Chapter 1
Acute Myeloid Leukemia with Recurrent Genetic Abnormalities: Part I Cytogenetic Abnormalities

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Introduction

Recurrent cytogenetic abnormalities have been used to subtype AML for many years, providing important prognostic information, as well as identifying potential molecular targets to guide therapy. The advent of new technology has generated abundant data, revealing a large number of genetic abnormalities existing in different AML cases. These findings provide ever-increasing evidence for subtyping in AML and include, but are not limited to, t(15;17)(q24.1;q21.2);*PML-RARA*, t(8;21)(q22;q22);*RUNX1-RUNXT1*, inv(16)(p13q22);*CBFB-MYH11*, t(9;11)(p22;q23);*KMT2A-MLLT3*, t(6;9)(p23;q34);*DEK-NUP214*,inv(3)(q21;q26.2);*RPN1-MECOM*,andt(1;22)(p13;q13);*RBM15-MKL1* [1–3] (please see Table 1.1 for a summary). Another example is the *BCR-ABL1* translocation, the disease-defining genetic alteration in chronic myeloid leukemia (CML), which has also been described in acute lymphocytic leukemia (ALL) and was later reported in AML. Now, the evidence indicates that de novo AML with a *BCR-ABL1* translocation should be considered as a provisional category of AML [2]. Many other translocations and inversions have been found in AML, which are sometimes recurring. These recurring cytogenetic abnormalities are less frequent, more often seen in pediatric patients with uncertain prognostic or therapeutic significance. AML with such cytogenetic abnormalities are not included in this category at this time [2]. Some translocations and inversions are seen in therapy-related myeloid neoplasms and are also excluded from this category [1]. Typically, a recurrent cytogenetic abnormality will create a fusion gene encoding a chimeric protein, with one exception that has been recognized: AML with inv(3)(q21.3;q26.2) [2, 4, 5]. This chapter will describe the recurrent genetic abnormalities in AML.

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C.-C. Chang, R.S. Ohgami (eds.), *Precision Molecular Pathology of Myeloid Neoplasms*, Molecular Pathology Library, DOI 10.1007/978-3-319-62146-3_1

Table 1.1 Recurrent cytogenetic abnormalities in AML

AML subtype	Cytogenetic abnormality	Molecular background	Clinical implications
AML with t(15;17) (q24.1;q21.2)	Forming PML-RARA fusion gene	PML-RARA may repress differentiation and apoptosis	Sensitive to all-trans-retinoic acid (ATRA) treatment
AML with t(8;21) (q22;q22)	Forming RUNX1-RUNXT1 fusion gene	RUNX1-RUNXT1 may inhibit transcription	Associated with favorable prognosis
AML with inv(16) (p13.1q22) or t(16;16) (p13.1;q22)	Forming CBFB-MYH11 fusion gene	CBFB-MYH11 may inhibit transcription	Associated with favorable prognosis
AML with t(9;11) (p22;q23)	Forming MLLT-MLL (KMT2A) fusion gene	MLLT-MLL may affect gene expression	Associated with unfavorable prognosis
AML with t(6;9) (p23;q34)	Forming DEK-NUP214 fusion gene	DEK-NUP214 may increase translation	Associated with unfavorable prognosis
AML with inv(3) (q21q26.2) or t(3;3) (q21;q26.2)	Bringing a GATA2 distal hematopoietic enhancer (G2DHE) to the vicinity of the EVI1 gene	Causing aberrant EVI1 expression, GATA2 haploinsufficiency, and transcription inhibition	Associated with unfavorable prognosis
AML with t(1;22) (p13;q13)	Forming RBM15-MKL1 fusion gene	RBM15-MKL1 may alter epigenetic regulation	Associated with unfavorable prognosis
AML with t(9;22) (q34;q11.2)	Forming a BCR-ABL1 fusion gene	BCR-ABL1 may increase cell proliferation	May benefit from TKI therapy

Acute Myeloid Leukemia with t(15;17)(q24.1;q21.2);*PML-RARA*

AML with *PML-RARA* is also called acute promyelocytic leukemia (APL) and comprises 5–8% of all AML cases. The key cytogenetic abnormality is formation of the *PML-RARA* fusion gene, most commonly caused by a t(15;17)(q24.1;q21.2), although cryptic and variant translocations also account for a minority of cases [2].

APL presents with myeloblasts and abnormal promyelocytes (which are considered blast equivalents) in the bone marrow and peripheral blood. On occasion, the blast count may be less than 20%, but the presence of a *PML-RARA* is sufficient for the diagnosis of APL. There are two morphologic variants of APL: the hypergranular (classic) and the microgranular variant. The abnormal promyelocytes in the hypergranular variant of APL typically show indented or bilobated "butterfly-shaped" nuclei and intense azurophilic granulation. Single or bundles of Auer rods may also be appreciated (Fig. 1.1). This contrasts with the microgranular variant of APL, in which the abnormal promyelocytes have very small and indistinct azurophilic granules and show

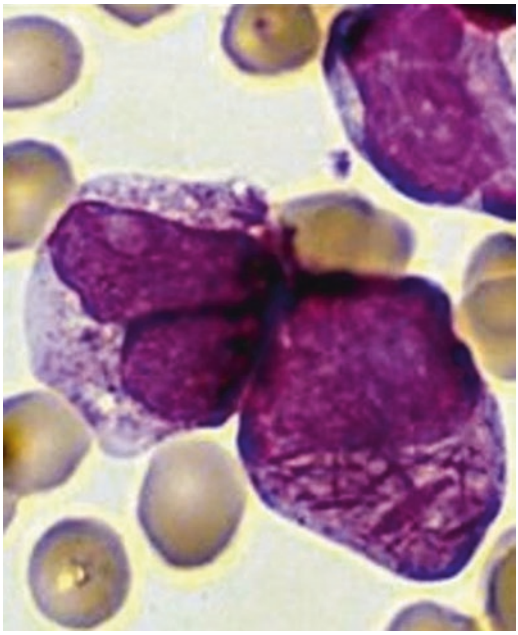

Fig. 1.1 Acute promyelocytic leukemia, hypergranular variant showing prominent azurophilic granules bilobed nuclei and bundles of Auer rods

predominantly bilobed nuclei. The blast/blast equivalent count in the microgranular variant of APL also tends to be higher than in the hypergranular variant of APL. The azurophilic granules in both types are positive for myeloperoxidase (MPO) [1].

