Atlas of Genetics and Cytogenetics in Oncology and Haematology

OPEN ACCESS JOURNAL AT INIST-CNRS

Leukaemia Section

Review

M3/M3v acute myeloid leukemia (AML M3/M3v)

Acute promyelocytic leukemia (APL)

Acute promyelocytic leukemia (APL) PML/RARA

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Published in Atlas Database: April 2017

Online updated version : http://AtlasGeneticsOncology.org/Anomalies/M3ANLLID1240.html
Printable original version : http://documents.irevues.inist.fr/bitstream/handle/2042/68887/04-2017-M3ANLLID1240.pdf
DOI: 10.4267/2042/68887

This article is an update of :
Schoch C. M3/M3v acute non lymphocytic leukemia (M3-ANLL). Atlas Genet Cytogenet Oncol Haematol 2006;10(3)

Abstract

ABSTRACT
Review on Acute promyelocytic leukemia, with data on clinics and the genes involved.

KEYWORDS
Acute promyelocytic leukemia; M3 acute myeloid leukemia

Identity

Note

FAB criteria AML M3

at least 20% of bone marrow cells are abnormal promyelocytes, with a characteristic pattern of heavy granulation

characteristic cells contain bundles of Auer rods ("faggots")

FAB criteria AML M3v

As other AML, at least 20% of bone marrow cells are blast cells. Blast cells have minimal granulation, relative scarcity of cells with heavy granulation and cells containing multiple Auer rods. The nucleus of blast cells is bilobed, multilobed or reniform, but the majority of cells are either devoid of granules or contain only a few fine azurophil granules. However, at least a few cells with all the cytoplasmic features of typical AML M3 are present. If these are overlooked, the cases are likely misdiagnosed as monocytic leukemia. The M3v morphology is mainly a feature of the peripheral blood cells - bone marrow morphology is closer to that of typical AML M3.

both subtypes show a very strong myeloperoxidase reaction and a negative reaction for non-specific esterase. Exceptional APL PML/RARA cases with basophilic granulations, metachromatic with

toluidine blue, can be MPO negative (Invernizzi R et al , 1995).

Immunophenotype

mature myeloid phenotype, characteristic but not diagnostic:

CD34 negative, HLA-DR negative, CD33 positive, CD13 positive.

Aberrant expression of CD56 is observed in approximately 10% of APL patients and is associated with a higher WBC count, a more frequent bcr3 isoform and, in some series of patients, a shorter event free survival (Testa and Lo-Coco, 2016).

in M3 but not M3v: characteristic light scatter pattern, strong unspecific fluorescence signal

WHO classification (2008, updated 2016)

APL with t(15;17)(q24;q21) PML/ RARA (WHO 2008) is now reported as APL with PML/RARA (WHO 2016)

Other very rare AML M3/M3v harbouring recurring translocations involving RARA with variant genes other than PML previously mentioned as AML with variant partners of RARA (WHO 2008) should be reported accordingly:

- AML with t(11;17)(q23;q21) ZBTB16/RARA (previously named PLZF/RARA),
- AML with t(11;17)(q13;q21) NUMA1/RARA,
- AML with t(5;17)(q35;q21) NPM1/RARA,
- AML with der(17); STAT5B/RARA (either cryptic abnormality leading to normal chromosome 17 on karyotype or abnormal der(17) both due to complex rearrangements involving STAT5B located downstream to RARA on band 17q21.2 and oriented 5'-3' telomere to centromere).

Clinics and pathology

Disease

Epidemiology

Rare: 5 - 8 % of AML, incidence higher in Spain, Italy and Latinos; occurs at any age, predominantly adults in mid-life.

Account for approximatively. 5% of treatment related leukemias (t-AML); 5-22% of APL are t-APL, mostly after breast cancer treated by epirubicine or mitoxantrone (chemotherapy targeting topo 2 isomerase); short-delayed (median inferior to 3 years) ; favorable prognosis among t-AML: benefit from ATRA-ATO treatment thus avoiding anthracyclins (reviewed in Lo Coco F, 2013).

Clinics

Low WBC in AML M3, high WBC in AML M3v; frequently associated with disseminated intravascular coagulation (DIC) and hyperfibrinolysis which are the main factors of immediate bad prognosis and death when diagnostic and treatment are delayed.

Biological and clinical data favour a crucial role of the leukemic cells in the development of the coagulopathy which is a multifactorial event, involving DIC and primary hyperfibrinolysis (Mantha et al., 2016).

