Expression profiling of leukemia patients: Key lessons and future directions

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Gene expression profiling (GEP) is a well-established indispensable tool used to study hematologic malignancies, including leukemias. Here, we summarize the insights into the molecular basis of leukemias obtained by means of GEP, focusing especially on acute myeloid leukemia (AML), one of the first diseases to be extensively studied by GEP. Profiling mRNA and microRNA expression are discussed in view of their applicability to class prediction, class discovery, and comparison, as well as outcome prediction, and special attention is paid to the recent advances in our understanding of the role of alternative RNA splicing in AML. In addition to microarray-based GEP approaches, over the last few years RNA sequencing based on next-generation sequencing technology is gaining wider recognition as an advanced tool for transcriptome profiling. Therefore, the advantages of RNA sequencing-based GEP and its current and potential implications in AML are discussed. Finally, we also highlight recent efforts to integrate already available and newly acquired omics data sets so that a more precise understanding of AML biology and clinical behavior can be achieved, which ultimately will contribute to further refine leukemia management.

At the end of the 20th century a seminal study by Todd Golub and colleagues \cite{1} demonstrated the usefulness of a new microarray-based technology for gene expression profiling (GEP) that for the first time allowed the comprehensive study of human acute leukemias. For the next decade, GEP became an invaluable research tool in the field of hematology, provided crucial insights in the biology of hematologic malignancies, and led to some clinical implications. In the last several years, the newly emerging next-generation sequencing (NGS) techniques have shifted the research focus to a genomic level. As the leukemia transcriptome is the immediate outcome of various genetic and epigenetic abnormalities \cite{2}, its continued precise investigation will remain an indispensable prerequisite for understanding hematologic malignancies, especially leukemias, at a more individual level. In that respect, the rapidly developing NGS platforms also provide the basis for novel approaches to quantitative assessment of the human transcriptome and the possibilities for integration of several levels of molecular profiling. In this review, we aim to briefly recapitulate the last 15 years of experience in transcriptome profiling. This is done using the prime example of acute myeloid leukemia, which was one of the first cancers to be extensively studied by GEP and, later, the first to be analyzed by NGS \cite{3}. We also discuss the future prospects for transcriptome analysis in the field.

**Gene expression profiling in acute myeloid leukemia: Key findings**

In their seminal work, Golub et al. used supervised gene expression profiling (GEP) analysis to successfully discriminate between acute myeloid leukemia (AML) and acute lymphoblastic leukemia (ALL) samples and developed a gene expression-based predictor that assigned new leukemia cases to each of the two leukemia types with high accuracy \cite{1}. In principle, this study suggested three main implications of GEP in the study of acute leukemia, and cancer in general: (i) class discovery, (ii) class prediction and class comparison, as well as later studies adding clinically oriented implications such as (iii) outcome prediction. Although several recent reviews provide an in-depth discussion of the main GEP studies in AML \cite{4–7}, here we briefly
summarize some of the key findings and conclusions obtained over the last decade.

Class discovery in AML through mRNA profiling
Several GEP studies in adult AML patients were able to distinguish new subgroups based on characteristic gene expression patterns, some of which are exemplified in this section. For example, two novel subgroups within the cytogenetically normal (CN) AML cases could be identified that were in part associated with FLT3-ITD mutations or myelomonocytic/monocytic differentiation, as defined by the French–American–British (FAB) classification, respectively [8]. Similarly, core binding factor (CBF) leukemias could be subdivided based on GEP analysis [8,9], and it was later found that a distinct gene expression profile in CBF-AML correlates also with the presence of KIT mutations [10]. In accordance, a pivotal study analyzing 285 AML cases reported 16 GEP-defined subgroups, which comprised novel leukemia subgroups that were also associated with different outcomes [11]. In line, Wilson and colleagues found novel clinically meaningful leukemia subgroups within older AML patients [12]. After these observations, the first studies also started to dig further into the molecular mechanisms underlying novel GEP-defined subtypes of AML, which, for example, can reflect a specific epigenetic abnormality, as illustrated by the epigenetic silencing of CEBPA [13]. Thus, all of the aforementioned studies clearly illustrated the power of GEP to discover novel leukemia classes of clinical importance. Furthermore, these early analyses suggested that GEP was a valuable tool with which to define known tumor classes and to gain further insights via the respective predictive gene signatures.