The flow immunophenotype of APL is also characteristic and often aids in the diagnosis, especially in cases in which the morphologic features are less developed. The hypergranular variant of APL is classically CD34 and HLA-DR negative, shows high side scatter (where granulocytes typically are seen) and bright MPO, and will often show dim CD64 expression, without CD16 expression. The microgranular variant of APL, on the other hand, frequently expresses CD34 and occasionally HLA-DR (usually subset of neoplastic cells and week), shows a lower side scatter than the hypergranular variant of APL (residing in the normal blast area on the CD45/SSC plots), and frequently shows dim CD2 expression.

Cytogenetic Abnormality

At the genome level, APL with *PML-RARA* is characterized by a t(15;17)(q24.1;q21.2) translocation (Fig. 1.2) [1]. The breakpoint in the *RARA* gene is within intron 2. The breakpoints in the *PML* gene can occur at three different locations resulting in different sized *PML-RARA* transcripts. Breakpoint 1 (BCR1) at intron 6 will result in a long transcript; breakpoint 2 (BCR2) at exon 6 will result in variable transcripts, which can be of different sizes in different patients; and

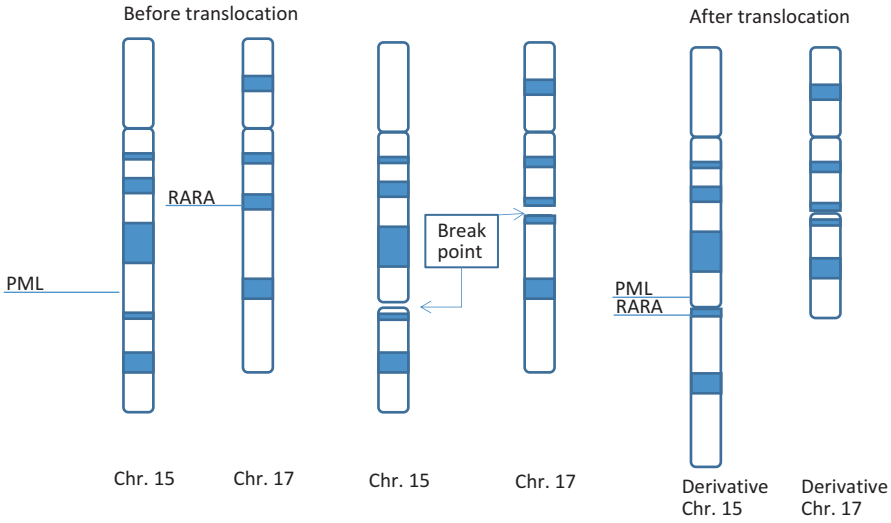

Fig. 1.2 APL with PML-RARA is characterized by a t(15;17)(q24.1;q21.2) translocation

breakpoint 3 (BCR3) at intron 3 will result in short transcript [6, 7]. As a result, a nuclear regulatory gene (promyelocytic leukemia or *PML* gene) on 15q24.1 is translocated to the vicinity of the retinoic acid receptor alpha gene on 17q21.2, forming a *PML-RARA* fusion gene.

The translocation can be detected by karyotyping and FISH assay. Different FISH assay designs have been used. One assay is called dual-color dual-fusion fluorescence FISH assay [8]. In this assay, two probes are designed to hybridize to *PML* at 15q24 and *RARA* at 17q21, respectively, overlapping the breakpoints on each chromosome (Fig. 1.3). The two probes can be labeled with different fluorescent dyes. For example, the probe specific for *PML* at 15q24 can be labeled with red fluorescent dye and the probe for *RARA* at 17q21 green. A normal cell will show two red and two green dots (Fig. 1.3). A cell with t(15;17)(q24.1;q21.2) will show one red, one green, and two yellow dots (Fig. 1.3). This assay works very well in detecting t(15;17)(q24.1;q21.2) *PML-RARA* translocation. This translocation is the most common change found in APL with *PML-RARA*. However, the *RARA* gene has been found to have other translocation partner genes in some cases. Such translocations are called variant *RARA* translocations. These variants include t(5;17)(q35;q21) *NPM1-RAR*A [9], t(11;17)(q23;q21) *PLZF* (also known as *ZBTB16)-RARA* [10, 11], t(11;17)(q13;q21) *NUMA-RARA* [12], t(4;17)(q12;q21) *FIP1L1-RARA* [13, 14], t(2;17)(q32;q21) *OBFC2A-RARA* [15], t(7;17)(q11;q21) *GTF2I-RARA* [16], t(1;**17**)(q42;q21) *IRF2BP2-RARA* [17, 18], der(17) with duplication of 17q21.3-q23 *STAT5b-RARA* [19], and der(17) *PRKAR1A-RARA* with del(17)(q21) [20]. Generally speaking, the dual-color dual-fusion FISH assay does not work well for these variants. Therefore, a *RARA* dual-color break-apart FISH assay has been designed to address this issue. This assay uses dual-color probe, red and green. The red part of the probe hybridizes to *RARA* centromeric to the breaking point and the green part of the probe telemetric to the breaking point (Fig. 1.4). A normal interphase cell will