Cytology

The cytomorphology of APL blasts is obviously different in the two subtypes: in AML M3, the abnormal promyelocytes show a heavy granulation and bundles of Auer rods; in AML M3v blasts have a non- or hypogranular cytoplasm or contain fine dustlike cytoplasmic granules that may not be apparent by light microscopy. Furthermore, M3v blasts show a typical bilobed nuclear configuration. This latter morphologic phenotype, together with missing granulation, often resulted in the misleading diagnosis of acute monocytic or myelo-monocytic leukemia before the cytogenetic correlation of both AML M3 and M3v with t(15;17)(q24;q21) was observed. AML M3v accounts for approximately 1/3 of APL cases.

Treatment

Previous chemotherapies which associated anthracyclines and cytosine arabinoside allowed long-lived remission in less than 50% of patients. From 1985 retinoic acid (RA) isomers were introduced with success as differentiation agents in several series of APL patients. The first molecule tested was 13-cis RA. In parallel all-trans RA (ATRA) showed a quite better efficacy. Then another molecule, arsenic trioxide (ATO), acting on differentiation and apoptosis, was tested with good results. With the current treatments, involving anthracycline chemotherapy associated with ATRA and/or ATO, a long-lived remission is obtained in more than 90% of cases, and bone marrow transplantation has been restricted to the sole cases of first relapse. Particularly the introduction of ATRA and/or ATO therapy at diagnosis, has largely reduced the rate of early death due to the coagulopathy associated with APL first stage (Wang Z-Y and Chen Z, 2008). Patients with low WBC (less than 10G/L) can be treated at diagnosis with ATRA and ATO thus avoiding the toxicity of chemotherapy especially anthracyclins (Lo Coco et al, NEJM, 2013).

To be noted that some variant translocations, i.e. t(11;17) with ZBTB1/RARA fusion and der(17) with STAT5B/RARA fusion do not respond to ATRA and ATO therapy, consequently their prognosis is quite more pejorative.

Evolution

APL PML/RARA have to be monitored by RQ-PCR on bone marrow samples every 3 months after

diagnosis for 18 months as patients with molecular relapse can benefit from preemptive therapy . Patients with hyperleucocytosis at diagnosis (more than 10 G/L WBC) or slow responding to treatment more especially benefit from this survey .(reviewed in Grimade D et al, BPRCH, 2014)

Prognosis

Adverse prognosis factors

High WB (more than 10 G/L).

The previously reported FLT3- internal tandem duplication (ITD).

Bad prognosis value is correlated with hyperleucocytosis asit is the case for the bcr3 molecular breakpoint and the M3v form.

Bleeding episodes.

Cytogenetics

Cytogenetics morphological

t(15;17)(q24.1;q21.2) leading to a PML (promyelocytic leukaemia)/RARA(retinoic acid receptor α) on der(15) and RARA/PML on der(17) rearrangements at the molecular level. Complex translocations involving one or more chromosomes in addition to 15 and 17 are found in 2-5% of cases with PML/RARA rearrangement. Cytogenetically cryptic PML/RARA rearrangements are observed in 2-3% of APL cases. Cases lacking PML/RARA rearrangement account for less than 2% M3/M3v AML cases and mainly involve RARA with partner genes different from PML (Grimwade D et al, Blood 2000).

Cytogenetics molecular

When karyotype fails to give an informative response, either for absence or poor quality metaphases or in case of complex or cryptic rearrangement, FISH analysis may be useful to validate PML/RARA fusion and initiate without delay or maintain specific APL therapy. Furthermore as cytogenetic diagnosis is urgent, FISH analysis can be performed urgently in parallel with karyotype analysis, preferably on cultured and fixed cells.

Among cryptic rearrangements, they are mainly insertions:

mainly insertion of 3'RARA into the PML gene: ins(15;17)(q24;q21q21)

but insertions of 5'PML into RARA can also be seen: ins(17;15)(q21;q24q24)

Probes

Different type of probes are commercially available:

PML/RARA fusion probes leading to either double or single fusion signals in positive cells

RARA break-apart probes leading to separated 5'RARA and 3'RARA signals signal in positive cells but failing to identify the RARA partner in nuclei. They identify the partner chromosome band if metaphases are present on the hybridized slide. Among these probes, some are less informative in case of cryptic insertions which accounts for approximately 5% of APL cases

Very large probes can fail to detect very small insertions.

RARA probes cannot detect PML insertions.

Flanking probes can fail to detect very small insertions.

The choice of informative probes has to take into account the probe maps and the critical fusion gene to be detected (PML/RARA rather than RARA/PML), thus covering 5'PML and 3'RARA. In order to obtain an optimal diagnosis (rapid and informative) multiple slides, each with a different type of probe can be hybridized. Furthermore, small insertions are easier to be detected on metaphases rather than on nuclei and the chromosomal location of the specific signals is very helpful for diagnosis.

Other diagnosis tools are:

PML immunofluorescence leading to a specific microspeckled pattern in nuclei of PML/RARA positive cases, related to the disruption of PML nuclear bodies.

PML/RARA RT-PCR analysis, very specific and sensitive, mandatory at diagnosis.

