



Chronic myeloid leukemia: a type of MPN

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Abstract

This review article classifies chronic myeloid leukemia (CML) based on cytogenetic analyses and different mutations detected in CML patients. The use of advanced technologies, such as karyotyping, fluorescent in situ hybridization, and comparative genomic hybridization, has allowed us to study CML in detail and observe the different biochemical changes that occur in different CML types. This review also highlights the different types of receptor and signaling pathway mutations that occur in CML.

Key Words Chronic myeloid leukemia, Cytogenetics, Philadelphia chromosome, Myeloproliferative neoplasms

INTRODUCTION

The World Health Organization (WHO) defines myeloproliferative disorders as a class of disorders in which the bone marrow (BM) produces an excess of red blood cells [polycythemia vera (PV)], platelets [essential thrombocytosis (ET)], or granulocytes [chronic myeloid leukemia (CML)]. In PV, there is an increase in the number of red blood cells and hemoglobin levels, which causes the blood to become more viscous, causing hypoxemia. ET is categorized by the multiplication of megakaryocytes, which are precursors to platelets. This leads to abnormal clotting and bleeding. Primary myelofibrosis (PMF), another type of myeloproliferative disorder, is caused by the production of many new cells that ultimately scar the BM and disrupt healthy cell production.

CML, also known as chronic myelogenous leukemia, is an abnormality of the blood marrow typified by extensive growth of granulocytes without compromising their ability to become specialized cells. This results in an increase in the number of granulocytes and blast cells, which are precursors of granulocytes, in the blood smear. One-fifth of all leukemia cases in adults are caused by CML [1]. Reciprocal translocation t(9;22) (q34;q11.2) leads to 9q+ and a small 22q- being the distinguishable factor for CML. This chromosome is known as the Philadelphia chromosome, and leads

to the formation of breakpoint cluster region (BCR)-tyrosine-protein kinase ABL1 fusion genes and proteins that are characteristically observed in patients with CML [2].

BCR-ABL fusion genes and proteins present in CML can be detected using fluorescent in situ hybridization and polymerase chain reaction, respectively. This helps in diagnosing CML in special cases (less than 10 percent), where the chromosome is difficult to locate owing to encrypted translocation [3].

CML PHILADELPHIA POSITIVE

In CML, the genetic translocation between ABL1 on the long arm of chromosome 9 and the BCR gene on the long arm of chromosome 22 is commonly observed, as shown by molecular studies. As a result, a BCR-ABL1 fusion gene is formed [4].

The position of the breaking point is highly irregular [5]; however, recombination usually follows a similar pattern. Two combinations are possible, as shown in Fig. 1A. The first is between intron 1 and intron 13/14 or exon 19 of BCR with ABL1 (140 kb region) and the second is when p210^{BCR-ABL1} is formed by fusion of BCR exon 13 and ABL1 exon 2 (e13a2) or e14a2. Both recombinations lead to the formation of a hybrid 210-kDa protein [6].

Fusion of the entire BCR and ABL1 gene results in the

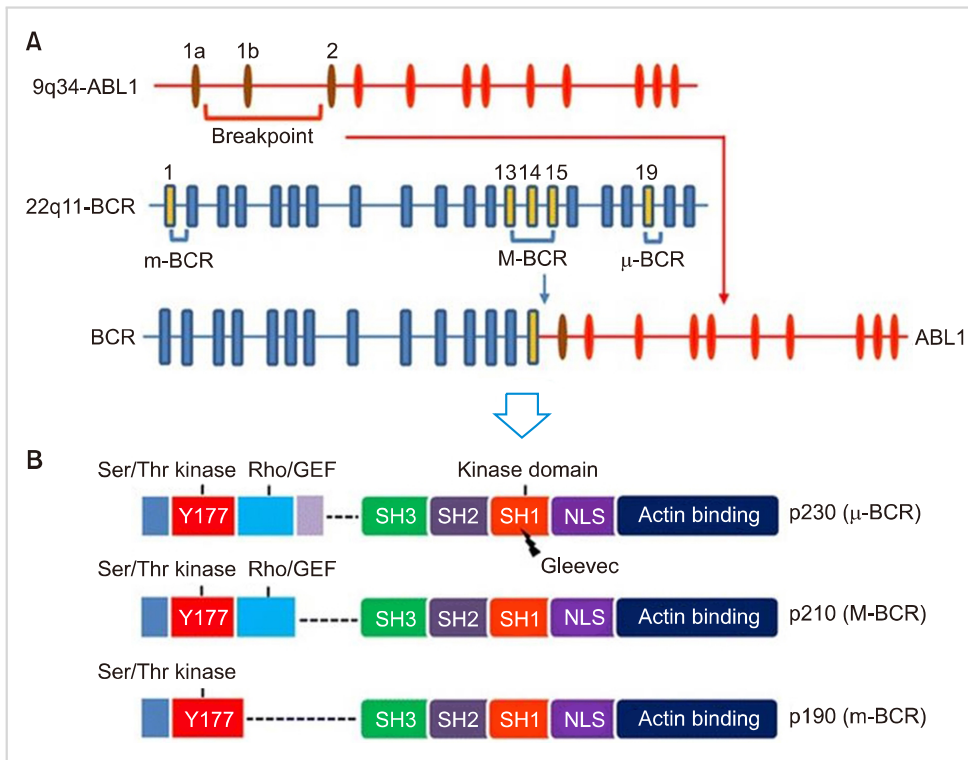


Fig. 1. Translocation of the breakpoint cluster region (*BCR*) gene.

