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Different Mechanisms of Drug Resistance in Myelodysplastic Syndromes and Acute Myeloid Leukemia

Lucia Messingerova, Denisa Imrichova,
Martina Coculova, Marian Zelina, Lucia Pavlikova,
Helena Kavcova, Mario Seres, Viera Bohacova,
Boris Lakatos, Zdena Sulova and Albert Breier

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Abstract

Myelodysplastic syndromes (MDSs) represent clonal hematopoietic stem cell (HSC) disorders in which genetic and/or epigenetic alteration are involved in the normal function of hematopoietic stem and progenitor cells. This results in the development of blood cytopenias and bone marrow dysplasia. In recent years, therapy with hypomethylating agents (HMAs) in combination with supportive therapies is recommended as frontline treatment for patients with high-risk MDSs according to International Prognostic Scoring System (IPSS HR-MDS). Therapy with HMAs is essential namely for IPSS HR-MDS patients who do not proceed to immediate allogeneic stem cell transplantation (al-loSCT). For IPSS LR-MDS (International Prognostic Scoring System, low-risk MDSs) patients, however, supportive therapies and growth factors are the mainstay of treatment. Some patients in this group are treated with immunomodulatory agents derived from thalidomide (lenalidomide) or using immunosuppressive therapy (IST). The therapeutic decisions can change during the course of the disease based on changes in risk-category and the functional status of patients, in response to prior therapies, changes in patient preferences, and other factors.

Resistance to chemotherapy is a serious obstacle to the successful treatment of overall malignancies, including AML and MDS. The failure of therapeutic treatment may be due to the development of multidrug resistance (MDR) phenotype. MDR represents the induction of large-scale defensive mechanisms from which the upregulation of membrane transporters (like P-glycoprotein – P-gp) effluxing chemotherapeutic drugs from tumor cells represents the most observed molecular causality. Other mechanisms of MDR include drug metabolism, alterations in drug-induced apoptosis, epigenetic changes, epithelial-

mesenchymal transition, alteration in drug targets structures, and acceleration of DNA repair.

The present contribution represents a state-of-the-art review of available knowledge about this issue.

Keywords: myelodysplastic syndromes, acute myeloid leukemia, multidrug resistance, lenalidomide, 5-azacytidine, 5-aza-2-deoxyazacytidine

1. Introduction

Myelodysplastic syndromes (MDSs) represent the group of disorders associated with altered hematopoietic stem cells (HSCs) that lead to inefficient hematopoiesis [1]. This clinically results in dysplasia in one or more myeloid cell lineages, and variable degrees of cytopenias. The mean age of MDS patients' diagnosis ranges from 60 to 70 years. The incidence of MDS varied from 4.3 to 1.8 per 100,000 individuals per year in the US and Europe, respectively. Incidence slightly favors Caucasian males. MDS can lead to acute myeloid leukemia (AML) in 10–15% of patients.

Improvements in cytogenetic analysis techniques enable predicting the risk of MDS patients lapse into AML and the selection of optimal therapy [2]. The International Prognostic Scoring System (IPSS) described in the 1990s [3] is still commonly used. This scoring system defines how to measure the risk of patients' development from MDS to AML, and recommends dividing patients into four groups (low, intermediate 1, intermediate 2, and high risk). In lower risk patients, a combination of supportive care (includes transfusions of blood products, antibiotics) with substance improving erythropoiesis, immunosuppressive therapy, immunomodulatory therapy, and stem cell transplantation has been used. Treatment options for patients diagnosed as higher risk include demethylating agents, cytotoxic chemotherapy, bone marrow HSC transplantation, and experimental treatments in clinical trials [4].

2. Treatment options

The only curative option for patients with MDS represents hematopoietic bone marrow stem cell transplantations. However, alloSCT is not available for all patients because of the comorbidities of elderly patients [1]. Therefore some patients cannot be treated with alloSCT and other treatment options have to be used.

