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Precision Molecular Pathology of Myeloid Neoplasms

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Precision Molecular Pathology of Myeloid Neoplasms

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Preface

Our understanding of myeloid neoplasms has been radically transformed by notable molecular developments over the past 10 years. In large part, this rapid advancement specifically has been centered on profound studies based on emerging and newer genetic technologies. Additionally, as our molecular pathology knowledge has grown, the ability to treat diseases with molecularly targeted therapies has become a simple reality. Yet, keeping abreast of all these advancements has become increasingly difficult.

The primary goal of this book is to provide the necessary and foundational molecular and diagnostic knowledge of myeloid neoplasms and further increase the readers' awareness and understanding of specific targeted therapies, where applicable. Critical myeloid neoplasms are covered here in this book and separated into well-defined and organized chapters. Authors are experts with special interest in their relative areas, and important literature and guidelines are consolidated into this comprehensive book. Figures and tables are made accessible, allowing easy access to critical information for diagnoses and understanding of prognosis and treatment.

This textbook serves as a useful resource for clinicians and pathologists who diagnose, treat, and study myeloid neoplasms. The information provided here will not only guide accurate diagnoses, appropriate ancillary molecular tests, and patient management but also vastly stimulate investigative efforts.

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

Jenny Hoffmann and Dahui Qin

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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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

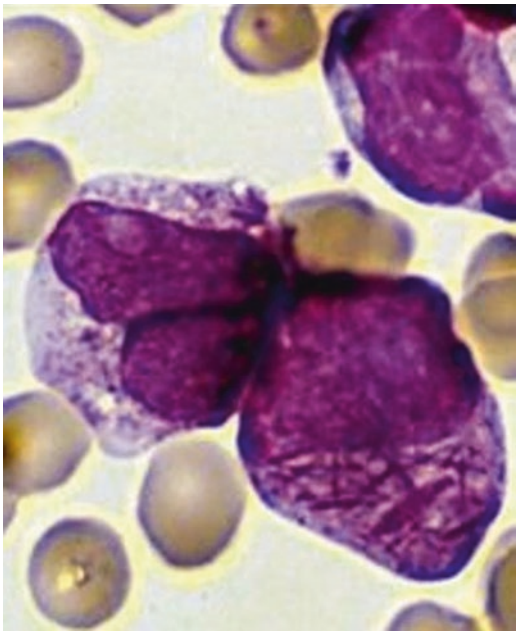

Fig. 1.1 Acute promyelocytic leukemia, hypergranular variant showing prominent azurophilic granules bilobed nuclei and bundles of Auer rods

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

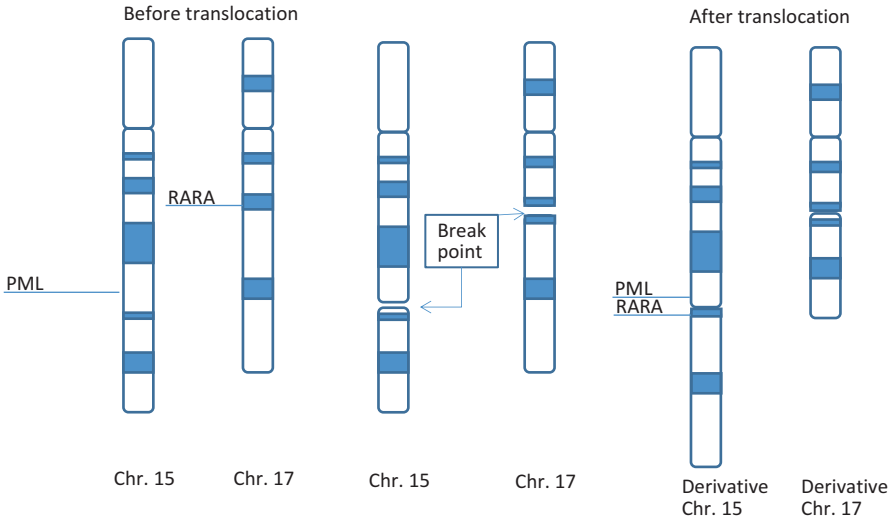

Fig. 1.2 APL with PML-RARA is characterized by a t(15;17)(q24.1;q21.2) translocation