Class prediction and class comparison in AML through mRNA profiling
Association of gene expression profiles with specific cytogenetically defined subtypes of AML, such as AML with t(15;17), inv(16), t(8;21), and t(11q23) has been addressed by all major studies because such associations, on one hand, confirm the true biological difference between these entities and, on the other hand, might be very useful in clinical settings if novel surrogate biomarkers are identified. In accordance, all major studies identified gene expression profiles associated with the cytogenetically defined adult AML subtypes, which also exhibited very strong concordance [5,6]. To predict the respective subgroups, a large international multicenter research program, the Microarray Innovations in Leukemia (MILE) Study, sought to identify biomarkers based on GEP in 2143 patients with leukemia and myeloid dysplasia [14]. The retrospective phase I of this study included 542 AML cases and classified, with a specificity of >99%, all subtypes of leukemia. The second prospective phase II of the study with 1152 cases comprising a large cohort of AMLs also reached that high specificity level to distinguish between AML with complex aberrant karyotype, CN-AML/AML with other abnormalities, t(15;17), inv(16), t(8;21), and t(11q23) [14].

Notably, several studies reported that homeobox genes (such as HOXA5, HOXA7, HOXA9, HOXA10, and also MEIS1) are frequently overexpressed in MLL-rearranged leukemias, but can also be found in CN-AMLS and in cases with trisomy 8 [15–17]. Later, it could be illustrated that this signature is frequently associated with NPM1 mutations, which also harbor a distinct gene and microRNA (miRNA) expression signature [18]. More recent studies also identified specific profiles in CN-AML cases defined by mutations in different genes such as CEBPA [13,19], FLT3 [20], IDH2 [21], TET2 [22,23], ASXL1 [24], and RUNX1 [25]. However, these efforts also indicated that not all aberrations tend to have strong profiles, which seem to be seen mainly in known “founder” mutations such as CEBPA and NPM1. Notably, the presence of CEBPA and NPM1 mutations can be reliably predicted, even within multicenter GEP studies [26]. Importantly, driver mutation-associated signatures can capture unknown molecular aberrations associated with identical phenotypes and patient outcome. In line, a FLT3-ITD gene expression profile performed better than the FLT3-ITD mutational status in terms of overall survival (OS) prediction [20].

Outcome prediction in AML through mRNA profiling
Besides the identification of distinct leukemia subgroups and the prediction of known aberrations, the prediction of outcome based on GEP is also of obvious clinical relevance, especially in a disease such as AML, in which even “well-defined” cytogenetic subgroups exhibit considerable heterogeneity, at both the molecular and clinical levels. A first study examining the feasibility of outcome prediction in AML stratified AML cases into two outcome groups based on the differential expression of 149 probes [16]. The prognostic power of this gene signature was confirmed in an independent study of CN-AML cases [27]. More recent studies could further narrow down the number of genes needed to delineate groups of prognostic relevance [28], and a meta-analysis including data from five different studies identified a prognostic score signature consisting of only 24 genes [29]. In accordance, GEP could potentially predict outcome in childhood myeloid leukemia as well. For example, Lacayo et al. were able to subdivide pediatric AML cases with FLT3-ITD into prognostic subgroups based on GEP [30], and Bresolin et al. identified an AML-like signature associated with poor prognosis in juvenile myelomonocytic leukemia [31].

