Chapter

Roads of Drug Resistance in Acute Myeloid Leukemia—Is It Dead End?

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Abstract

Acute myeloid leukemia (AML) is a biologically and clinically heterogeneous neoplasm, which is characterized by abnormal proliferation, impaired apoptosis, and differentiation of leukemic immature cells. Nowadays, the first line treatment of AML is the chemotherapy regimen, which combines both cytosine arabinoside and anthracycline. Despite that complete remission (CR) can be achieved in 40–80% of patients depending on age, a considerable number will eventually relapse (acquired resistance) or have refractory disease (primary resistance). Finally, the estimated 5-year overall survival (OS) is less than 30%. Recent investigations reveal various mechanisms, responsible for drug resistance leading to AML persistence and recurrence. In order to improve clinical outcomes and develop successful therapeutic strategies, it is necessary to better explore the major adverse factors for escape from treatment, as well as to explore ways to predict and prevent or target drug resistance.

Keywords: acute myeloid leukemia, chemotherapy, drug resistance

1. Introduction

Acute myeloid leukemia (AML) comprises approximately 80% of all acute leukemias in adults [1]. The median age of the disease is about 69 years with a progressing incidence with advanced age [2]. AML represents a clinically and genetically heterogeneous disease, which is characterized by uncontrolled clonal proliferation, impaired apoptosis, and differentiation of leukemic immature cells. It has poor survival and fatal outcome in those who are untreated. Despite the progress in understanding the pathophysiology of AML and the discovery of novel therapeutic agents, the treatment approach has not changed essentially since the 1970s. According to the European LeukemiaNet (ELN) recommendations for the diagnosis and management of AML in adults (2017), the first-line therapy for the patients, eligible for intensive chemotherapy remains the conventional "3 + 7 regimen" (3 days of anthracycline + 7 days of cytarabine) [3]. Unfortunately, recent studies report that the disease is curable in only 5–15% of patients above 60 years and 35–40% in younger adults with intensive chemotherapy, which is unsatisfactory [4, 5]. Additionally, research data reveals that 10–40% of newly diagnosed AMLs fail to achieve CR after frontline therapy and are classified as primary refractory AML [6–8]. Lately, ELN determines primary refractory AML as a lack of obtaining CR or complete remission

with incomplete hematologic recovery (CRi) after at least 2 courses of intensive induction chemotherapy [3]. One of the major reasons for treatment failure is considered to be drug resistance. Besides, chemoresistance is basically divided into two groups intrinsic (primary) and acquired (secondary). The primary drug-resistant leukemic cells are present already at diagnosis, while the secondary resistance emerges during or after therapy, probably as a result of additionally occurring genetic disorders. In 30–40% of relapsed AML patients, newly developed gene mutations occur, yet in about 25% no molecular alterations emerge [9]. So, these data raise the question that gene alterations are drivers of relapse or refractoriness of AML and whether mutational status is the only cause of disease progression. This review will discuss the potential molecular pathways underlying drug resistance in blast cells and the interactions with the leukemic microenvironment.

2. Leukemogenesis in AML

Two decades ago, Gilliland and Griffin introduced the "two-hit model". In this model, the collaboration of two lesions of two different classes of mutations contributes synergistically to inflict AML [10]. Class I mutations (FLT3, c-KIT, NRAS) comprise mutations that activate signal-transduction pathways and thereby give a proliferation advantage to the hematopoietic progenitor cells. Class II mutations (recurring chromosomal aberrations, which produce fusion transcripts) affect transcription factors and cause impaired differentiation and following apoptosis. However, recent studies report that it is difficult to divide functions between the two classes of mutations [11]. Further, in the last years, genomic sequencing research has encountered new epigenetic genes associated with AML (chromatin-modifying genes: MLL fusions, ASXL1, and EZH2 mutations; methylation-related genes: DNMT3A, TET2, IDH1/2 mutations), which expands the complexity and heterogeneity of AML [12, 13]. Thus, the "two-hit model" of Gilliland and Griffin turns out to be insufficient to explain AML leukemogenesis.

