Pediatric acute myeloid leukemia and drug resistance in the context of microarray studies

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Abstract

Genome and transcriptome profiling methods are increasingly used in studies of pediatric acute myeloid leukemia (AML). AML can be distinguished from acute lymphoblastic leukemia (ALL) on the basis of gene expression profiles; so too can the various subclasses of these two forms be further distinguished and genetically characterized. Genome-wide analysis studies (GWAS) have also contributed to new insights into the biological basis of the mechanisms of drug resistance, and allow the identification of new prognostic factors and the potential for targeted therapy. On the basis of changes in gene expression level, it is also possible to predict the risk of early recurrence and prognosis at the time of diagnosis of de novo leukemia. Although the possibility to analyze gene expression profiles already presents significant progress in our understanding of the complex pathobiology of pediatric AML, the introduction of new microarrays formats, such as CGH, SNP, CpG islands or antibodies, should be considered to build a complete picture of the cells in this form of cancer.


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Acute myeloid leukemia in children

Childhood acute myeloid leukemia (AML) is a heterogeneous disease, accounting for 15-20% of acute leukemias in this age group [1-2]. It is classified on the basis of the presence of cytogenetic rearrangements. Treatment of patients with AML relies on genetic tests that inform the diagnosis and prognosis, predict response to therapy, and measure minimal residual disease. Currently employed therapeutic programs achieve complete remission in about 90% of patients [3]. Further improvement of treatment outcomes requires a better understanding of both the molecular abnormalities responsible for the creation and growth of leukemic cells, and the mechanisms underlying drug resistance.

New cases of AML are currently diagnosed using cytomorphology and immunophenotyping. Further characterization needed for risk stratification includes detection of chromosomal aberrations using conventional immunophenotyping and specific genetic changes using molecular cytogenetics techniques (e.g. FISH or qPCR). Cytogenetic aberrations have prognostic significance in pediatric AML. They can be divided into random, and non-random abnormalities. Their presence or absence may determine stratification into risk groups and inform the choice of treatment protocol. However, in general, it is unknown why certain genetic subgroups respond better to chemotherapy than others, or whether this is related to differences in cellular drug resistance. Genetic subtypes are used to determine risk
stratification in most current treatment protocols and are part of the current WHO classification system [4]. Differences in prognosis may reflect differences in treatment, cellular drug resistance, pharmacokinetics, and potential relapse of residual disease. Response to induction therapy and cytogenetic and molecular subtypes are among the most important prognostic factors for pediatric AML [5-6]. In general, in childhood AML, favorable outcomes occur in the presence of three rearrangements: t(8;21)(q22;q22) [AML1-ETO], inv(16)(p13q22) [CBFβ-MYH11], and t(15;17)(q21;q22) [PML-RARa], and the normal karyotype. Patients who have 11q23/MLL rearrangements are stratified to the intermediate risk group. In cases with -5/del(5q), -7/del(7q), inv(3)(t(3;3)) +8, and a complex karyotype, the prognosis is unfavorable [2,5-9]. Cellular resistance in pediatric AML could have prognostic importance, e.g. cases with inv(16) show increased cytarabine sensitivity [10-11].

Chromosomal alterations can be detected in approximately 80% of de novo childhood AML cases. For the remaining 20% of pediatric AML cases, specific cytogenetic and genetic changes have not yet been identified, and the molecular abnormalities underlying leukemogenesis are still unknown. These patients are currently treated as a homogeneous group of intermediate risk. In basic cytogenetic analyses, 40-50% of AML patients show a normal karyotype; this is a clinically and molecularly heterogeneous group with gene expression changes.

Recently, a number of new molecular aberrations, including NPM1, CEBPA, and ML(-PTD), were determined for a subgroup of "cytogenetically normal" patients. The heterogeneity of pediatric AML is also illustrated by molecular aberrations detected for the other cytogenetic subgroups such as FLT3-ITD, KIT, and mutations in the pathway associated with the RAS gene [12-16].

Treatment of AML in children has limited efficacy and significant toxicity. Failures in the treatment of this cancer are the consequence of not only disease recurrence, but also the complications of intensive polychemotherapy. This indicates the need to match the therapy to both subtypes of the disease and the patient’s age [17]. Further progress is needed in terms of increasing the effectiveness of therapy while reducing its toxicity. Prognostic factors in this disease in children are less clearly defined. Currently, it is believed that the most important factors involved in long-term prognosis of childhood AML are: cytogenetic abnormalities of blasts, early response to therapy, and morphology according to the French-American-British (FAB) classification [18-20].