Another recent key study indicated that AML cellular subpopulations enriched for leukemia stem cells (LSCs) have a distinct gene expression signature, which shares core “stemness” features with profiles of hematopoietic stem cells (HSCs) [32]. Notably, both signatures were predictive of patient survival. In an independent study,
Metzeler et al. [33] reported that the LSC-like gene expression profile was associated with clinical outcomes of CN-AML patients. Furthermore, this LSC signature was also associated with a specific microRNA expression profile. These studies support the LSC theory for AML pathogenesis and indicate that true biological features at the cellular and transcriptomic levels are of particular relevance to the clinical outcome.

Initially, a major criticism of the application of GEP in AML in clinical settings was the poor reproducibility of gene expression findings in early studies, which was due to poorly designed studies, platform differences, and overfitting of the data. However, over the years, investigators have learned how to use GEP, and since the early days, it has become a very reliable tool to measure gene expression differences in leukemias and cancer in general. This is reflected by a growing number of multicenter clinical trials and meta-analyses, some of which were mentioned above [14,26,29], and a very recent meta-analysis of 25 studies indicated a high level of concordance between independently defined gene expression profiles of prognostic relevance [34].

**Alternative splicing in AML as revealed by microarray technologies**

In the last 2 years, several groups identified coding mutations in the genes involved in the RNA splicing processes [35,36]. Notably, these mutations appeared to be a common feature of many myeloid malignancies including AML [37,38]. Although it has been known for many years that alternative splicing is affecting some leukemia-associated genes, such as WT1 [39], the recent identification of splicingosomal machinery mutations spurred interest in the evaluation of the presence and general features of alternative splicing events in AML, as this might be a significant source of phenotypic variation and may have a direct role in leukemia pathogenesis. Some microarray platforms such as exon arrays and high-density tiling arrays provide the tools needed to address this issue. Very recently, Adamia et al. reported a genome-wide profiling of alternative splicing in adult AML patients [40]. They found that 29% of expressed transcripts were differentially spliced in AML patients compared with normal donors at a significance level of 0.05. They found a high rate of partial or complete intron retention events, which is surprising because intron retention is the least frequent alternative splicing event in mammals [41]. Also of note, up to 21% of the differential splicing events mapped to 5’ and 3’ untranslated regions, suggesting a high likelihood for affected posttranscriptional regulation of these genes. More than half of the identified alternatively spliced variants had not been reported before, and a large number of alternatively spliced oncogenes (RUNXI, PML, BRAF, FOS, JUN) and tumor suppressor genes (ATM, TP53) were identified. Furthermore, NOTCH2 missplicing and FLT3 gene missplicing were found to be common events in AML cases, pointing to new potential targets [42]. Overall these studies nicely illustrate the importance of investigating alternative splicing further in AML. However, the usefulness of microarray-based approaches in identifying novel alternative splicing events is limited and requires functional investigation. But obviously, the novel NGS-based approach to the study of alternative splicing events on a genome-wide level is a promising field for future research, as has recently been found for U2AF1 mutated cases [43,44]. In myeloid malignancies (including AML), it was reported that U2AF1 mutated cases, wild-type cases with lower U2AF1 expression, and wild-type cases with normal U2AF1 expression levels had distinct patterns of alternative splicing events [44]. Similarly, another recent study reported an impact of U2AF1 mutations on alternative splicing in lung cancer and AML [43] by identifying 30 splicing events common to the two malignancies and by verifying a causative effect of U2AF1 mutations on the respective splicing pattern.

**The noncoding transcriptome**

A key breakthrough in our knowledge of the molecular biology of the eukaryotic cell during the last decade was the recognition that the protein-coding transcriptome comprises only a small proportion of the entire RNA content of the cell [45]. In accordance, most of the noncoding RNAs appeared to be of functional relevance as they exert modulation of the expression and translation of the coding mRNAs through a number of mechanisms. The best-studied noncoding RNA species are the miRNAs. Mature miRNAs are single stranded RNAs of about 22 nucleotides of length that are processed endogenously through processing of pre-miRNA transcribed from polymerase II-dependent loci [46]. MicroRNAs are complementary to short stretches of the 3’ untranslated regions of the coding mRNAs and suppress their translation or trigger their degradation [47]. So far, more than 2500 well-annotated human miRNAs have been identified, some of which were experimentally verified as participating in important mRNA–miRNA regulatory networks in hematopoiesis [48]. MicroRNA expression profiling was also used to study AML for the same purposes as the mRNA expression profiling described above. Here we focus on key findings only because more extensive reviews were published recently [49–51].