A novel hypothesis for AML development has been formulated recently, based on three types of AML-associated mutations, investigated in mouse models [14]. According to this functional classification the first "type A mutations" (fusion genes) are necessary to initiate or maintain the leukemic phenotype, "type B mutations" (ABL, PDGFR, KIT, FLT3, etc.) support the proliferation and survival of leukemic cells and "type C mutations" (epigenetic modifiers), also called "seed mutations", provide a growth advantage, but still not sufficient to induce leukemia. The study research demonstrates that the combination of mutations of any two types, that is, A + B or A + C or B + C, may result in AML [14]. The model of clonal evolution of AML is considered to be a process of losing specific mutations or gaining a feature, which leads toward resistance. Emerging evidence has revealed that a relapse may present with re-occurring of the initial leukemic clone, assuming that the chemotherapy was unsuccessful or due to further clonal evolution following AML treatment [15, 16].

3. Mechanisms underlying drug resistance

Relapsed and refractory AML (R/R AML) is associated with unfavorable prognosis, due to poor response to conventional antileukemic therapy [17, 18]. Thus, a better understanding of the mechanisms, underlying drug resistance, would improve the therapeutic approach using novel strategies. Data are available showing that

Factors	Molecules	Function	Reference
Proteins and enzymes	P-gp, GST, MCL-1, MRP1/ LRP, Topo II, PKC	Affect the drug transport; altered enzyme function	[19–43]
Signal pathways	NF/kB, PI3K/Akt/mTOR	Coordinate complex cellular changes	[44, 45]
Genes and epigenetics	ASLX1, DNMT3, EZH2, FLT3, IDH1/2, TET2, WT1	Cell proliferation and differentiation; regulation of DNA and histones	[46–53]
microRNA	miRNA-155, miRNA-125, miRNA-100, miRNA-223	Control of cell division, self-renewal, DNA damage	[54–56]
Microenvironment	CD44, FGF2/FGFR1, SDF1/ CXCR4, VCAM/VLA4	Cell-to-cell, cell-to-matrix interactions	[57–66]

ASXL1, additional sex combs-like 1; CXCR4, C-X-C motif chemokine receptor 4; DNMT3A, DNA methyltransferase 3A; EZH2, enhancer of zeste homolog 2; IDH1/2, isocitrate dehydrogenase 1/2; FGF2, fibroblast growth factor 2; FGFR1, fibroblast growth factor receptor 1; FLT3, FMS-like tyrosine kinase 3; GST, glutathione S-transferase; LRP, lung resistance protein; MCL-1, myeloid cell leukemia 1; microRNA, microribonucleic acid; MRP1, multidrug resistance-related protein; mTOR, mammalian target of rapamycin; NF/kB, nuclear factor kappa B; P-gp, P-glycoprotein; PI3K, phosphatidylinositol-3-kinase; PKC, protein kinase C; SDF-1, stromal cell-derived factor 1; TET2, ten eleven translocation methylcytosine dioxygenase 2; Topo II, topoisomerase II; VLA-4, very late antigen-4.

Table 1.
Different mechanisms of drug resistance.

chemoresistance in AML may be due to numerous factors, which include proteins and enzymes with altered function, dysregulation of signaling pathways, mutations in cell cycle control genes, epigenetic modifiers, microRNA as well as impaired interactions with the bone marrow environment, and changes in the immune tolerance (**Table 1**).