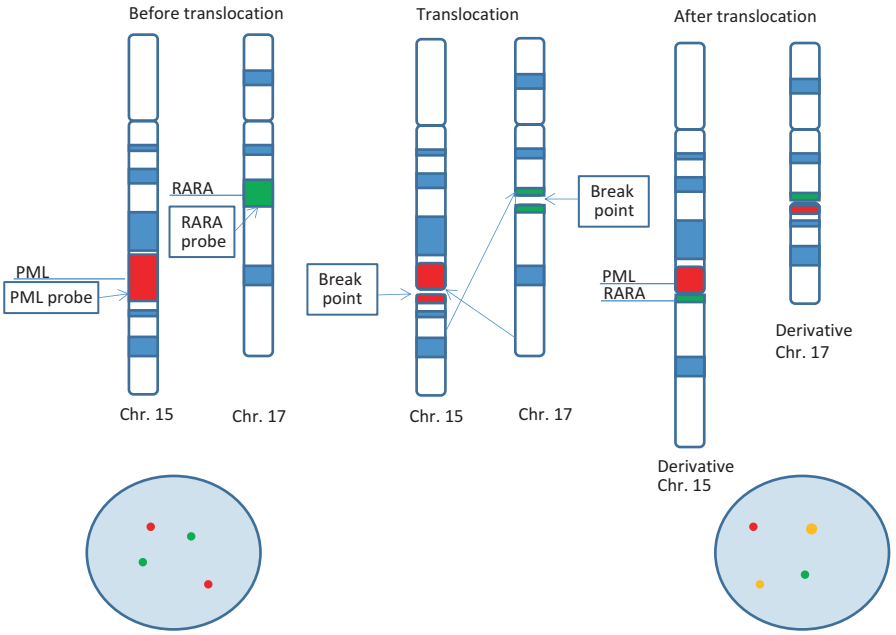

Fig. 1.3 Two probes are designed to hybridize to PML at 15q24 and RARA at 17q21, respectively, overlapping the breakpoints on each chromosome. The two probes can be labeled with different fluorescent dyes. For example, the probe specific for PML at 15q24 can be labeled with red fluorescent dye and the probe for RARA at 17q21 green. A normal cell will show two red and two green dots. A cell with t(15;17)(q24.1;q21.2) will show one red, one green, and two yellow dots

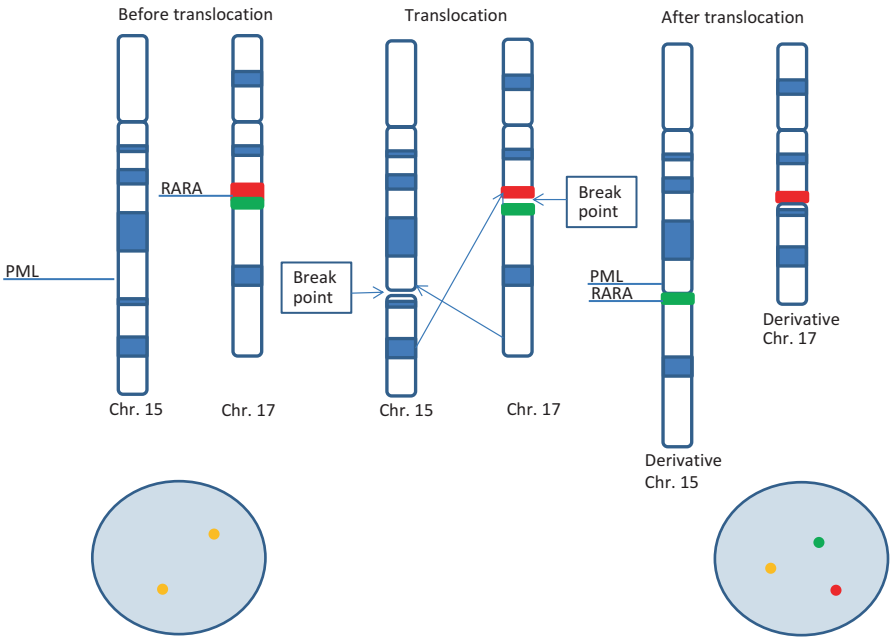

Fig. 1.4 A normal interphase cell will show two yellow dots. A cell with translocation will show one green, one red and one yellow dots

show two yellow dots (Fig. 1.4). An interphase cell with *RARA* translocation, regardless of the partner genes, will show one yellow, one red, and one green dot (Fig. 1.4). This assay works better than dual-color dual-fusion assay for different *RARA* translocation variants. In rare cases, cryptic *PML-RARA* translocations cannot be detected by karyotyping or FISH assay. For such cases, RT-PCR assay can be used to detect translocations [21, 22]. RT-PCR assay can also be used to detect minimal residual disease (MRD) with *PML-RARA* [23]. Different RT-PCR designs have been used to improve the performance [7, 24]. In general, RNA is extracted from patients' blood or bone marrow samples. Random hexamer primers are used to make cDNA. The target in cDNA is amplified using different primer pairs flanking fusion site of *PML-RARA* [23].

Somatic Gene Mutations

Somatic gene mutations in *FLT3* are relatively common (50% of cases) with the *FLT3*-ITD being most common (30–40%) and the *FLT3*-D835 mutation occurring in approximately 10% of cases. However, the prognostic significance of *FLT3* mutation remains unclear [25, 26].

Other gene mutations including alterations in *KIT* and *RAS* genes have been described relatively rare (generally reported frequency of 5% or less) [27].

Clinical Significance

Clinically, patients with APL often develop disseminated intravascular coagulation (DIC), which may result in rapidly unfavorable consequences (e.g., intracranial hemorrhage). However, APL is generally sensitive to the treatment using all-trans-retinoic acid (ATRA). Therefore, early diagnosis of APL is critical for improving patient outcome. Morphologic and immunophenotypic evaluation, in combination with FISH and PCR assays, plays an important role in patient care. Resistance to ATRA can occur due to genetic mutations in the retinoic acid receptor alpha (RARA) ligand-binding domain or the PML-B2 domain of PML-RARA. Additionally, cases of APL with variant RARA translocations may exhibit ATRA resistance [28].

Acute Myeloid Leukemia with t(8;21)(q22;q22);*RUNX1-RUNX1T1*

AML with t(8;21)(q22;q22);*RUNX1-RUNX1T1* is one of the AMLs with recurrent genetic abnormalities, first described in 1973 [29, 30], and comprises approximately 5% of all AML cases. The key cytogenetic abnormality is a *RUNX1-RUNX1T1* fusion gene [1]. *RUNX1* (runt-related transcription factor 1) is also known as acute myeloid

leukemia 1 gene (*AML1*), polyomavirus enhancer binding protein 2 subunit a (*PEBP2a*), and core-binding factor subunit A2 (*CBFA2*) [29]. *RUNX1T1* (runt-related transcription factor 1 translocated to 1) is also known as *ETO* (eight twenty one).