**Class discovery in AML through microRNA expression profiling**

A landmark study by Lu et al. made use of a custom bead-based suspension array to analyze the miRNA expression profiles of more than 334 cancer samples [52]. Notably, miRNA expression profiles correctly classified cancer types including AML and ALL samples. In a later study, Li and
colleagues reported discrimination between AML and ALL samples based on the expression of 28 miRNAs, and 4 of these were the most discriminatory [53]. Using a multiplex quantitative polymerase chain reaction platform, Jongen-Lavrencic et al. profiled the expression of 178 miRNAs in a total of 215 adult AML patients [54]. They were able to identify 22 clusters based on differential miRNA expression profiles, many of which correlated well with known cytogenetic abnormalities.

Class prediction and class comparison in AML through microRNA expression profiling

Most of the studies on miRNA expression profiling in AML were focused on the identification of expression signatures associated with different AML subtypes. Notably, studies from different groups exhibited only partial overlap, mostly because of differences in microarray platforms, but nevertheless, common signatures could be identified. For instance, AML with t(15;17) is characterized by expression of miR-127, miR-299, miR-323, miR-368, and miR-382 and downregulation of miR-17 and miR-126 [54,55]. Other studies focused primarily on the identification of specific miRNA expression signatures in CN-AMLs with specific molecular features [56]. CN-AMLs with NPM1 mutations miRNA expression signatures in CN-AMLs with specific molecular subgroups of AML are summarized in Table 1. In pediatric AML, several studies also reported that miRNA expression profiling correlated well with cytogenetically defined subgroups and could distinguish between AML and ALL cases [65].

Outcome prediction in AML through microRNA expression profiling

In the last decade it became clear that miRNA gene expression profiling, similar to mRNA-based GEP, can also add prognostic information in AML. Much of the work in this respect was done on CN-AML cases, most of which are expression of miR-204, miR-128, miR-126, miR-130a, and miR-451 [56,57]. Although FLT3-ITD mutations were found to be associated with overexpression of miR-155 [58], the more favorable CEBPA mutation genotype was characterized by miR-181a and miR-335 expression and downregulation of miR-34a [59,60]. Other molecular aberrations with specific miRNA expression profiles were IDH2 [21], TET2 [22], and RUNX1 [61] mutations, as well as DNMT3A mutations [62], whereas other mutations of epigenetic modifiers, like ASXL1 mutations, did not exhibit a characteristic miRNA expression signature [24]. Notably, deregulation of leukemia-relevant genes such as overexpression of MNI [63], BAALC [64], and ERG [64] was also found to be associated with distinct miRNA patterns. The miRNA signatures associated with the main molecular/cytogenetic subgroups of AML are summarized in Table 1. In pediatric AML, several studies also reported that miRNA expression profiling correlated well with cytogenetically defined subgroups and could distinguish between AML and ALL cases [65].

Table 1. Characteristic microRNA expression signatures in different adult acute myeloid leukemia subtypes