**Resistance to therapy**

An important issue in the treatment of AML is resistance to cytostatic drugs. Many patients do not respond to chemotherapy, while others suffer from recurrent refractory leukemia. Even with aggressive chemotherapy, complete remission is achieved in only about 60% of cases. The molecular mechanisms involved in the development of drug resistance are not fully understood. Moreover, recurrent chromosomal alterations have not yet been precisely defined in childhood AML. Therapy results in pediatric AML are different from those of acute lymphoblastic leukemia (ALL); no drug is more effective at treating AML as it is at treating ALL.

Clinical resistance to chemotherapeutic drugs is a limiting factor in the therapy of AML. Cancer cells are characterized by a kind of metabolism that allows them to survive in the presence of cytostatics. Leukemic cells have several drug resistance mechanisms. A possible cause of recurrence of acute leukemia may be differences in the susceptibility of cancer cells. Resistance to treatment is associated with poor prognosis in ALL. Both de novo drug resistance (associated with a lack of response to therapy) and that arising during the course of cytostatic treatment (a consequence of the acquisition of resistance by leukemic blasts initially sensitive to chemotherapy) are a significant clinical problem [21-22].

Resistance to therapy is a complex multi-factorial problem. So far, several mechanisms of resistance to chemotherapeutics have been identified. One of these is the overexpression of ATP-binding cassette family (ABC) transporters, which work as pumps to actively remove the drug from the cell [23-26]. Overexpression of the ABC family genes can be used as a measure of chemotherapeutic response, and thus becomes an important prognostic factor [27-28]. Multi-drug
resistance genes (e.g. BCRP, PGP and MRPs) are differentially expressed in pediatric AML, making leukemic stem cells drug resistant. Expression of the best-characterized member of this family, P-glycoprotein (MDR1, ABCB1), has been regarded as an independent adverse prognostic factor of complete remission and survival in adult patients with AML [24-26]. However, the clinical significance of P-glycoprotein is significantly lower in pediatric AML [29-30]. The genetic properties of cancer cells, whether primary or acquired during the development of leukemia, also affect the chemoresistance of leukemic blasts. Among those most frequently mentioned are: the expression of oncogenes (such as BCL-2, MYC, RAS), mutations of tumor suppressor genes (e.g. TP53), the presence of specific cytogenetic changes leading to fusions and gene rearrangements, and the polyclonality and heterogeneity of tumor cells. As well as multi-cytostatic drug-resistant resistance genes, the expression of genes involved in cell cycle regulation, DNA repair, drug metabolism, is also linked [31-34]. For example, overexpression of genes encoding anti-apoptotic proteins leads to the survival of leukemia cells by inhibition of programmed death mechanisms and thus failure of therapy [35]. However, the role of most of these genes in the evolution of resistance to various cytotoxic drugs is still poorly understood. Cellular drug resistance in AML cells seems to be similar across all other age groups, although generally speaking, the older the patient, the worse the outcome [36]. Specific cytogenetic abnormalities predict prognosis in pediatric AML. However, it is unknown why they are predictive, or whether this is related to drug resistance [11].

Previous research has demonstrated that the above-mentioned mechanisms coexist in cancer cells, and that there is cross-talk between them. However, detailed analysis of the impact of these mechanisms on treatment outcome has not led to a breakthrough in cancer therapy [37]. For example, the use of ABC family inhibitors has not provided rational benefits in the treatment of cancer [22,38].

Research focusing on the simultaneous examination of thousands of cancer cell genes has been ongoing for many years. The objective of such analyses is the selection of genes associated with cancer’s developmental course, as well as that associated with sensitivity or resistance of cancer cells to drugs [39-41]. One of the most important directions for current cancer research is to explore the processes that enable multi-drug resistance to develop; in doing so, we hope to find ways to avoid or prevent those processes from occurring, and to discover new and effective resistance gene inhibitors. One promising direction of research could be the development of new methods and diagnostic techniques that would allow monitoring of the full sensitivity state of tumor cells in individual patients, and the choice of an effective chemotherapy program. Recent studies indicate that the drug resistance profile associated with cancer cells is one of the strongest prognostic factors in acute childhood leukemias [42-43].

Genetic profiling with microarray technology

Assaying panels of transcript mRNAs can survey numerous biochemical pathways, while DNA arrays can simultaneously detect mutations, deletions or amplifications, epigenetic changes and sometimes even balanced translocations [44].