2.1. IPSS low-risk MDS patients' treatment option

Supportive care is an important therapy for the management of patients with low-risk MDS, as well as patients with poor disease prognosis which due to age or physical condition could

not be treated with more intensive forms of therapy [5]. Several low-risk patients are dependent on blood transfusions. However, patients treated with blood transfusions may be overloaded with iron ions, so that iron chelation therapy is required [6]. Due to the partial dysfunction of immunity, antibiotics are needed for treatable infections [7].

There are three commonly used therapies for low-risk MDS patients: i. erythropoiesis-stimulating agents (ESAs); ii. immunosuppressive therapy; and iii. immunomodulatory therapy with thalidomide derivative lenalidomide (revlimid). The treatment of patients with ESAs leads to significant erythroid response in 20–70% of unselected patients with MDS [1]. A median response for treatment with erythropoietin and colony-stimulating factor (CSF) applied together was 2 years and improves life quality [8]. Several immunosuppressants such as antithymocyte globulin and cyclosporine A were studied in a randomized phase III clinical trial. This treatment seems to be associated with hematologic responses in a subset of patients, however, it was not found to reduce the 2-year transformation and overall survival [9].

Over recent years, attention has been paid to immunomodulatory-acting drugs (IMIDs). The anti-MDS activity of these drugs involves antiproliferative effects, downregulation of crucial cytokines, and costimulatory effects on T and NK cells [10]. The IMIDs are thalidomide analogs which have greater immunological and anticancer properties, but lack the toxicity associated with thalidomide [11]. Lenalidomide (LEN) was proven to be effective in the treatment of patients with low-risk MDS, particularly in cases with special molecular feature, i.e., deletions in the long arm of chromosome 5 [12, 13].

2.2. IPSS high-risk MDS patients' treatment option

Patients with high-risk MDS have poor outcomes, high probability of AML development, and without intensive treatment or alloSCT their median survival is limited to 1 year [3]. The treatment of high-risk MDS patients is based on three commonly used therapies: i. alloSCT; ii. intensive chemotherapy; and iii. drugs with epigenetic mechanism of action such as deme-thylation agents and histone deacetylase inhibitors (HDACi) [1]. Similarly as in low-risk patients, the application of alloSCT is limited by the patients' age and overall condition.

About 50% of patients with high-risk MDS achieve complete remission with standard anti-leukemic chemotherapy with fludarabine, idarubicin, or topotecan. This therapy could be improved by a combination of these drugs with intermediate- or high-dose cytosine arabino-side [14]. The combination of such therapy with granulocyte colony-stimulating factor (G-CSF) is well tolerated and highly effective in the remission of both high-risk MDS patients and AML patients [15].

Inhibitors of histone deacetylase block the deacetylation of histones molecules, i.e., they protect histones in acetylation forms. The acetylation of histones occurs in the replication- and transcription-active euchromatin. HDACi could protect euchromatin against formation changes to heterochromatin that is replication and transcription inactive. The effect of HDACi could be pleiotropic, leading to the induction of differentiation, growth arrest, and finally to the apoptosis of tumor cells. The mechanisms of HDACi's effectiveness are under intensive

debate, and it may be p53 dependent or independent [16]. Valproic acid, entinostat, vorinostat, and other HDCAi are under intensive research with the aim of characterizing their effectiveness against MDS [16].

The cytosine analogs 4-amino-1-(β-D-ribofuranosyl)-1,3,5-triazin-2(1H)-one – azacytidine (AzaC) and 4-amino-1-(β-D-deoxyribofuranosyl)-1,3,5-triazin-2(1H)-one – deoxyazacytidine (decitabine, DAC), which were described as cancerostatic agents in the late 1960s and the early 1970s [17, 18], were found to effectively block DNA methylation [19]. Their effectiveness in inducing beneficial effects in the treatment of MDS [20] and AML [21] was already proven. The downregulation of DNA methylation induced by AzaC and DAC is related to the ability of this substance to be artificially incorporated into DNA instead of cytosine, which has to be methylated by DNA methyltransferase [22]. This could be considered as a major principle of DAC action. In contrast to DAC's effects, AzaC is more complex and also involves incorporation into mRNA, tRNA, and rRNA, which disrupts nucleic acid and protein metabolism leading to apoptosis in addition to the incorporation of substances into DNA [22, 23]. Consistently AzaC induced more pronounced cell damage effects than DAC [24].