<table>
<thead>
<tr>
<th>Genetic/molecular subtype</th>
<th>Upregulated</th>
<th>Downregulated</th>
<th>Study</th>
</tr>
</thead>
<tbody>
<tr>
<td>t(15;17)</td>
<td>miR-193b/379/382/4855p/134/376a/2995p/452/127/424/370/170/323/125b/154/424/181a,b/d</td>
<td>miR-96a,b/151/10b/let-7c</td>
<td>[54]</td>
</tr>
<tr>
<td>NPM1 mutated</td>
<td>miR-127/175/154*/299/323/368/370</td>
<td>miR-173p/185/187/194/200a,b,c/330/339</td>
<td>[55]</td>
</tr>
<tr>
<td>FLT3-ITD mutated</td>
<td>miR-126*</td>
<td>miR-126*/150/17-5p/20a/422b/10a/124a</td>
<td>[55]</td>
</tr>
<tr>
<td>inv(16)</td>
<td>miR-126*</td>
<td>miR-126*/150/17-5p/20a/422b/10a/124a</td>
<td>[55]</td>
</tr>
<tr>
<td>11q23 rearrangements</td>
<td>miR-326/219/194/301/324/339/99b/328</td>
<td>miR-34b/15/29a/29c/37/30/39/b/99/10a/b/196a,b/133a</td>
<td>[54]</td>
</tr>
<tr>
<td>Cytogenetically normal AML</td>
<td>miR-196b/17-3p,5p/18a/19a,b/20a/92/93/10a,b/124a</td>
<td>miR-126b/220/295</td>
<td>[53]</td>
</tr>
<tr>
<td>t(8;21)</td>
<td>miR-124a/30d/337/18/302/b/105/157/d/153/215/1/194</td>
<td>miR-126b/120/597</td>
<td>[58]</td>
</tr>
<tr>
<td>CEBPA mutated</td>
<td>miR-30b/126/126*/30a/30b/146a/146/199a/363</td>
<td>miR-223/99a/100/let-7f</td>
<td>[61]</td>
</tr>
<tr>
<td>RUNX1 mutated</td>
<td>miR-221/220</td>
<td>miR-223/99a/100/let-7f</td>
<td>[61]</td>
</tr>
<tr>
<td>ASXL1 mutated</td>
<td>None</td>
<td>None</td>
<td>[24]</td>
</tr>
<tr>
<td>DNMT3A mutated</td>
<td>miR-10a*</td>
<td>miR-30c-1/181/504/365/2410/626/640</td>
<td>[62]</td>
</tr>
<tr>
<td>IDH2 R172 mutated</td>
<td>miR-131a/125b/125a/5p/421/374a/361-5p/26a/30d</td>
<td>miR-7/345/129-5p/632/615-5p/130/163/548/b/520a-3p/526a/194-1</td>
<td>[21]</td>
</tr>
<tr>
<td>TET2 mutated</td>
<td>miR-148a, 148b, 24, 640, 107</td>
<td>miR-135a/186</td>
<td>[22]</td>
</tr>
</tbody>
</table>

Adapted from Havelange et al. [49], Wieser et al. [50], and Marcucci et al. [51].

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MicroRNA as circulating biomarkers in AML

Recently, it was recognized that, because of their small size, miRNAs might be less amenable to degradation in body fluids (e.g., plasma) than mRNAs [68], and indeed, miRNAs are stable in human serum even after long-term storage for up to 10 years [69]. Several studies have addressed the feasibility of miRNA expression profiling in serum/plasma samples derived from AML patients [70]. Tanaka et al. reported that miR-638 was stably present in human AML plasma, whereas miR-92 was downregulated in plasma of AML patients [71]. Therefore, the expression ratio miR-92a/miR-638 could be valuable for diagnostic purposes. Similarly, Fayyad-Kazan et al. found that let-7b and miR-523 were upregulated, whereas let-7d, miR-150, miR-339, and miR-342 were downregulated, in AML patients compared with normal controls [70]. Furthermore, the combination of the expression of miR-150 and miR-342 exhibited good discriminatory power between AML cases and normal controls. Finally, Zhi et al. reported that miR-93, miR-129, miR-155, and miR-320 were upregulated in all AML subtypes compared with normal controls, and the level of expression of miR-181b in serum appeared to be of prognostic relevance [72]. Obviously, these first studies on the clinical relevance of circulating miRNAs in AML patients require independent verification in larger cohorts (and, ideally, in a prospective fashion), but nevertheless they indicate future directions for “liquid biopsies” that will play a role in the management of not only leukemia, but also solid tumors.