Morphologically, AML with t(8;21)(q22;q22) typically shows large myeloblasts with abundant basophilic cytoplasm, perinuclear hofs, and azurophilic granules, some of which coalesce into larger salmon-colored granules (pseudo-Chédiak-Higashi granules) (Fig. 1.5). In rare cases, the blasts may be less than 20%, which should not invalidate the diagnosis of AML with t(8;21)(q22;q22). Apart from myeloid blasts, the granulocytic lineage may show features of dysplasia, including hypogranulation or irregular lobation. Dysplasia in other lineages is not typically seen. Eosinophilic precursors are often increased, and occasionally basophils and mast cells are present in excess. [1, 30, 31].

By immunophenotypic evaluation, AML with t(8;21)(q22;q22)is positive for CD34, HLA-DR, MPO, and CD13, with dim CD33. Of note, these leukemias can often express lymphoid markers, including CD19, CD79a, PAX5, and on occasion dim Tdt. A diagnosis of biphenotypic leukemia should not be made in this setting.

Cytogenetic Abnormality

At the genome level, AML with t(8;21)(q22;q22) is characterized by a chromosome 8 and 21 translocation (Fig. 1.6) resulting in a *RUNX1-RUNX1T1* fusion gene [1]. The *RUNX1* gene is on chromosome 21 and *RUNX1T1* gene on chromosome 8. The breakpoint in the *RUNX1T1* gene is within an intron at the 5′ end of *RUNX1T1*. The breakpoint in the *RUNX1* gene occurs between the exon 5 and 6. Therefore, the fusion gene contains the N-terminal 177 amino acids of *RUNX1*, which is fused in frame to nearly full-length *RUNX1T1* protein [6, 32]. The fusion gene resides on derivative chromosome 8 after the translocation [6] (Fig. 1.6).

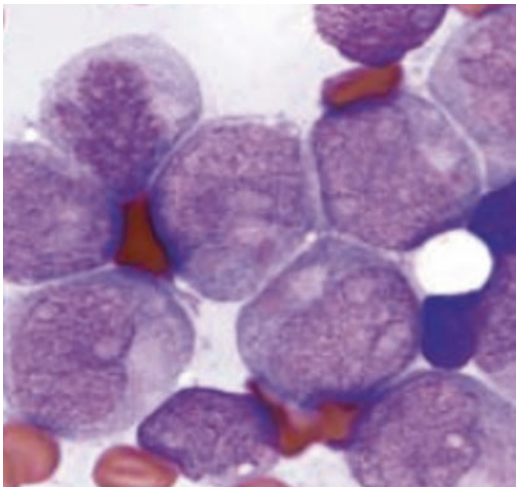

Fig. 1.5 Acute myeloid leukemia with t(8;21) showing basophilic cytoplasm and paranuclear hofs

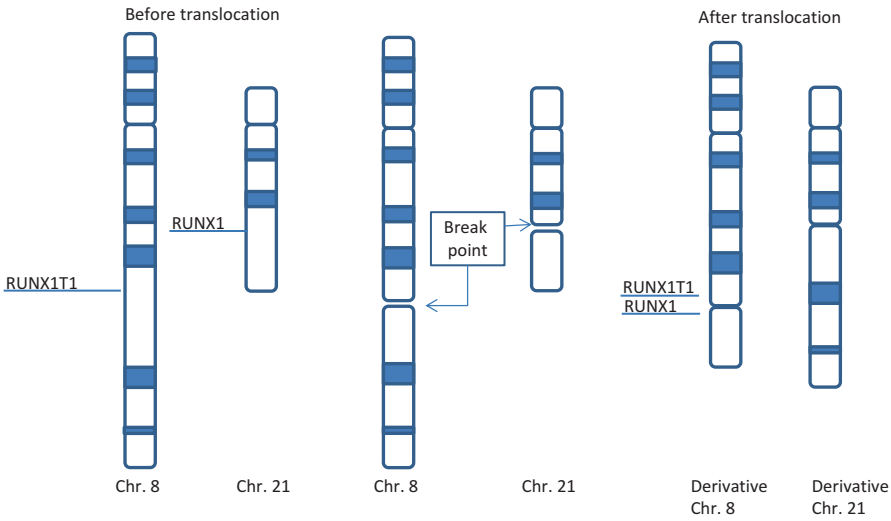

Fig. 1.6 At the genome level, AML with t(8;21)(q22;q22) is characterized by a chromosome 8 and 21 translocation

RUNX1-RUNX1T1 translocation can be detected using karyotyping and FISH. One of the FISH assay designs is a dual-color dual-fusion FISH assay. In this assay, two probes are designed to hybridize to *RUNX1T1* at 8q22 and *RUNX1* at 21q22, respectively, overlapping the breakpoints on each chromosome. The two probes can be labeled with different fluorescent dyes. For example, the probe specific for *RUNX1T1* can be labeled with red fluorescent dye and the probe for *RUNX1* green. A normal cell will show two red and two green dots (Fig. 1.7). A cell with t(8;21)(q22;q22) will show one red, one green, and two yellow dots (Fig. 1.7). This assay is typically more sensitive than karyotyping.

In rare cases, cryptic *RUNX1-RUNX1T1* translocations may not be detected by karyotyping or FISH assays. RT-PCR assays in these instances can be used to detect such cryptic translocations [30]. RT-PCR assays may also be used to detect minimal residual disease (MRD) of *RUNX1-RUNX1T1* with a sensitivity of 10^{-3} to 10^{-4}. A nested PCR design can reach a sensitivity of 10^{-4} to 10^{-5} [30].

Somatic Mutations

Several genes additionally deserve discussion here with regard to AML with t(8;21)(q22;q22): *KIT*, *FLT3*, and the *RAS* genes, *NRAS* and *KRAS*. *KIT* mutations are the most frequent and vary in incidence in these AMLs (5–30%); most mutations are within the tyrosine kinase domain [33–44].