3. Drug resistance of MDS and AML patients

3.1. Mechanisms of drug resistance of neoplastic cells

The multidrug resistance (MDR) of neoplastic cells represents a real obstacle in the effective treatment of neoplastic diseases [25]. MDR could be an inherent property of tissue from which neoplastic cells were developed – primary (intrinsic) MDR, or could be induced by prior treatment with anticancer drugs – secondary (acquired) MDR (reviewed in [26]). In both cases the neoplastic cells exert reduced sensitivity to more than one drug that differs in structure and pharmacological efficiency. In many cases, cells with resistance to a large scale of diverse drugs are present in cancer tissue. Several mechanisms are involved in the mediation of MDR, which can be divided into seven groups (**Figure 1**, reviewed in [27]):

i.   Potentiating drug metabolism via the induction/activation of phase I and phase II detoxification enzymes

ii.   Potentiating cell drug efflux via the induction/activation of membrane drug transporter predominantly members of the ABC family

iii.   Alteration in drug target structures

iv.   Acceleration of DNA-repair

v.   Changes in epigenetic regulation

vi.   Programmed cell death inhibition
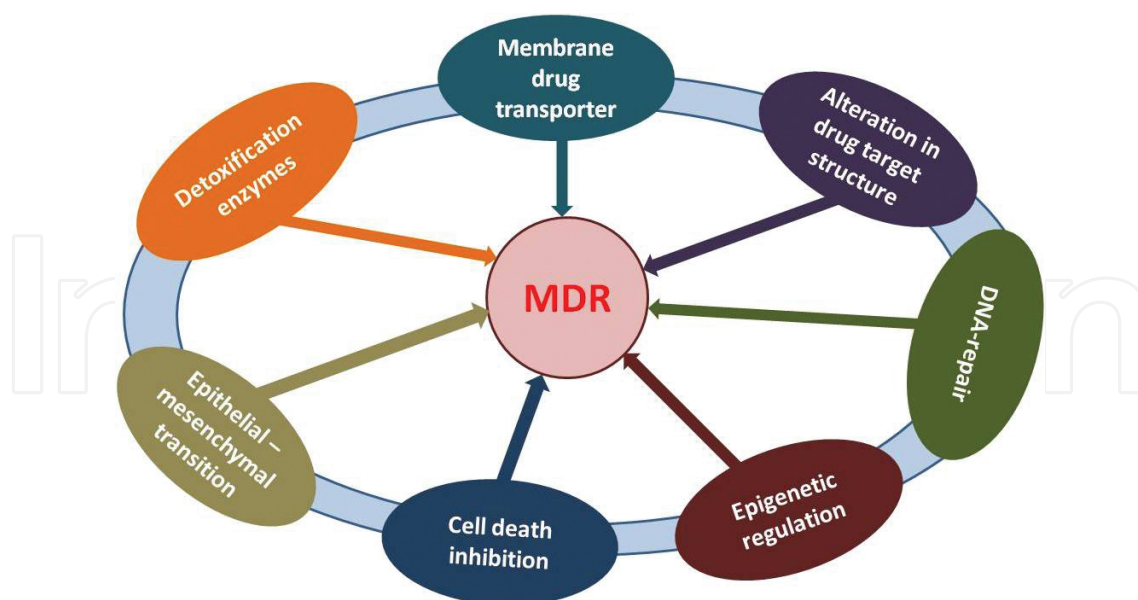
vii.   Epithelial-mesenchymal transition

Figure 1. Mechanisms of multidrug resistance.