Gene expression profiling in AML in the era of deep sequencing

Like for microarray technology, leukemia researchers were also pioneering the application of whole-genome sequencing to study human cancer. After the first sequencing of an entire tumor genome, an AML genome, in 2009 [78], we are witnessing a large number of studies identifying various previously unknown somatic mutations in AML and cancer in general. This chasm after the
disease-causing mutations, however, is not sufficient to explain the leukemogenic process in its entirety [2]. Fortunately, massively parallel sequencing has also been adapted to sequence cDNA libraries derived after reverse transcription from various RNA sources (poly(A) RNAs, size-fractionated RNAs, ribosomal RNA-depleted RNA, etc.), a technique usually referred to as RNA sequencing (RNA-Seq) [79,80]. The advantages of RNA-Seq, compared with standard hybridization-based microarray methods, are the ability to identify unknown transcripts as well as transcripts not present in the reference genome (e.g., fusion transcripts, viral sequences), short- or long-range splicing events, and sequence variations at the RNA level (e.g., RNA editing-associated single-nucleotide variations). RNA-Seq can achieve very high coverage of the reference genome (80–90%) at a relatively affordable cost based on the depth of sequencing (e.g., several million uniquely mapped reads can yield coverage of 80%).

RNA sequencing is very suitable for digital quantification of RNA expression based on the number of reads per kilobase per million reads (RPKM), with a very high dynamic range of almost ten-thousandfold. However, today, RNA-Seq still poses some hurdles with respect to technical variation based on library preparation and bioinformatic analysis. Although library preparation is easier for short RNA species (miRNAs, piRNAs, etc.), for mRNAs, it requires additional steps of fragmentation, which may confer bias toward specific regions. Similarly, bioinformatic challenges are due to the requirements to process, store, and analyze large amounts of data and to align multiple short reads to unique genomic regions, as well as to account for sequence variants. Detailed discussion of these issues is beyond the scope of the present review, but continuous efforts in this field will make RNA-Seq the future GEP technology. On the other hand, large microarray-based data sets are already available. Thus, it will be crucial to integrate gene expression data from various sources including RNA-Seq experiments. In that respect, the first bioinformatic tools have already been developed and are going to address the integration of gene expression data [81].

Integration of gene expression profiling with other omics data

The most prominent example illustrating the power of a combined analysis of several omics data set layers stems also from the analysis of AML cases. As part of The Cancer Genome Atlas project [82] 200 adult AML cases were studied by whole-genome and whole-exome sequencing, and these data sets were integrated with microarray-based GEP data, mRNA and microRNA expression RNA-Seq data, microarray-based DNA methylation data, and single-nucleotide polymorphism microarray profiling data. This pivotal integrative study provided many insights in addition to capturing the genomic landscape of AML. For example, as RNA-Seq data provide a single-base resolution of the expressed transcripts, the RNA-Seq analysis in The Cancer Genome Atlas study revealed increased or exclusive expression of the mutant allele for several genes such as DNMT3A, RUNX1, PHF6, and TP53, that was most commonly caused by loss of heterozygosity or partial uniparental disomy. The unsupervised clustering analysis of the data derived from RNA-Seq allowed identification of seven RNA-Seq groups and five miRNA sequencing groups, which, for example, were associated with leukemia differentiation and with mutations of NPM1, DNMT3A, FLT3, and cohesin complex genes. Furthermore, univariate analysis indicated that these mRNA/miRNA expression-defined groups correlated with overall survival, which is in line with previous GEP reports [8,11]. In addition, the comparison of the clustering based on RNA-Seq with microarray data indicated a high level of concordance between the platforms, further supporting the possible integration of these two technologies.

