class I HDACs are almost exclusively nuclear, while class II HDACs shuttle in and out of the nucleus in response to specific cellular signals. HDACs are associated with specific chromatin loci in pairs and triplets. DNA methylation is followed by recruitment of histone deacetylases, histone methylases, and eventually, a si-

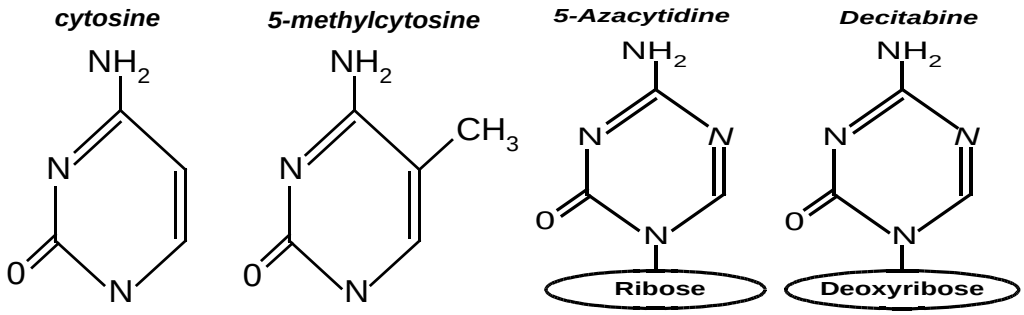

Figure 2. Chemical structure of cytosine, 5-methylcytosine and the demethylating cytosine analogs 5-azacytidine (AZA) and decitabine (DAC)

lencing complex of proteins including heterochromatin protein 1 (HP1). By this mechanism, chromatin is remodeled such that it becomes "invisible" to transcription factors, achieving a stable silenced state[3,15].

Therapeutic implications of epigenetic changes in MDS and AML. Several compounds targeting DNA methylation and histone modifications have so far proven beneficial in high-risk MDS and AML patients:

1. DNA Methylation Inhibitors in MDS and AML treatment

Two cytosine analogs, 5-azacytidine (AZA) and 5-aza-2'-deoxycitidine or *decitabine* (DAC) were found 25 years ago to specifically inhibit DNA methylation by trapping DNA-methyltransferases (MTases). AZA can incorporate into RNA and also is a pro-drug of DAC. DAC is phosphorylated by deoxycytidine kinase and incorporates efficiently into DNA. The structure of these compounds is presented in *Figure 2*. MTases, upon encountering DAC, form irreversible covalent bonds with the incorporated base and are then targeted for degradation in the proteosome. Cells then divide in the absence of MTases, which results in progressive DNA hypomethylation and reactivation of previously silenced genes. The covalent binding of MTases to DNA can also result in cytotoxicity at high doses of DAC and/or high levels of MTases[10].

Clinical trials with **azacytidine (AZA)** were initiated two decades ago and revealed efficacy at high doses in AML and at lower doses in MDS. In a recent report, data from three sequential trials comprising 309 patients were recollected and reanalyzed. The dosage used was 75 mg/m^2/d for 7 days every 28 days. Complete remissions were seen in 10% to 17% of azacytidine-treated patients and 23% to 36% of patients had hematologic improvement. The median number of cycles to first response was three, and 90% of responses were seen by cycle 6. The most frequent side effects consisted of transient worsening of preexisting cytopenias. However, azacytidine did not increase the rate of infection or bleeding above the rate caused by the underlying disease[18].

Decitabine (DAC) is more active than AZA in vitro at equimolar doses and may have a different spectrum of activity and side effects compared to AZA because it does not incorporate into RNA. Clinical trials with DAC were also initiated two decades ago and revealed promising efficacy in hematologic malignancies[20]. In a phase III trial, 170 patients with MDS were randomized to receive either DAC at a dose of 15 mg/m2, i.v., every 8 hours for 3 days and repeated every 6 weeks, or best supportive care. Patients who were treated with DAC achieved a significantly higher overall response rate (17%), including 9% complete responses, compared with supportive care (0%). Patients treated with decitabine had a trend toward a longer median time to AML progression or death compared with patients who received supportive care alone[11]. The most common adverse effects of decitabine, are due to myelosuppression with transient grade 4 neutropenia and thrombocytopenia being experienced by a majority of patients. Several studies are currently under way to test the effects of DAC in AML. A study on 51 elderly (median 72 years) AML patients, using the same dosage, showed objective responses occurring in 31 % of patients including complete remission in 14%. Besides the antileukemic effect, limited hospitalization times, few adverse effects and the good feasibility of outpatient treatment, support DAC treatment in this subset of AML patients[14].

Currently the hypomethylating agents azacytidine (Vidaza ^{R}) and decitabine (Dacogen ^{R}) are commercially available. They have been approved for MDS treatment in USA and are under clinical investigation in Europe and other parts of the world. There are also several ongoing trials assessing the role of DAC in AML However, it is still unclear which MDS and AML patients benefit most from the treatment

and which are the optimal drug schedules. The response and survival data from the above mentioned clinical trials seems to indicate that it is the higher risk MDS patients, with unfavourable and intermediate karyotype and the elderly AML patients with unfavourable cytogenetics who benefit mostly from hypomethylating agents. In fact in this groups of patients the other available therapeutic options are extremely disappointing[2].