These mechanisms could act independently or cooperate in the development of MDR in relation to cancer cells' specific character. The expression of drug transporters represents the most observed molecular causality of MDR (reviewed in [28, 29]). At least three transporters are involved in the reduction of drug sensitivity of neoplastic cells. The best known is P-glycoprotein (P-gp) that represents an ABCB1 member of the ABC transporter family and was discovered as the first ABC transporter in 1976 [30]. P-gp could efflux a large scale of different uncharged substances from cells. Drugs such as colchicine, tacrolimus, and quinidine; chemotherapeutic agents such as etoposide, doxorubicin, and vinblastine; different lipids and steroids; xenobiotics; DNA-intercalators such as ethidium bromide; linear or circular peptides like valinomycin and gramicidin; bilirubin; cardiac glycosides like digoxin; different immu-nosuppressive agents; glucocorticoids like dexamethasone; HIV-type 1 antiretroviral therapy agents like protease inhibitors and nonnucleoside reverse transcriptase inhibitors; and many others are known to be P-gp substrates. When P-gp is expressed in neoplastic tissue it can depress cell sensitivity to its substrates several hundred times [31]. Besides this generally accepted role as a drug transporter, this protein may also play another role as an antiapoptotic regulatory protein and this role is independent of P-gp efflux activity [29, 32]. This additional role also enables P-gp to reduce cell sensitivity to substances that are not its substrates, such as cisplatin several times [33, 34].

Other important transporters involved in MDR are ABCC1-3 members of the ABC transport-ers' family, also known as multidrug-resistant associate proteins 1–3 (MRP1-MRP3) that in contrast to P-gp are specific to negatively charged organic anions (reviewed in [35]). They are also specific for drug conjugates with glucuronic acid and glutathione as a product of phase II enzyme drug detoxification.

One more transporter ABCG2 member of the ABC transporter family is often described to be involved in MDR [36]. This transporter also known as breast cancer-resistant protein (BCRP)

may efflux substances such as mitoxantrone, methotrexate, topotecan, imatinib, and others. The substrate specificity of P-gp, MRP1-3, and BCRP overlaps, and each could be responsible for the efflux of common substrate.

The drug could be detoxified by phase I and phase II detoxification enzymes that secure oxidative and conjugative ways of drug modification which are mediated by cytochrome P450 (CYP) monooxygenases and conjugating enzymes (glutathione S-transferases [GSTs] and UDP-glucuronyl-transferase), respectively [37]. The CYP family, particularly the CYP3A members of the CYP family, may be involved in the reduction of cell sensitivity to several drugs. The transcriptional control of the CYP family is mediated by pregnane X nuclear receptor, i.e., the same nuclear receptor involved in P-gp expression [38].

GSTs represent a group of enzymes that are often involved in the protection of cells against toxic stress [39]. These enzymes catalyze the conjugation of several xenobiotics with reduced glutathione [40]. The actions of GSTs are often coordinated with MRPs that transport several conjugates of drugs and reduced glutathione [41]. While P-gp cannot transport glutathione conjugates, coordinated coexpression of P-gp and GST was observed in vitro using AML cell lines [42].

Alteration in drug target structures such as the mechanism of MDR represents a large group of diverse changes in regulatory pathways, which is finally responsible for the downregulation or upregulation of drug molecular target. An example of this behavior alteration of topoisomerase II, such as the molecular causality of neoplastic cell resistance to topoisomerase poisons, could be performed (reviewed in [28]).

The repair of DNA primarily damaged by drugs' direct action, or secondarily by the elevation of oxygen reactive species formation, clearly yields to the drug resistance of neoplastic cells [27]. The therapeutic effects of DNA-damaging drugs in cancer treatment are given by the equilibrium between drug-induced DNA damage and the effectiveness of DNA repair mechanisms. The inhibition of repair pathways used in conjunction with DNA damaging chemotherapy could sensitize cancer cells and therefore increase the efficacy of therapy [27].

Epithelial-mesenchymal transition is a mechanism predominantly taking part in solid tumor metastatic processes. This mechanism could play only a minor role (if any) in drug resistance development in MDS and AML patients.