Additional anomalies

(ACA) are observed in 35-45% of cases at diagnosis (62% at relapse), most frequent: +8(or trisomy 8q), del(9q), ider(17)(q10)t(15;17) or del(17p) Classically, ACA do not impact the prognosis, however the loss of one copy of TP53 gene generated by ider(17q) or del(17p) could have a prognostic value. Complex karyotype at diagnosis, defined as ≥ 2 anomalies in addition to t(15;17), could be associated too with a reduced overall survival (Poiré X et al., 2014).

Variants

Approximately 1-2% of M3/M3v AML cases harbor a variant translocation which fuses RARA with an other partner gene than PML (Grimwade D et al. 2000; Redner RL, 2002):

t(11;17)(q23;q21) ZBTB16/RARA (also named PLZF/RARA), is the most frequent

t(5;17)(q23;q21) NPM1/RARA,

t(11;17)(q13;q21) NUMA1/RARA,

der(17) STAT5B /RARA,

der(17) PRKAR1A /RARA,

t(X;17)(p11;q12) BCOR /RARA,

t(4;17)(q12;q21) FIP1L1 /RARA

t(1;17)(p34;q21) IRF2BP2 /RARA

t(2;17)(q32;q21) NABP1 /RARA

t(3;17)(q26;q21) TBL1XR1 /RARA

t(7;17)(q11;q21) GTF2I /RARA

The cases with variant translocation have initially been reported as having APL morphology. However, morphological differences exist. Clinically important is that APL variant with t(5;17)(q12;q12)

NPM1/RARA, respond to ATRA, while M3/M3v AML with t(11;17)(q23;q12) ZBTB16/RARA or der(17) STAT5B/RARA do not respond to ATRA or ATO.

Genes involved and proteins

PML (promyelocytic leukemia)

Location

15q24.1

DNA/RNA

9 exons, 53 kb; alternative splicing on side of the transcript coding for the c-terminal part of the protein, leads to 6 nuclear and 1 cytoplasmic isoforms.

Protein

The PML protein is a tumor suppressor implicated in a wide variety of cellular activities: apoptosis, differentiation, genome stability. It has 3 zinc binding domains, BB1 (b-box1) and BB2 (b-box2) regions, and a coil-coiled region (CC). It contains also a C-terminal SUMO interaction motif (SIM) and 3 lysine residues able to be modified by SUMOylation.

PML organises and is located in PML-nuclear bodies (PML-NBs) which are 0.2 to 1.0 m organelles, mainly located in the nucleus of many cell types. Their number varies from 5 to 30 per nucleus, according to the cell cycle and the differentiation stage. PML-NBs are actively involved in transcriptional regulation of a number of loci active in cell growth and differentiation. Proteins are stored or sequestered inside PML-NBs, until they are required. PML-NBs mediate protein modifications such as SUMOylation, acetylation, ubiquitination and phosphorylation (Bernardi and Pandolfi, 2007).

RARA (Retinoic acid receptor, alpha)

Location

17q21.2

Protein

RARα protein is a member of nuclear steroid receptors and it presents homologies with nuclear receptors of vitamin D and thyroid hormone. It contains 2 zinc finger motifs which constitute the domain of linkage to the DNA (DBD, DNA binding domain). This link needs the formation of a heterodimer with the cofactor RXRα to be stable.

Result of the chromosomal anomaly

Hybrid gene

Description

t(15;17) leads to 2 fusion genes PML/RARA and RARA/PML. The latter is not critical; indeed it is lacking in 30% of cases either due to a submicroscopic deletion or, less frequently, to a mechanism of insertion. Variant or alternative translocations all involve RARA with another partner than PML; no variants are known involving PML with a partner different from RARA.

Fusion protein

Description

The PML/RARA fusion oncoprotein is expressed in 100% of APL cases with t(15;17) and is crucial for the leukemic phenotype, while RARA/PML is expressed in no more than 80-90% of cases and probably plays a secondary role. PML/RARA is able to homodimerise via the coiled-coil domain of PML, forming a complex which binds to the DNA on RARE sites. It acts as a dominant-negative transcriptional repressor of both target and non-target genes of retinoic acid. The main consequence is the arrest of myeloid cells differentiation at the promyelocytic stage. To achieve the gene silencing process, PML/RARA recruits various nuclear receptors with co-repressor properties, out of which RXRα, polycomb complex or DAXX (ZHU et al.; 2007, Lo-Coco and Hasan, 2014).

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This article should be referenced as such:

Lafage M, Viguié F. M3/M3v acute myeloid leukemia (AML M3/M3v); Acute promyelocytic leukemia (APL); Acute promyelocytic leukemia (APL) PML/RARA Atlas Genet Cytogenet Oncol Haematol. 2018; 22(4):148-152.