These mutations in *KIT* are activating mutations which increase cell proliferation and survival.

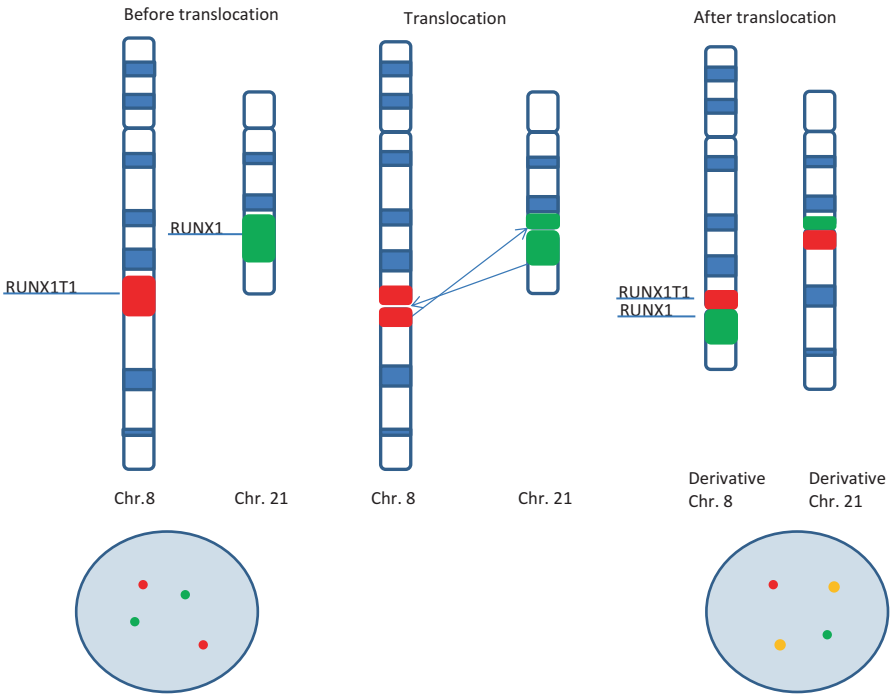

Fig. 1.7 The probe specific for RUNX1T1 can be labeled with red fluorescent dye and the probe for RUNX1 green. A normal cell will show two red and two green dots. A cell with t(8;21)(q22;q22) will show one red, one green, and two yellow dots

Three other genes infrequently mutated in AML with t(8;21)(q22;q22) are *FLT3* and the *RAS* genes *KRAS* and *NRAS*. As in many other AML subtypes, *FLT3* internal tandem duplication (ITD) mutations may be seen, though at a low frequency (~5%) in AML with t(8;21)(q22;q22) [36].

In vivo mouse models have shown a cooperative effect in *FLT3*-ITD mutations in leukemogenesis [45].

FLT3 D835 mutations are additionally rare though the significance of these mutations in AML with t(8;21)(q22;q22) is even less certain. Similarly, *NRAS* and *KRAS* mutations are relatively rare and like *FLT3-ITD* mutations may have a role in leukemogenesis without impacting chemosensitivity [36].

Clinical Significance

AML with the *RUNX1-RUNX1T1* translocation is considered to have a favorable prognosis when compared with other AML subtypes [46, 47]. Somatic gene mutations in *KIT* and *FLT3-ITD* mutations may modify the prognostic outcomes of patients. Several studies have shown that patients with AML with the *RUNX1-RUNX1T1*

translocation and *KIT* mutations may have an adverse outcome though some studies have shown no prognostic impact [33–37, 39, 41, 42].

FLT3-ITD mutations are rare but are associated with poor outcomes in patients with t(8;21)(q22;q22) [38].

Acute Myeloid Leukemia with inv(16)(p13.1q22);*CBFB-MYH11* or t(16;16)(p13.1;q22);*CBFB-MYH11*

AML with inv(16)(p13.1q22);*CBFB-MYH11* comprises 5–8% of AML cases and is predominantly seen in younger patients [1]. Morphologically, these leukemias demonstrate myelomonocytic differentiation, but more uniquely, the marrow shows increased eosinophils, which can show dysplastic features, including prominent granulation with aberrant purple-blue coloration and, on occasion, nuclear hyposegmentation (Fig. 1.8). While most cases show eosinophilia, occasionally, eosinophils are not increased, and myelomonocytic differentiation may be the only feature present. Like APL with *PML-RARA* and AML with t(8;21)(q22;q22), the translocation is diagnostic, and a blast count of greater than or equal to 20% is not required.

The immunophenotype of these leukemias may be heterogeneous, reflecting its myelomonocytic differentiation. It can include an immature blast population expression CD34 and CD117, as maturing granulocytes expressing CD13, CD33, CD65, and MPO and a monocyte population expressing CD4, CD14, and CD64.

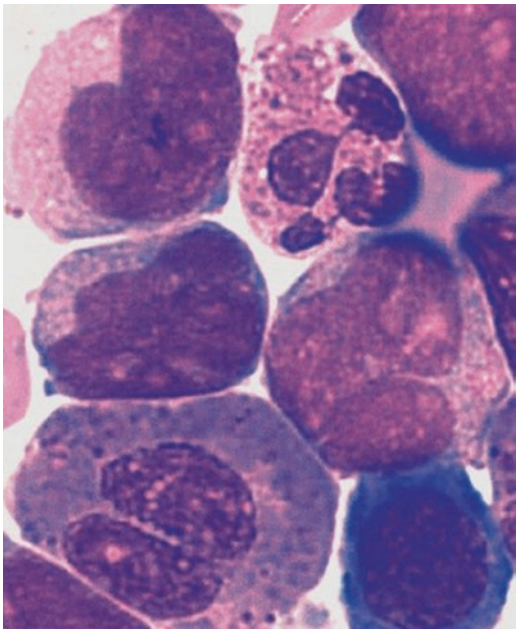

Fig. 1.8 Acute myeloid leukemia with inv(16) showing abnormal eosinophils with violet-blue granules