The high expression levels of antiapoptotic proto-oncogene of the Bcl-2 family (such as BCL-2, Bcl-XL, Mcl-1, Bcl-w, and Bfl-1) were often reported to be associated with in vitro resistance to chemotherapeutic agents, poor clinical outcomes in cases of AML [43], and in cases of adults with acute lymphoblastic leukemia [44]. Bcl-2 was shown to be restricted in tissues characterized by apoptotic cell death [45]. Antiapoptotic proteins of the Bcl-2 family hetero-oligomerize in vivo with a conserved homolog – proapoptotic member of the Bcl-2 family (such as Bax), and this process is known to modulate apoptosis [46]. The translocation of the Bax (or other proapoptotic protein) monomer from the cytosol to the mitochondria followed by the formation of BAX homo-oligomers represents a physiological death stimulus, which may be prevented by the presence of the Bcl-2 protein (or other antiapoptotic proteins) [47]. Therefore for apoptosis progression, an equilibrium between anti- and proapoptotic proteins plays a

crucial role. This is molecularly regulated by the p53 known as the central regulator of apoptosis [48]. This is consistent with known data about the role of the mutated form of TP53 in cancer [49, 50].

Epigenetic regulations are involved in the development of MDR directly by the downregulation or upregulation of important genes responsible for cell death or survival. For example, tumor-suppressor genes are often silenced via hypermethylation, and oncogenes are overexpressed via hypomethylation [27]. Epigenetics could also play an indirect role in cell drug sensitivity by the following mechanism: the opening of the chromatin structure, which is prerequisite for DNA replication and transcription, and to produce uncovered DNA that is more accessible for drug-induced DNA damage. This is consistent with more pronounced DNA damage induced with drugs in more proliferating and/or transcriptionally active cells.

Hypermethylation of the MDR1 promoter was associated with transcriptional repression and chromatin structural changes [51]. The demethylation of this promoter in cancer cell lines was found to elevate the multidrug-resistant phenotype [52].

Epigenetic mechanisms can also influence DNA damage repair. For example, DNA mismatch repair processes can be lost due to the hypermethylation of the human mutL homolog 1 (hMLH1) gene promoter, which can lead to cancer development [27].

Demethylation by DAC may have a role in increasing the efficacy of chemotherapy for patients with tumors, as characterized by high hMLH1 promoter methylation and low hMLH1 expression [53].

3.2. Resistance to immunomodulatory drugs

Over recent years, attention has been paid to exploiting the immunomodulatory effects primarily obtained for thalidomide [54], which has resulted in novel IMIDs. The anti-MDS activity of these drugs (namely LEN) was proven for low-risk MDS, particularly with 5q deletion (del[5q]) [55]. This action is attributed to several mechanisms that involve antiproliferative effects, downregulation of crucial cytokines, and costimulatory effects on T and NK cells [10]. However, the exact mechanism of IMIDs' action in MDS treatment is still not fully understood. The IMIDs' immunomodulatory compounds derived from the thalidomide structure have greater immunological and anticancer properties, but lack the toxicity associated with thalidomide [11]. LEN (Revlimid) was approved for use in low- and intermediate-1-risk MDS patients who have the deletion 5q chromosome and no other chromosomal abnormalities, are dependent on red blood cell transfusions, and for whom other treatment options have been found to be insufficient or inadequate by EMA (European Medicines Agency) and the FDA (Food and Drug Administration). After LEN treatment, blood transfusion-independent rates were 56–67%, and median response duration was longer than 104 weeks [1]. Additionally, a significant proportion of these responders achieved cytogenetic responses (50–76%), indicating a direct cytotoxic effect of LEN on the neoplastic clones, although a significant proportion of patients develop resistance to this treatment. The study of cytogenetics and molecular predictors of responses in patients with myeloid malignancies without del[5q] treated with LEN indicated that treatment could be effective in patients with

normal karyotype and a gain of 8 chromosome is present [55]. The LEN response was achieved by one quarter of MDS patients lacking the 5q abnormality. Ebert et al. [56] found that mononuclear cells from bone marrow aspirates of patients who respond to LEN have a decreased expression of genes, which are specific to terminal erythroid differentiation, regardless of the presence or absence of a 5q deletion. Moreover, LEN acts directly on hematopoietic progenitor cells to increase erythropoiesis relative to other lineages.

The mechanism of LEN's therapeutic effects and the mechanisms that depress its effectiveness in MDS treatment are not fully understood, but could be related to TP53 mutation (reviewed in [57]).