3.1 Proteins and enzymes with altered function

Overexpression of the transporter proteins P-glycoprotein (P-gp) and multidrug resistance-related protein (MRP) 1 plays an important role in cross-resistance to drugs. In 1976 Juliano and Ling firstly described the ATP-dependent membrane P-gp, which acts as an efflux pump conferring resistance [19]. P-gp is a 170-kDa protein, encoded by the MDR1 gene (multidrug resistance gene 1) and belongs to the ABC transporter family. It pumps out chemotherapy drugs, maintaining lower drug concentrations intracellularly continuously, so that drug resistance is developed [20]. P-gp is considered to be an independent adverse prognostic factor for response and survival in newly diagnosed or R/R AML [21, 22]. However, according to other research studies, no correlation was identified between the MDR parameters and overall survival of the AML patients [23] and also P-gp activity is not consistently upregulated in relapsed AML [24]. Broxterman et al. found no correlation between the expression of P-gp and the complete response rate, event-free survival, or overall survival after idarubicine-containing induction [25]. The steady-state cellular accumulation of lipophilic idarubicine may circumvent the P-gp-mediated drug resistance in AML patients. The increased expression of P-gp and MRP1 are associated with advanced age, leukocytosis, poor chromosomal abnormalities, shorter overall survival, and also are detected with higher incidence in R/R and secondary AML comparing with de novo cases [26, 27]. Interestingly, an association has been observed between the expression of P-gp and MRP1 and the flow cytometric antigens (CD34 and CD7) and the FAB (French-American-British) classification of AML morphology

(M2, M5a, and M7 types) [26]. Moreover, a recommendation to observe the higher MRP1 expression by flow cytometry as an adverse prognostic marker in AML was proposed by Legrand et al. [28]. In contrast, another study revealed that AML patients relapse despite the lower expression of MRP1, suggesting the involvement of other intracellular mechanisms, possibly leading to cytarabine resistance [29].

LRP (lung resistance protein), a drug efflux transporter, is also assumed to play a role in drug resistance. However, the published data show conflicting evidence of the involvement of LRP in the process of chemoresistance. The overexpression of LRP is found to predict an inferior response in AML, but another study described that higher bone marrow expression of LRP predicts significant favorable therapeutic outcome with increased CR rate and 1-year DFS (disease-free survival) and OS [22, 30, 31].

Glutathione S-transferase (GST) is a drug-metabolizing enzyme, consisting of α, μ, θ, and π-types. It is responsible for controlling cellular oxidative balance, catalyzing the reduced glutathione which leads to diminished cytotoxic drug effects. The decreased enzymatic activity due to GST polymorphisms is associated with cancerogenesis and AML [32]. Indeed, changed detoxification contributes toward drug resistance in AML. Furthermore, patients with higher expression of GSTμ tend to have also MRP1 overexpression which results in increasing the survival of tumor cells and protects them from apoptosis [33, 34]. Recent investigation by Pei et al. demonstrated that primitive leukemic cells acquire aberrant glutathione metabolism and may be selectively eliminated by target therapy against the glutathione pathway [35].

Topoisomerase II (Topo II) is an essential ribozyme that alters the topological properties of DNA. The inhibitors of Topo II may trigger chromosomal translocations that are associated with therapy-related secondary leukemia, often bearing 11q23 translocations involving the *MLL* gene [36]. Decreased or increased expression or mutation in the topoisomerase II genes may lead to chemoresistance to topoisomerase II inhibitors [37, 38].

Somatic mutations in protein kinase genes play a significant role in proliferation, resistance, and apoptosis. The overexpression of PKC (protein kinase C) in AML results in a decline in CR induction and DFS by diminishing intracellular concentration of daunorubicine [39]. The connection between the activation of PKC and the upregulation of P-gp further contributes to chemoresistance in AML [40].

As a BCL-2 family protein member, MCL-1 (myeloid cell leukemia 1) prevents apoptosis. It is upregulated in several hematologic malignancies such as multiple myeloma, AML, and non-Hodgkin lymphoma and is associated with treatment resistance and inferior prognosis [41]. The increased expression or amplification of MCL-1 gene protects tumor cells from programmed cell death and decreases their sensitivity to conventional chemotherapy which appears to be a potential drug resistance mechanism [42]. Besides, the overexpression of MCL-1 correlates with resistance to venetoclax [41]. MCL-1 is described to be regulated by cyclin-dependent kinase (CDK). Recent studies reveal that the treatment with both MCL-1 inhibitors and BCL-2 inhibitors may overcome the acquired resistance to BCL-2 inhibition [43].