The most common technique used to determine a given gene expression profile is to monitor the levels of mRNA using microarray technology. In this way, it is possible to analyze the expression levels of many genes in one experiment - even the entire known sequence of the genome, as well as identify numerous, functionally interconnected groups of genes. Often, data concerning the selected genes are sufficient for further analysis (e.g. classification, prediction) and they can be tested with other, cheaper, faster, molecular biology methods (e.g. qRT-PCR). This technique is currently widely used in studies of molecular methods for the analysis of gene expression at the entire genome and transcriptome scale [28,45].

A second genomic technology is microarray-based comparative genomic hybridization (aCGH). aCGH measures differences in the DNA copy number between a test and reference genome. It is primarily used to map genomic copy number alterations at the submicroscopic level, thereby directly linking disease phenotypes to gene dosage alterations [46-47]. In comparison to traditional karyotyping, aCGH...
technology can help to identify smaller copy number changes, mutations, subtle deletions, and/or gene amplifications. As well as revealing the extent and complexity of copy number variation in the human genome, high-resolution aCGH has rapidly emerged as the method of choice for molecular cytogenetic detection of chromosomal abnormalities associated with complex phenotypes. Identification of gene expression profiles, which analyzes a pool of microdeletions or duplications as well as cryptic translocations, may add value when interpreted in combination with \textit{in vitro} drug resistance results.

These results indicate that aCGH could be a valuable tool for cytogenetic and genomic diagnostics in cancer. The advent of genome-wide microarray-based methods for assessing copy number changes has made it possible to search for cytogenetically invisible regions of chromosome imbalance. aCGH technology opens the door for the comprehensive identification of functional elements in the human genome, which could facilitate an understanding of sequence features affecting the drug resistance of leukemic cells. High-density aCGH has already made a significant impact on cancer cytogenetics and could be the way to identify drug resistance-related genetic aberrations that have not yet been detected by other technologies. aCGH promises the opportunity for genome-wide analysis at a level of resolution not previously achievable by conventional cytogenetics. Using cytogenetic and molecular data, along with morphology and immunophenotype data, acute myeloid leukemia can now be classified into improved, more clinically relevant categories.

**Genome and transcriptome profiling in AML**

For several years, gene expression profiling using microarrays has been a means to a better understanding of the etiology, diagnosis, prognosis and pathobiology of several cancers, including AML [48-50]. Gene expression profiles correlating characteristic (and newly identified) clinical phenotypes with specific responses to treatment have particular clinical importance. Results of molecular studies suggest the possibility for subgroup-directed chemotherapy in certain karyotypic or molecular genetic subgroups in \textit{de novo} childhood AML and may be useful in the rational design of future treatment protocols [11]. Based on gene expression profiling using microarray technology, it has been demonstrated that AML in children and adults can be accurately classified according to cytogenetic subtype [51-56]. These studies have shown that gene expression profiles determined by DNA microarrays are identical in certain genetic subtypes of AML in children and adults, suggesting a common leukemogenesis.

The current classification of AML risk groups is based on cytogenetic data and early response to therapy [3]. From a therapeutic point of view, the most important group, comprising almost half of diagnosed cases, is that of AML patients with standard risk. The main difficulty is that in this group there is large variation in cytogenetics, with many cases having a normal karyotype [57-58]. Many researchers have tried to create a new AML classification based on genetic profiles [59-61]. Gene expression profiling chiefly allows individual cases to be assigned to the relevant AML subtypes according to FAB classification [59,62]. In early studies, researchers showed that gene expression profiling of the karyotype can distinguish AML from the presence of chromosome 8 trisomy [63]. Subsequent analyses have led to the development of expression patterns for three major subtypes of AML: t(15;17)(PML-RAR), t(8;21)(AML1-ETO), and inv(16)(CBFβ-MYH11) [79]. Based on the expression pattern of a specific set of genes, the researchers were also able to predict the prognosis of a specific group of patients (cases of inv(16), t(8;21), t(15;17), mutations in CEBPA) with high accuracy [51,54-55,59,64-66]. Based on selected groups of genes, Ross et al. [54] were able to predict subtypes of AML with 93% accuracy. Such molecular classifiers were developed for the five subtypes of pediatric AML: \textit{PML-RAR}, AML1-ETO, CBFβ-MYH11, and MLL rearrangement of subtype M7 FAB classification [54]. After analyzing a diverse cytogenetic group of 285 patients, Valk et al. [55] reported the presence of 16 different gene expression patterns within AML. The study shows that the greatest diversity of AML expression profiles is found among patients with a normal karyotype, those with 11q23 rearrangement,
chromosome 8 trisomy, or monosomy of chromosome 7 \[51,55,63\].