Cytogenetic Abnormality

At the genome level, AML with inv(16)(p13.1q22) is characterized by an inversion of a segment of chromosome 16 resulting in *CBFB-MYH11* fusion gene. *CBFB* is the core binding factor beta subunit located at 16q22. The *MYH11* gene encodes a smooth muscle myosin heavy chain, which is normally located at 16p13.1 [1, 48]. The breakpoints at *CBFB* are near the 3-prime end of the coding region of *CBFB* and are found to be the same in different cases, though the breakpoints at *MYH11* are more variable. The N-terminus and most of *CBFB* gene are fused to the C-terminus of *MYH11* gene with its multimerization domain. All rearrangements maintain the reading frame of the fusion transcript [49, 50]. This chromosome 16 inversion disrupts *CBFB* resulting in impaired differentiation [51]. A similar fusion gene can also result from t(16;16)(p13.1;q22) and bears a similar consequence. The common chromosome 16 inversion is depicted in Fig. 1.9. Such an inversion comprises about 95% of cases generating a *CBFB-MYH11* fusion gene. Karyotyping is a common method used and is capable of detecting most cases. A FISH assay has been used in detecting AML with inv(16)(p13.1q22) with higher sensitivity when compared to karyotyping. Generally speaking, RT-PCR assay has the highest sensitivity since it can detect subtle *CBFB-MYH11* fusion transcripts [52–55].

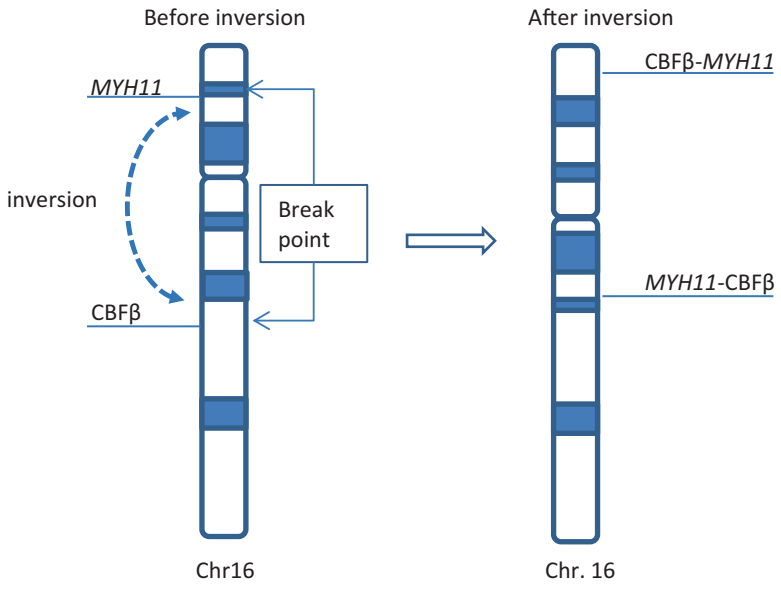

Fig. 1.9 The common chromosome 16 inversion

Somatic Gene Mutations

Similar to AML with t(8;21)(q22;q22), AML with inv(16)(p13.1q22) also has frequent mutations in the *KIT* gene (10–40%); additionally mutations in *FLT3* are seen with lesser frequency (10%) [34, 36, 42, 43, 56, 57].

RAS mutations are frequently seen in AML with inv(16)(p13.1q22) (35–50%); mutations in the RAS family of genes may function cooperatively with *RUNX1-RUNX1T1* to promote leukemogenesis [36, 56, 58].

Clinical Significance

Like AML with *RUNX1-RUNX1T1*, AML with *CBFB-MYH11* has a favorable prognosis when compared with other AML subtypes [59]. The significance of *KIT* and *RAS* mutations in these AMLs is uncertain; however, *FLT3*-ITD mutations have been shown in some studies to be associated with poorer prognosis within this category [34, 36, 42, 43, 56, 57].

Acute Myeloid Leukemia with t(9;11)(p22;q23);*KMT2A-MLLT3*

AML with t(9;11)(p22;q23) results in the fusion of KMT2A, also known as MLL, to MLLT3 and is more often seen in the pediatric setting, comprising 9–12% of pediatric AMLs and 2% of adult AMLs [1]. Morphologically, AML with t(9;11)(p22;q23) is characterized primarily by monocytic differentiation. AML with *KMT2A-MLLT3* is often associated with acute monocytic and myelomonocytic leukemias [1]. Monoblasts, as well as promonocytes (considered blast equivalents), predominate. Monoblasts demonstrate round/oval nuclei, delicate chromatin with prominent nucleoli, basophilic cytoplasm, and rare azurophilic granules. Promonocytes, on the hand, have more convoluted or indented nuclei and less basophilic cytoplasm but can also demonstrate delicate chromatin and visible nucleoli.

In terms of the immunophenotype, the most commonly expressed markers in AML with t(9;11)(p22;q23) include CD13, CD33, CD15, CD38, CD64, CD4, and HLA-DR, whereas CD14, CD117, and MPO are less frequent. Interestingly, pediatric patients will often lack CD14 and CD34 expression, while adult patients will more frequently show CD14 expression and variable CD34.

Cytogenetic Abnormality

At the genome level, AML with t(9;11)(p22;q23) involves *MLLT3* (mixed-lineage leukemia translocated to 3) and *KMT2A* genes. *MLLT3* is located at 9p22 and *KMT2A* gene at 11q23. The breakpoints of *KMT2A* gene are clustered in a region

that spans the 3′-portion of exon 8, exons 9–13 and the 5′-portion of exon 14 [60, 61]. The breakpoints of *MLLT3* are clustered in introns 4, 7, and 8 [60, 62]. The translocation results in a *MLLT-KMT2A* fusion gene, which consists of the 5′ portion of *KMT2A* gene and the 3′ portion of *MLLT3* gene [60]. Although *MLLT3* is a major partner gene in the translocation associated with *KMT2A*, it is well known that *KMT2A* has many other translocation partner genes. About 80 of all partner genes have been identified [63–65]. In general, karyotyping can detect the majority of translocations. However, since *KMT2A* may have different partner genes and may have cryptic translocations, a significant portion of the translocations may be missed by karyotyping. FISH assays are a good compliment in detecting those cryptic or variant translocations. Considering the fact that *KMT2A* has many possible partner genes, FISH assays usually use dual-color, break-apart designs. The probes span over the *KMT2A* gene and cover the region on either side of *KMT2A* breakpoints. The probe on one side of the breakpoints will be labeled with red florescent dye, and the probe on the other side of the breakpoints will be labeled with green florescent dye (Fig. 1.10). Normal interphase cells will show two yellow dots. The cells with *KMT2A* translocation will show one yellow dot, one green dot, and one red dot. Next-generation sequencing (NGS) offers a new opportunity for *KMT2A* translocation testing as well.