3.2 Signaling pathways

Knowledge of aberrantly regulated signal pathways in AML allowed the identification of novel therapy targets. The combination of conventional chemotherapy with targeted agents may potentially overcome resistance. An example is the PI3K/Akt/mTOR signal pathway which is responsible for cell metabolism, proliferation, differentiation, and survival. The upregulation of the PI3K/Akt/mTOR pathway in AML is caused by

mutations in the receptor tyrosine kinases. The FLT3 mutation leads to deregulation of PI3K/Akt/mTOR signaling which results in cytokine-independent survival and proliferation of hematopoietic cells and myeloproliferative neoplasms [44]. In addition, according to lately published data the PI3K/Akt/mTOR pathway plays a significant role in the regulation of therapy-resistant leukemic cells through the pro-inflammatory transcription factor NF-κB (nuclear factor-kappa-light-chain-enhancer of activated B cells). Thus, the mediated chemoresistance is caused by upregulation of anti-apoptotic genes, which leads to tumor cell growth and resistance of apoptosis [45].

3.3 Genes and epigenetic modifiers

FLT3 is normally expressed by hematopoietic stem and progenitor cells, but in AML acts as a protooncogene that stimulates cell proliferation, differentiation, and survival. In approximately 30% de novo AML patients mutations in FLT3 gene emerge. There are two types of mutations, that is, internal tandem duplication (ITD), which is present in about 25% of cases with adverse prognostic impact, and the tyrosine kinase domain (TKD) in about 5%, which prognostic value remains disputable [46]. According to the 2016 revision of the WHO classification of myeloid neoplasms and acute leukemia, the potent significance of the FLT3-ITD mutation depends on the allelic ratio and the presence of NPM1 (nucleophosmin) gene mutations [47]. Published data demonstrated that FLT3-ITD mutation can constitutively activate the receptor and force uncontrollable cell proliferaton, which turns leukemic cells resistant to conventional chemotherapeutic agents [48, 49]. In addition, authors suggested that the mechanisms of drug resistance consist of clonal evolution of resistant leukemic cells, adaptive cellular mechanisms and a protective leukemic microenvironment [50]. FLT3-inhibitors the relapse may occur due to leukemic cells harboring FLT3-TKD mutations or non-FLT3 clones, carrying epigenetic mutations such as IDH1/2, ASXL1, or TET2 [50]. By the advanced methods of whole genome or exome sequencing, several epigenetic modifiers have been determined in AML, regulating DNA methylation (DNMT3A, IDH1/2, TET2) and histone modification (EZH2 and ASXL1) [51]. Research data suggest that epigenetics-modifying gene mutations promote genetic instability and induce FLT3-ITD, leading to drug resistance and relapse [52]. However, the prognostic impact and the precise contribution of these genes to leukemogenesis have not been fully elucidated yet [53].

3.4 microRNA

MicroRNAs are small, 19–24 nucleotide-long, non-coding single-stranded RNAs which play a key role in the control of the expression of several genes involved in the differentiation of hematopoietic stem cells and the development of cancers [54, 55]. The impaired regulation of microRNAs may contribute to the chemoresistance of tumor cells by affecting cell survival and apoptosis-related signaling pathways [56]. Research efforts in the last decade have demonstrated the unquestionable role of microRNAs in reversing drug resistance. However, their implementation into clinical practice is hampered by the inability to ensure sufficient safe and specific entry into tumor cells and further studies are needed [56].