As described in Bullinger et al. \[51\] such a method of genetic testing, combined with appropriate data analysis algorithms can lead to the identification of new subgroups of adult AML. Gene expression profiling can also help to identify patients with states often presenting the same prognostic markers for groups of standard and high risk \[67\]. Bullinger et al. \[51\] identified 133 genes whose changes in expression profile corresponded to risk groups designated by cytogenetic methods, while Yagi et al. \[68\] then used a set of 35 genes to assign pediatric patients to groups of low or high risk. The researchers pointed out that most of the identified genes were not previously recognized as prognostic markers, were not related to the FAB classification and/or known chromosome rearrangements. Expression profiles for these two age groups, developed by these research teams, were largely consistent \[51,68\]. As is clear from numerous studies, strong correlation of genetic patterns with prognosis and outcome should be the basis of reclassification \[51,55,68-69\].

The Microarray Innovations in LEukemia (MILE) study, which had a cohort of 3000 patients, confirmed that specific gene expression profiles allow the precise classification of acute and chronic leukemias \[70\]. A set of 75 gene classifiers allows accurate (99% accuracy, 100% predictive value) identification of cytogenetic and molecular subtypes of pediatric AML \[12\]. Several attempts to obtain molecular prognostic patterns for AML, have also been made, for instance, a set of 22 gene predictors for AML relapse as proposed by Ross et al \[54\]. The first approach taken was comparative genomic hybridization (CGH) of chromosomes; nearly 300 cases studied by this technique have already been reported. Two major groups (those with normal karyotypes and cases with complex karyotypes with multiple structurally altered chromosomes) are yet to be better characterized at the genetic and genomic level. aCGH-based studies have confirmed that recurrent genomic losses and gains can be found in cases with complex karyotypes. Moreover, for patients with a normal karyotype (where relapse is very low), researchers have been able to unveil small DNA copy number changes \[71\]. Similarly, in Kim et al.’s study, it was demonstrated that in cases with very complex alterations, CGH can detect true gain and loss of critical chromosome regions more accurately than conventional karyotyping, and can thus prove useful in predicting prognosis \[72\]. Expression profiling is helpful for describing new genes associated with subtypes and/or prognosis of AML. Such investigations may provide important information on the biology of the disease. This knowledge is needed for the rational development and optimization of therapeutic protocols. Previous studies, however, have not specified a transcriptional background of resistance to therapy, or a lack of AML cell sensitivity to various drugs used in chemotherapy.

Chromosomal changes identified in AML are associated with clinical, morphological and immunophenotypic specificity for a particular AML subtype. Specific cytogenetic AML subgroups may show different resistance profiles. Classification according to the gene expression profile may reduce the number of cases in which conducting multiple simultaneous diagnostic procedures (cytomorphology, FISH, RT-PCR, karyotyping) is required. Importantly, gene expression profiles may provide a better insight into the biology and pathophysiology of different AML subtypes, which may then provide new and/or more effective treatments. DNA- or RNA-level profiling are widely considered to be the most powerful tools for predicting AML cell behavior in response to therapy. Results could not only help to identify important drug response-specific genetic alterations, but could also provide a way to monitor acute leukemia in response to drugs used in therapy, which in turn could predict the occurrence of relapse. Expression profiling does not currently have a role in monitoring residual disease; however, this does not exclude a role for gene expression profiles in monitoring response to treatment.

**Conclusions**

Microarray technology raises hopes of understanding the complex, heterogeneous genome and transcriptome of leukemia cells such that the development of novel therapies and assays to predict and track therapy efficacy may be driven forwards.
Although microarray technology is often used to explore the genetics of leukemia cells, relatively few studies have been conducted for pediatric AML. Most research focuses on the potential modernization of the existing classification, the search for new prognostic factors, and the evaluation of prognosis. Very few studies have been carried out to assess genetic resistance to specific cytostatic drugs in pediatric AML.

The value of genetics was reinforced in the revised 2008 World Health Organization AML classification scheme, which took into account various analytic procedures (karyotype, FISH, RT-PCR, DNA sequencing, and microarray technology) [44]. New biomarkers and pharmacogenetic tests are emerging. Better understanding of the biology, pathogenesis and response to therapy of leukemic cells is essential to AML prevention, as is the need to design novel treatment protocols that are personalized to the genotype.

The identification of specific cytogenetic abnormalities (genomic profile) and gene expression profiles (transcriptomic profile) can be powerful predictors of prognosis and response to therapy.

References