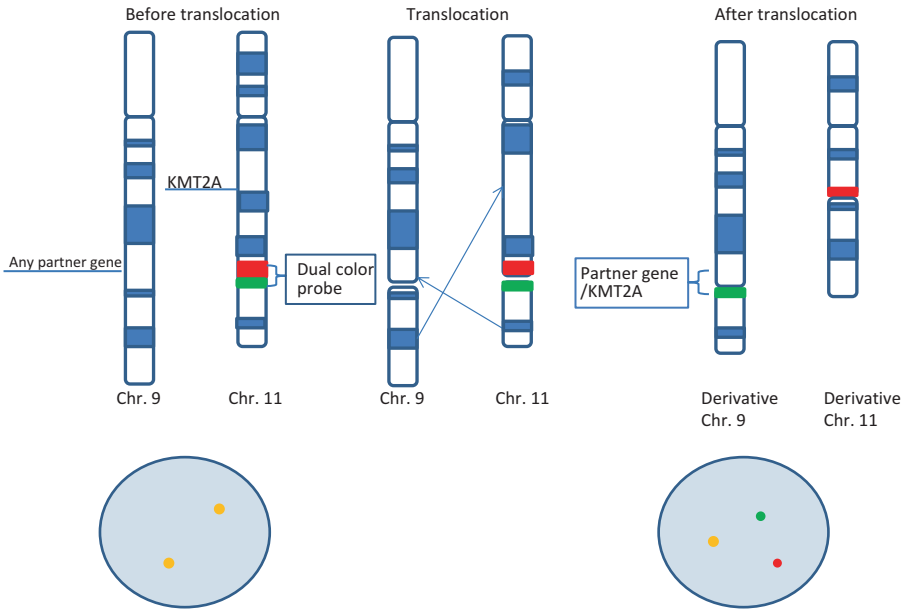

Fig. 1.10 FISH assays usually use dual-color, break-apart designs. The probes span over the KMT2A gene and cover the region on either side of KMT2A breakpoints. The probe on one side of the breakpoints will be labeled with red florescent dye, and the probe on the other side of the breakpoints will be labeled with green florescent dye

Clinical Significance

AML with *KMT2A* translocations are often associated with an unfavorable prognosis when compared to other AML subtypes; however, AML with 11q23 translocations can also be seen in therapy-related leukemias after topoisomerase II inhibitor therapy [66, 67]; but such cases should be diagnosed as therapy-related leukemias [1].

Acute Myeloid Leukemia with t(6;9)(p23;q34);*DEK-NUP214*

AML with t(6;9)(p23;q34) can be seen in both children and adults, consisting of approximately 1% of total AML [1]. AML with t(6;9)(p23;q34) can be seen in any FAB subtype of AML. Patients usually present with anemia, pancytopenia, and thrombocytopenia. In addition to general AML morphology, this subtype is often associated with basophilia (often >2%) and multilineage dysplasia [1].

Morphologically, AML with t(6;9)(p23;q34) can be seen in any FAB subtype of AML, except APL and acute megakaryoblastic leukemia. In addition, this subtype is often associated with marrow and peripheral blood basophilia (defined as >2%). Multilineage dysplasia is often seen, with granulocytic and erythroid dysplasia being more common than megakaryocytic dysplasia. Ringed sideroblasts are present in a subset of cases [1].

The immunophenotype of AML with t(6;9)(p23;q34) is fairly nonspecific, showing typical myeloblast markers including CD34, CD117, MPO, CD13, CD33, and CD15. A subset of cases also express CD64, and Tdt expression is not uncommon.

Cytogenetic Abnormality

At the genome level, AML with t(6;9)(p23;q34) translocation results in *DEK-NUP214* fusion gene on derivative chromosome 6 [6, 68, 69]. The *DEK* gene is located at 6p23 and the *NUP214* (nuclear pore complex protein 214 kDa) gene at 9q34. The *NUP214* gene is also known as the *CAN* gene. The breakpoints in the *DEK* and *NUP214* genes occur in the intron of each gene. The *DEK-NUP214* fusion gene consists of the 3′ portion of the *NUP214* gene from chromosome 9 and the 5′ portion of the *DEK* gene from chromosome 6. The fusion gene contains the open reading frames of both genes [69–71]. Since most translocation breakpoints occur in a single intron of the *NUP214* gene and in a single intron of the DEK gene, most translocations can be detected using FISH and PCR assays. PCR assays usually have higher sensitivity and real-time PCR assays can be used to follow patients for minimal residual disease [72–75].

Somatic Gene Mutations

AML with t(6;9)(p23;q34) is frequently associated with a *FLT3* mutation, often *FLT3*-ITD (as high as 70% of cases); other mutations in myeloid mutation-associated genes are much rarer in this AML [76].

Clinical Significance

Overall, AMLs with t(6;9)(p23;q34) usually have an unfavorable prognosis, and *FLT3*-ITD mutations confer even faster relapse than those patients without *FLT3*-ITD mutation [76].