TP53-mutated populations seem to be associated with the early stage of low-risk MDS in patients with del[5q] [58]. However, these authors stated that TP53 mutations could not be predicted by general clinical features but were associated with p53 overexpression. Specific R72P polymorphism of TP53 results in two molecular forms of p53. Molecular variant p53-R72 with better mitochondrial localization activates apoptosis more efficiently (by direct induction of cytochrome c release) than p53-P72 variant [59]. McGraw et al. [60] underscore the distribution of R72P in MDS and highlight differences between del(5q) and non-del(5q) subtypes by gene polymorphism and the relationship to LEN response. However, to prove the potential interaction of R72P variants with germline variants in other key regulators or effectors of the p53 pathway that may modify MDS risk and LEN treatment response, further research will be necessary.

Allelic deletion of the RPS14 gene is a key effector of the hypoplastic anemia in patients with MDS and chromosome 5q deletion [61]. Disruption of ribosome integrity liberates free ribosomal proteins to bind to and trigger the degradation of E3 ubiquitin-protein ligase MDM2 (a negative regulator of p53), with consequent p53 transactivation. Consistently, p53 is overexpressed in erythroid precursors of primary bone marrow del(5q) MDS specimens accompanied by reduced cellular MDM2. LEN may act in the stabilization of MDM2 that leads to p53 degradation [61].

When LEN was used in establishing human AML cell lines SKM-1 and MOLM-13 for resistance, only SKM-1 but not MOLM-13 cells developed MDR phenotype with massive expression of P-gp [62]. Both these cell lines were derived from AML patients, whose disease developed from MDS. In contrast to MOLM-13 with wild type of p53 [63, 64], SKM-1 represents cells expressing a mutated p53 form [65]. Thus cells with mutated TP53 could express P-gp under long-term LEN treatment, which leads to typical P-gp mediated MDR.

3.3. Resistance to hypomethylating agents

In pathogenesis of MDS, both genetics and epigenetics alterations are cooperated. Disruption of genetic pathways regulating the processes of self-renewal, differentiation, quiescence, and stem cell-niche signaling contributes to AML transformation. The hypermethylation of different genes was discussed to be partially responsible for the poor prognosis of MDS patients [66]. Demethylating agents, such as AzaC and DAC, were shown to induce clinical responses in 40–70% of MDS patients [67, 68]. Although hypomethylation is considered the

dominant mode of therapeutic action of these drugs, it may also induce DNA damage and consequent apoptosis [69]. Interestingly, clinically significant responses to decitabine without significant toxicity can be seen in patients after nonsuccessful azacitidine therapy [70].

Changes in the expression profile of genes like CD9, GPNMB, FUCA1, ANGPT1, PLA2G7, TPM1, and ARHGEF3 were observed when CD34+ cells isolated from the bone marrow of high-risk MDS patients treated in vitro with DAC were compared with CD34+ cells isolated from patients with untreated early stage Hodgkin's lymphoma taken as a control [66].

AzaC resistance represents a real obstacle for the effective treatment of MDS patients, which focused the attention of scientists on alternative therapeutic strategies for nonresponsive patients. For this reason, AzaC-resistant MDS/AML cell lines are established. AzaC-resistant SKM-1 cells exhibited increased expression of the BCL2L10 member of the antiapoptotic Bcl-2 family that altered apoptosis progression [71]. Interestingly we described the downregulation and changed molecular form of the Bcl-2 protein identified by polyclonal antibody (sc-492, Santa Cruz Biotechnology, USA) in our variant of SKM-1 AzaC-resistant cells [42]. Moreover, other AzaC-resistant AML cells derived from the MOLM-13 cell line exerted similar changes in the Bcl-2 protein. Significant correlation of AzaC resistance with a percentage of MDS or AML cells expressing BCL2L10 was established on a group of 77 patients [71].

AzaC resistance could include impaired mitochondrial membrane permeabilization and caspase activation when AzaC resistant and sensitive SKM-1 myeloid were compared [72]. In our experiments, when the same cell model was used resistance to AzaC was associated with strong over expression of P-gp that secured additional resistance to P-gp substrates [42]. This is a rather interesting finding because AzaC is not a P-gp substrate, and P-gp was not responsible for AzaC resistance. We obtained similar results using MOLM-13 cells. The activity of GST was found to be elevated 8 times when AzaC resistant and sensitive SKM-1 and MOLM-13 cells were compared [42].