3.5 Tumor microenvironment

The bone marrow microenvironment supports normal hematopoiesis through signaling cascades and affects the evolution, progression, and chemotherapy resistance

of AML [57]. The bone marrow consists of two distinct niches, that is, the osteoblastic (endosteal) and the vascular (endothelial), which act synergistically in order to regulate cell self-renewal, proliferation, and differentiation [58]. Hematopoietic stem cells are maintained by stem cell factor (SCF), CXCL12 (C-X-C Motif Chemokine Receptor 4), Notch ligands, and transforming growth factor-β [59]. Mesenchymal stromal cells secrete SCF and CXCL12, which regulate leukocyte migration [60]. The binding of CXCL12 to its receptor CXCR4 initiates the phosphorylation of CXCR4 and activates prosurvival signaling pathways such as MEK/ERK, JAK/STAT, and PI3K/AKT cascades [58]. Of note, CXCR4 signaling is associated with increased retention to the bone marrow, enhanced chemoresistance of leukemic cells, and poor prognosis in AML [61]. The interaction between the very late antigen-4 (VLA-4) and fibronectin take part in chemokine-mediated homing and mobilization [62]. The adhesion receptor VLA-4 binds to the fibronectin and vascular cell adhesion molecule-1 (VCAM-1), resulting in the retention of the leukemic cells within the bone marrow niche [63]. Wang et al. observed that a higher level of expression of VLA-4 is characterized by poorer survival [58]. The surface marker CD44 is a glycoprotein, that mediates cell adhesion, migration, and homing of leukemic cells [64, 65]. The antigen CD44 is expressed on both hematopoietic and leukemic cells, binding to E-selectin and L-selectin. The inhibition of E-selectin strengthens the influence of the chemotherapeutics daunorubicin and cytarabine [66], and lowers the leukemia burden [57]. As components of the microenvironment have been shown to contribute to drug resistance in AML, novel targeted therapies have been advanced in order to overcome it.

4. Conclusion

The development of drug resistance has emerged as an insurmountable challenge in the treatment of patients with R/R AML. The incompletely understood molecular mechanisms which cause therapeutic failure remain as a major obstacle to the long-term success of leukemic therapy, inferior prognosis, and reduced survival. Further investigations are needed to delineate more precise, genomic-guided, individualized clinical approaches.

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Conflict of interest

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Acronyms and abbreviations

AML	acute myeloid leukemia
ASXL1	additional sex combs-like 1

ATP	adenosine triphosphate
CDK	cyclin-dependent kinase
CR	complete remission
CRi	incomplete hematologic recovery
CXCR4	C-X-C motif chemokine receptor 4
DFS	disease-free survival
DNMT3A	DNA methyltransferase 3A
ELN	European LeukemiaNet
EZH2	enhancer of zeste homolog 2
FAB	French-American-British
FGF2	fibroblast growth factor 2
FGFR1	fibroblast growth factor receptor 1
FLT3	FMS-like tyrosine kinase 3
GST	glutathione S-transferase
IDH1/2	isocitrate dehydrogenase 1/2
ITD	internal tandem duplication
LRP	lung resistance protein
MCL-1	myeloid cell leukemia 1
MDR	multidrug resistance
MRP1	multidrug resistance-related protein 1
mTOR	mammalian target of rapamycin
NF/kB	nuclear factor-kappa-light-chain-enhancer of activated B cells
NPM1	nucleophosmin
P-gp	P-glycoprotein
PI3K	phosphatidylinositol-3-kinase
PKC	protein kinase C
R/R AML	relapsed and refractory acute myeloid leukemia
RNA	ribonucleic acid
SCF	stem cell factor
SDF-1	stromal cell-derived factor 1
TET2	ten eleven translocation methylcytosine dioxygenase 2
TKD	tyrosine kinase domain
Topo II	topoisomerase II
VCAM-1	vascular cell adhesion molecule-1
VLA-4	very late antigen-4
WHO	World Health Organization

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