Acute Myeloid Leukemia with inv(3)(q21q26.2) or t(3;3)(q21;q26.2)

AMLs with inv(3)(q21q26.2) are rare (2% of AMLs) but usually seen adults [1]. Patients usually present with anemia, thrombocytopenia, or pancytopenia. Morphologically, AML with t(6;9)(p23;q34) can show variable features, but promyelocytic and megakaryoblastic differentiation are not typically seen. In addition, this subtype is often associated with marrow and peripheral blood basophilia (defined as >2%). Multilineage dysplasia is frequently present, with granulocytic and erythroid dysplasia being more common than megakaryocytic dysplasia. Ring sideroblasts can also be demonstrated in a subset of cases [1]. The immunophenotype of AML with t(6;9)(p23;q34) is fairly nonspecific, showing typical myeloblast markers including CD34, CD117, MPO, CD13, CD33, and CD15. A subset of cases also express CD64, and Tdt expression is not uncommon.

Cytogenetic Abnormality

At the genome level, the result of inv(3)(q21q26.2) brings a *GATA2* distal hematopoietic enhancer (G2DHE) to the vicinity of the *EVI1* gene, causing aberrant *EVI1* expression and *GATA2* haploinsufficiency [2, 4, 5]. This is unlike other AML translocations that usually result in fusion genes. The breakpoints in 3q26 are distributed over several kilobases [77–81]. The breakpoints in 3q21 are distributed over 100 kb [81–83]. The inv(3)(q21q26.2) and t(3;3)(q21;q26.2) can be detected using karyotyping; however, karyotyping may miss some cryptic changes. A dual-color, double-fusion FISH assay

has better sensitivity than that of karyotyping [84]. As in other translocations, RT-PCR assays can be used to detect the inversion and translocation [83, 85, 86]. Due to the wide spread of breakpoints in the inversion and translocation, designing a multiplex RT-PCR to reach a good sensitivity is difficult. However, RNA NGS assays may overcome such challenges and offer practical solutions with good sensitivity and specificity.

Somatic Gene Mutations

The somatic gene mutational landscape of AML with inv(3)(q21q26.2) has been preliminarily mapped, and mutations in *RAS* genes (*NRAS, KRAS*) and *RUNX1* are most common, with 40–50% and 20% of cases, respectively. *NF1* deletions, *FLT3*, and *CBL* and *IDH1* mutations are less frequent, with 10%, 10%, 10%, and 5% of cases, respectively [87, 88].

Clinical Significance

The AML patients with inv(3)(q21q26.2) and t(3;3)(q21;q26.2) tend to have unfavorable prognosis with short overall survival and poor response to conventional chemotherapy [77, 78, 87]. The role of other somatic gene mutations in AML patients with inv(3)(q21q26.2) and t(3;3)(q21;q26.2) such as those in *NRAS, KRAS*, *RUNX1*, *NF1*, *FLT3*, *CBL*, and *IDH1* is less certain.

Acute Myeloid Leukemia with t(1;22)(p13;q13);*RBM15-MKL1*

AML with t(1;22)(p13;q13) is usually seen in infants without Down syndrome, more often in female infants. It consists of less than 1% of all AML cases [1] and about 10% of pediatric AMLs [89–92]. Morphologically, the blasts in AML with t(1;22)(p13;q13) present as small or large megakaryoblasts resembling those of acute megakaryoblastic leukemia [1].

Cytogenetic Abnormality

At the genome level, t(1;22)(p13;q13) results in a fusion gene, *RBM15-MKL1*. *RBM15* (RNA-binding motif protein 15) is located at 1p13 and is also known as *OTT* (one twenty-two). *MKL1* (megakaryocyte leukemia 1) is located at 22q13. The breakpoints

on chromosome 1p13 are located at a 6 kb genomic region. The breakpoints on chromosome 22q13 are located in a 28 kb intron [93]. Although both reciprocal fusion genes are expressed, *RBM15-MKL1* fusion gene has all the functional motifs encoded by each partner gene [93]. The translocation can be detected by karyotyping, FISH, and PCR [93], with PCR assays being used to follow minimal residual disease after treatment [94].

Clinical Significance

The early studies suggested that AMLs with t(1;22)(p13;q13) carry an unfavorable prognosis [1, 95, 96]; however, more intensive therapy has improved the long-term survival of such patients [97, 98].

De Novo Acute Myeloid Leukemia with t(9;22)(q34;q11.2)

AML with t(9;22)(q34;q11.2) is seen in 0.5–3.0% of all AML cases . In the cases of de novo AML, the patients should not have a history of *BCR-ABL1* positive chronic myeloid leukemia (CML), as such splenomegaly and peripheral basophilia are less common than in CML in blast crisis. The translocation t(9;22)(q34;q11.2) results in a *BCR-ABL1* fusion gene and is often the specific Philadelphia chromosome [99–105]. It is now considered a provisional category of AML with recurrent genetic abnormalities [2].

As it is a newly defined provisional entity, our experience with AML with t(9;22)(q34;q11.2) is somewhat limited. To date, no specific histologic or immunophenotypic features which distinguish this entity from typical AML have been identified [99–105].

Cytogenetic Abnormality

At the genome level, the t(9;22)(q34;q11.2) in this AML is the same as that seen in CML. Karyotyping, FISH, and PCR assays can be used to detect the translocation. As one may expect, FISH has higher sensitivity than karyotyping. Quantitative PCR assay is the most sensitive among the three assays and can be used to monitor minimal residual disease. Differentiating between the blast phase of CML and AML with t(9;22)(q34;q11.2) has been difficult and heavily depends on the patient's history. However, genomic analysis of de novo cases in comparison to the blast phase of CML has demonstrated specific deletions in *IKZF1* and *CDNK1A/B* as well as loss of *IGH/VDJ* and *TARP* regions [104].

Clinical Significance

These cases are so rare that tailored therapies are still in their infancy. It is thought that, like CML in blast phase crisis, AML with t(9;22)(q34;q11.2) may benefit from TKI (tyrosine kinase inhibitor) therapy [103–106].

Conclusion

Understanding cytogenetic changes in acute myeloid leukemia is critical not only for diagnostic subtyping but also for understanding the pathogenesis and potential clinical progression of patients. While the majority of cytogenetically defined categories of AML have been elucidated, understanding how these genetic changes result in leukemia is still far from complete. Additionally, the significance and prevalence of other somatic gene mutations in these leukemias require further study.

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