AzaC induced the upregulation of LC3-II and elevation of cathepsin B activity (both autophagy markers). Increased basal autophagy was observed in SKM-1 AzaC-resistant cells, but these cells were resistant to AzaC-mediated autophagy [72]. Autophagy depression using a LC3 silencer revealed the protective function of autophagy in AzaC-sensitive and AzaC-resistant cells in basal condition [72]. Taking all the facts about apoptosis and autophagy progression in AML cell models together, it could be concluded that resistance to AzaC is associated with alterations of both processes via impaired homeostasis of its key regulators such as Bcl-2 family proteins and LC3. However, the exact mechanism of this feature is not fully understood and future research will be necessary.

Enzymes involved in cytidine metabolism such as cytidine deaminase (CDA) and deoxycytidine kinase (DCK) seem to be responsible for the primary (intrinsic) AzaC resistance, because nonresponders of AzaC have a 3-fold higher CDA/CDK ratio. There were no significant differences at relapse in DAC metabolism genes, and no CDK mutations were detected [73].

MDSs are characterized by mutations in genes encoding epigenetic modifiers and aberrant DNA methylation. Clonal mutation of TP53 and non-receptor type 11 protein tyrosine phosphatase were associated with shorter overall survival, but not the drug response of

patients. Clonal tet methylcytosine dioxygenase 2 (TET2) mutations predicted a response when subclones were treated as wild type. The highest response rate was observed in patients with a mutation in the TET2 gene without a clonal mutation of the trancriptional regulator encoded by ASXL1 gene. [74].

While somatic mutations did not differentiate responders from nonresponders for DAC treatment, differentially methylated regions of DNA at baseline distinguished responders from nonresponders. In responders, the upregulated genes included those that are associated with the cell cycle, potentially contributing to effective DAC incorporation [75].

While DAC is generally accepted as a hypomethylating agent, it may exert a therapeutic effect also in another way. The acceleration of reactive oxygen species induced with DAC could take place in the overall DAC effect. However, reactive oxygen species accumulation was not always present in the sample of AML patients after DAC treatment. Therefore, the relevance of reactive oxygen species generation in the mechanism of DAC pharmacological effectiveness should be studied more intensively in the future [76].

3.4. Resistance to intensive chemotherapy

MDS intermediate- or high-risk patients may be treated with an intensive chemotherapy regimen that is similar as used for AML treatment. A combination of drugs such as cytosine arabinoside, fludarabine, idarubicin, and topotecan, etc. could be used [14]. This chemotherapy is oriented on destroying abnormal blood cells or preventing their growth. For patients who are eligible, bone marrow transplantation is recommended after this therapy.

The development of MDR resistance during the application of this protocol is similar as for other types of neoplastic diseases, and may involve each of the mechanisms described in Chapter 3. The combination of drug-resistant mechanisms that are included in MDR pheno-type development during high intensive treatment depends on the specific patient molecular feature, previous therapeutic history, and drugs applied during previous treatment.

3.5. Resistance to CD33-targeted therapy

Progressive methods of MDS treatment represent antibody targeting therapy with cytotoxic agents linked to humanized antibodies against antigens specific to neoplastic cells. Both MDS and AML are characterized by the presence of undifferentiated CD33-positive myeloblast in peripheral blood and bone marrow, compared with healthy control [77]. CD33 is a 67 kDa glycoprotein present on the surface of myeloid cells, and is a member of the sialic acid binding immunoglobulin-like lectin family of proteins [78]. After binding to an appropriate antibody, CD33 is rapidly internalized into leukemia cells [79, 80]. This action enables the use of a humanized CD33 antibody, conjugated to cytotoxic agents for targeted immunotherapy [78, 79]. Gemtuzumab ozogamicin represents antibody drug (from a class of calicheamicins) conjugates [7, 78–81]. This therapy has proven to be effective, but resistance to treatment could be developed. Another immune-targeting preparation is AVE9633 (immunoconjugate of humanized monoclonal CD33 antibody, linked through a disulfide bond to the maytansine derivative DM4) that was used in the treatment of several leukemia cell lines [82]. The activity