An important issue is the fact that hypomethylating agents require 3 to 6 months of treatment before response is obtained, therefore one should not declare failure until at least 4 courses of hypomethylating agents have been delivered. Several trials also address the question of whether hypomethylating agents could improve the results of aggressive chemotherapy and allogeneic stem cell transplantation. It seems that azacytidine and decitabine given as maintenance therapy after intensive chemotherapy in high risk MDS may prolong the relapse-free survival with an acceptable toxicity profile[3].

2. Histone deacetylase (HDAC) inhibitors in the treatment of MDS and AML

A variety of HDAC inhibitors (HDACi) are under clinical investigation. Interest in the use of HDACi for the treatment of myeloid malignancies dates back to the recognition of the activity of butyrate derivatives and polar planar compounds to induce differentiation. Certain AML fusion genes, such as AML1-ETO, PML-RARα, and PLZF-RARα specifically recruit nuclear corepression complexes which include HDAC, thereby silencing expression of genes downstream from the promoters bound by the fusion proteins. In such leukemias, HDACi may be utilized to specifically reverse the transcriptional repression induced by the fusion proteins[16]. Among the several HDACi investigated, the following have been found most promising:

Small - chain fatty acids. A variety of small-chain fatty acids inhibit HDAC activity at submillimolar concentrations. These include **sodium and arginine butyrate, sodium phenylbutyrate, and valproic acid.** Phase I studies of continuous infusion **phenylbutyrate (PB)** demonstrated that the drug was well-tolerated. The dosage was 375 mg/kg/day 21 days every 28 days, for a total of 12 weeks (three cycles). Lineage responses were achieved in several patients with MDS and AML[5]. Recent data demonstrating that **valproic acid (VA)** has similar HDAC inhibitory activity has raised hopes that oral formulations of this drug, which is commonly used for neuropsychiatric disorders, could be used to modulate gene expression. The pharmacodynamic impact of VA on leukemic cells is similar to PB. VA is currently being investigated in monotherapy and in combination with other drugs in MDS and in AML[4].

Suberoylanilide hydroxamic acid (SAHA) also known as *vorinostat* is an oral HDAC inhibitor that modulates acetylation of histones and promotes apoptosis and differentiation in various leukemic cell lines in vitro. Clinical administration has also been associated with induction of histone acetylation. Several phase I and phase II clinical trials have been performed, in a variety of hematological malignancies, including patients with AML and MDS. The doses used in these trials were between 400 and 700 mg daily for 14 days, every 21 days. In about 15-20% of patients partial objective responses were observed, while the most common toxicities were fatigue, diarrhea and thrombocytopenia[17].

3. Combinations of drugs targeting epigenetic modifications in MDS and AML

Probably the best chance to improve clinical responses with epigenetic targeted therapy is by using combinations of active drugs. The recognition that HDAC recruitment accounted at least in part for the silencing of genes with methylated promoters led to the demonstration that optimal reexpression of such genes required sequential exposure to a methyl-

transferase inhibitor followed by an HDACi[1]. Following this encouraging in vitro data, several investigators are presently testing the idea of augmenting the clinical activity of AZA and DAC through the subsequent addition of an HDACi. Gore et al[6] evaluated a variety of doses and schedules of AZA, followed by a 7-day continuous infusion of PB. The combination has been well-tolerated, and significant sustained clinical responses were achieved in 50% of patients. The responders, who had pretreatment methylation of p15 or CDH-1 promoters, reversed methylation during the first cycle of therapy (methylation-specific PCR), whereas none of nonresponders showed any demethylation. Administration of both drugs was also associated with induction of acetylation of histones H3 and H4. Garcia-Manero et al[4] evaluated DAC plus VA in a cohort of AML and MDS patients, with an overall response rate of 53% (14% complete responses). Another direction is given by the fact that HDACi synergize with retinoids in a variety of systems. This has led to the concept of potentially combining these classes of agents to build on the modest single activity of retinoids in MDS as single agents or in combination with growth factors. Studies combining all *trans*-retinoic acid (ATRA) with PB and with VA are ongoing. In a recent phase I/II study, the combination AZA, VA and ATRA was tested in patients with AML or high-risk MDS. The overall response rate was 42% and median remission duration was 26 weeks[19].

In conclusion, epigenetic changes seem to be important events in MDS and AML pathogenesis and progression, especially in high risk patients. Targeting these mechanisms has become possible lately due to the incorporation in treatment regimens of the demethylating agents azacytidine and decitabine. Combining these agents with histone deacetylase inhibitors and possibly with other molecules may redefine the standard of care in high risk MDS and elderly high-risk AML patients, who until recently have benefited mainly from supportive measures.

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