Furthermore, The Cancer Genome Atlas consortium analyzed the correlation of the mRNA-Seq- and miRNA-Seq-defined leukemia subgroups with DNA methylation profiles and found that distinct methylation patterns are associated with distinct gene expression groups. This is in accordance with previous studies, which could indicate that DNA methylation signatures integrated with gene expression data can identify biologically distinct subtypes in AML [83], and that an integration of findings can improve outcome prediction [84]. Similarly, a very recent study by Marcucci et al. integrated DNA methylation profiling with GEP and microRNA expression profiling data to develop a prognostic score for CN-AML patients [85]. In a training set of 134 AML patients, they identified 7 genes (CD34, RHOC, SCRN1, F2RL1, FAM92A1, MIR155HG, and VW8), whose differentially methylated promoter regions correlated with outcome. The expression levels of these seven genes also correlated with prognosis: low expression (and higher methylation level) was associated with a better overall survival. The weighted summary score was then validated in four independent data sets, where its low levels were always associated with a better 3-year overall survival [85].

Overall, these studies point the way to integrate multiple omics data to gain additional insights into leukemia, thereby defining biologically more meaningful disease subtypes. Although of course further studies will have to elucidate the biological processes affected by these integrated molecular profiles and although their precise role in the leukemogenic process needs to be determined, these first integrative studies are nevertheless pioneering improved patient management in the future. In addition, the development of public platforms for integrative exploration of genome-wide molecular data offers novel perspectives. For example, the Leukemia Gene Atlas (LGA) provides a public platform that supports the research and analysis of
thousands of published GEP, DNA methylation, single-nucleotide polymorphism and NGS profiling data sets derived from leukemia and normal hematopoiesis samples [86]. Similarly, the Gene Set Control Analysis (GSCA) integrates publicly available chromatin immunoprecipitation with high-throughput sequencing data sets to identify likely combinatorial transcriptional control mechanisms, thereby complementing Gene Ontology and Gene Set Enrichment analyses to analyze gene sets of interest [87].

**Gene expression profiling at a single-cell level**

Another paradigm shift in our understanding of AML biology during the last decade was the recognition that at the individual level, the leukemic cell population comprises a mixture of hierarchically organized subclones that may evolve differently over time, especially on interaction with treatment [88–91]. Although recent advances in cellular and molecular techniques allow for interrogation of the clonal heterogeneity of AML, several techniques now also exist that can be used to shed light on the individual AML gene expression phenotype at the single-cell level. These include, for example, multiparameter flow cytometry, mass cytometry, single-cell sequencing, single-cell polymerase chain reaction for selected transcripts, single-cell RNA-Seq, and matrix-assisted laser desorption/ionization time of flight imaging [92]. In line, two very recent articles reported the applicability of single-cell expression profiling through multiplex high-throughput technologies to elucidate the hierarchy of early stages of normal hematopoiesis [93,94], and soon these tools will also be applied to study the hierarchy and clonal evolution of various AML subtypes at a single-cell level.

**Conclusions**

During the last 15 years, gene expression profiling has become an invaluable platform to investigate the coding and noncoding transcriptomes of AML (Fig. 1). A number of studies have identified novel subclasses of AML and characteristic gene and miRNA expression signatures associated with known AML subtypes. These signatures also contributed to the experimental elucidation of complex signaling and regulatory networks in AML. Gene and miRNA expression profiling have been also useful in the development of prognostic scores with relevance to clinical management of AML. However, we are just in the prelude of a novel boom of transcriptome profiling in AML because of the emerging RNA-Seq technologies. Important insights will be derived from the integration of multiple omics data, even at a single-cell level. Undoubtedly, the integration of genomic and RNA-Seq data will further the development of novel prognosis stratification schemes in AML, although this effort will highly depend on the quality of the patient sample annotation data, which optimally should stem from controlled clinical trials. Then, the great hope for the future is that advances in AML transcriptomics will support the development of effective therapeutic strategies for most AML subtypes, a challenge not conquered in the last decades.

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