of P-gp was attributed as a critical factor in depressing the success of this therapy. P-gp mediated the efflux of drug liberated from linkage with antibody due to intracellular enzymes was attributed as being responsible for this resistance [82]. However, a significant inverse correlation was determined for the expression of P-gp and CD33 in the AML blast obtained from patients [81]. We described the strong downregulation of CD33 on mRNA and the protein level in P-gp positive SKM-1 cells selected for resistance by LEN, vincristine, mitoxantrone, or in P-gp positive MOLM-13 cells selected for resistance by vincristine or mitoxantrone [62]. Upregulation of CD33 expression level was also observed in P-gp silenced cell lines. Therefore, the failure of CD33-targeted therapy of patients with AML blast overexpressing P-gp could be caused by a lack of CD33 as an antibody target structure on the cell surface.

3.6. Detection of drug-resistant markers in neoplastic cells

Cellular expression of drug-resistant markers could be monitored on mRNA level (RT-PCR methods) or protein level (Western blot and immunofluorescence flow cytometry) [83]. Proteins active in MDR development like ABC transporters, drug metabolizing enzymes, antiapoptotic proteins, and many others could be detected by these methods. Transport activity of drug transporters could be measured using depressed intracellular retention of their fluorescent substrates by flow cytometry. Retentions of calcein/AM, rhodamine 123, or doxorubicin were often used for P-gp efflux activity detection directly in cells isolated from patients [84]. Activities of detoxification enzymes could be monitored by appropriate substrates, which enzymatic modifications induce changes in either fluorescence or light absorbance properties. Conjugation of 1-chloro-2,4-dinitrobenzene with reduced glutathione as a result of GST activity could be taken as example [42]. Specific mutations of genes active in MDR development (like *TP53*) could be detected using mutation analysis including specifically designed PCR reaction, denaturing high-performance liquid chromatography, or DNA-sequencing techniques. Alteration in drug-induced apoptosis could be monitored as difference in drug-induced DNA-fragmentation by electrophoresis or using comet assay [85]. Application of oligonucleotide microarray for human genome represents available methods to obtain complex information about expression profiles of MDR-associated genes in patients [86]. Moreover, when nonresponders and responders will be compared using oligonucleotide microarrays, new information about involving different genes' expression in MDR development could be obtained. Cytotoxic effects of several drugs could be monitored directly in cells isolated from patient samples using assays based on reduction of formazan (like MTT assay) by intracellular dehydrogenases, or liberation of fluorescent label from its esters (like fluorescein diacetate) by intracellular esterases.

4. Perspectives

MDSs represent a very diverse group of hematological malignancies. Treatment of this disease involves several drugs such as LEN, AzaC, and DAC. Unfortunately, the exact mechanism of action is still not fully understood. Therefore, prediction of the response to this treatment is still very complicated. For this reason, a detailed knowledge of the molecular causality of MDS

and AML progression will be necessary. Moreover, unclear questions about the molecular mechanisms of these drugs should be answered. Resistance to treatment use for AML and MDS still represents serious problems with causality not fully understood. Research oriented on these topics will bring new knowledge and new predictive molecular markers that will enable the better selection of effective therapy for each patient.

Acknowledgements

Author details

Lucia Messingerova[1,2], Denisa Imrichova[2], Martina Coculova[1], Marian Zelina[2], Lucia Pavlikova[2], Helena Kavcova[2], Mario Seres[2], Viera Bohacova[2], Boris Lakatos[1], Zdena Sulova[2] and Albert Breier[1,2*]

*Address all correspondence to: albert.breier@stuba.sk

1 Institute of Biochemistry and Microbiology, Faculty of Chemical and Food Technology, Slovak University of Technology, Bratislava, Slovakia

2 Institute of Molecular Physiology and Genetics, Slovak Academy of Sciences, Bratislava, Slovakia

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