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

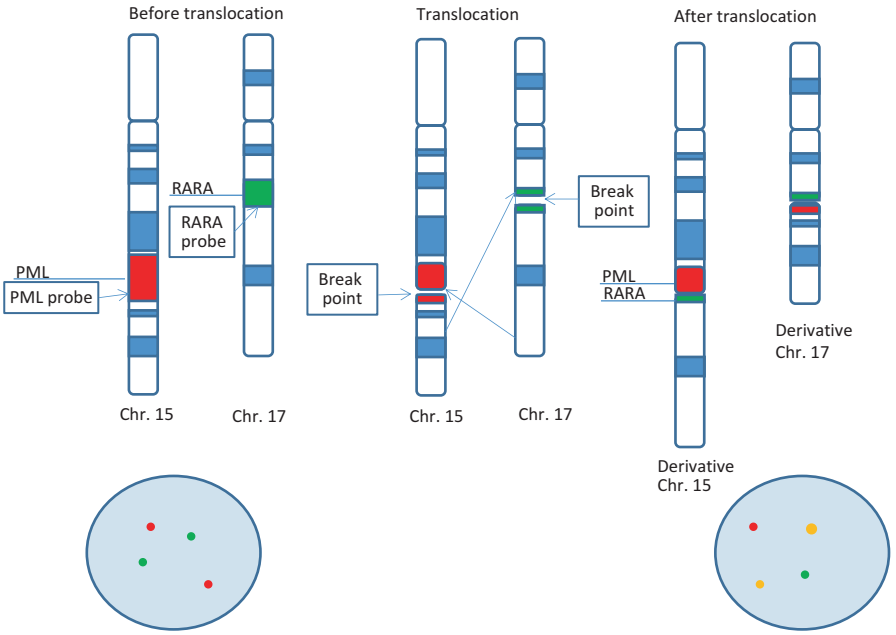

Fig. 1.3 Two probes are designed to hybridize to PML at 15q24 and RARA at 17q21, respectively, overlapping the breakpoints on each chromosome. The two probes can be labeled with different fluorescent dyes. For example, the probe specific for PML at 15q24 can be labeled with red fluorescent dye and the 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

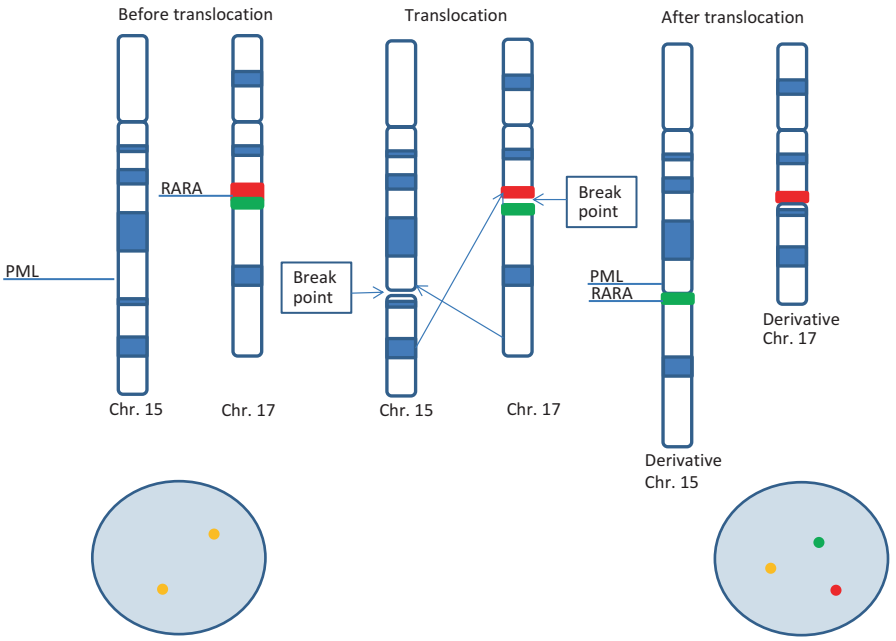

Fig. 1.4 A normal interphase cell will show two yellow dots. A cell with translocation will show one green, one red and one yellow dots