BCR-ABL1 transcript, p230^{BCR-ABL1} (e19a2). This transcript codes for a hybrid 230-kDa protein that is used in the diagnosis of neutrophilic-chronic myeloid leukemia (CML-N) [7], as explained later in the article.

PHILADELPHIA NEGATIVE CML

Less than 1 in 10 patients with CML are Philadelphia negative [8]. The mean age group of Philadelphia negative patients is greater than 65 years, and patients present with common CML abnormalities such as monocytosis and thrombocytopenia. They have a shorter life span than Philadelphia positive patients because of a less effective response to chemotherapy [9, 10]. In Philadelphia negative CML there is a mutation in the *JAK2* gene on chromosome 9p24 (V617F) instead of the *BML/ABL* gene. The *JAK2* gene encodes for JAK2 kinase [11]. Many signaling pathways are activated through phosphorylation by non-receptor tyrosine kinases, such as Janus kinases. One such pathway is JAK/STAT, which is overly active in response to JAK2 gene mutations [12, 13]. In most cases of PV, there is a mutation in *JAK2V617F* gene, which is expected to be added to the WHO classification for the diagnosis of PV [14].

CHRONIC NEUTROPHILIC LEUKEMIA

Chronic neutrophilic leukemia (CNL) is a rare myeloproliferative disease that presents with an elevated neutrophil count in the blood or BM and an enlarged spleen [15]. The

WHO classifies CNL according to the following characteristic features: sustained neutrophilia with minimal levels of circulating immature monocytes, basophils, and granulocytes in the peripheral blood or BM [16]. Along with a high percentage of neutrophils, CNL is also characterized by a defective Philadelphia chromosome that eventually translates into a novel *BCR/ABL1* protein [7]. A *BCR* is located on chromosome 22 between exons 17 and 20, where a large fusion protein, p230, is also encoded [17].

Clinically, CNL is significantly more benign than CML. The total white blood cell (WBC) count was lower in CNL patients, anemia was milder, splenomegaly was less noticeable, and blastic transformation occurred considerably later [18].

CNL has been referred to as a version of “classical” CML linked to a defective Philadelphia chromosome. Translocation (9;22) in CNL causes the e19/a2 type *BCR/ABL* mRNA to be transcribed, which codes for a 230-kD *BCR/ABL* protein (p230). The e19/a2 *BCR/ABL* gene fusion anomaly on Philadelphia chromosome is a reliable diagnostic criterion for CNL. In contrast to classical CML, CNL’s significantly benign course stems from a distinct molecular lesion [19]. Recent developments in molecular genetics have revealed significant similarities between CNL and other chronic myeloid disorders such as atypical CML. These include epigenetic, spliceosome, and signaling mutations [20].

CHRONIC EOSINOPHILIC LEUKEMIA

Chronic eosinophilic leukemia (CEL) is a myeloprolifer-

ative disorder characterized by sustained eosinophilia, organ involvement, increased blast cell count in the blood, and/or clonality, as demonstrated by cytogenetics and X-inactivation [21]. To the WHO classification system, CEL is characterized by the lack of a Philadelphia chromosome or PDGFRA/B and FGFR1 gene rearrangements and the ruling out of other acute or chronic primary marrow neoplasms linked to eosinophilia [22]. CEL is classified into two subtypes: CEL FIP1L1-PDGFR α rearrangement positive, included in the WHO's new subdivision, and CEL-NOS (not otherwise specified), which belongs to the WHO's classification of myeloproliferative neoplasms [23]. A cryptic loss of 800 kb pairs on chromosome 4q12 causes FIP1L1 to fuse with the PDGFRA protein, resulting in a constitutively active tyrosine kinase that promotes eosinophil growth, manifests as chronic eosinophilic leukemia in CEL FIP1L1-PDGFR α rearrangement positive patients [24]. FIP1L1-PDGFR α is a molecular indicator linked to a variant of hypereosinophilia, which is myeloproliferative in nature, that is, CEL [25, 26]. The characteristic findings in CEL-NOS include an absolute peripheral eosinophilia count $>1.5 \times 10^9$ and a missing Philadelphia chromosome or *BCR-ABL* fusion gene [23].

Several chromosomal abnormalities have validated the neoplastic nature of CEL, the most common of which are trisomy 15, trisomy 8, isochromosome 17, translocation t(2;5) (p23;q31), and translocation t(5;12) (q33;p13) [27-31].

CHRONIC MYELOMONOCYTIC LEUKEMIA

In most patients with chronic myelomonocytic leukemia (CMML) gene mutations found (Fig. 2) are due to the following reasons: involvement of genes regulating histone methylation like ASXL1 (40-50%); EZH2 (5-10%), genes causing methylation and hydroxymethylation like TET2 (50-60%); IDH1 (<5%), IDH2 (5-10%), and DNMT3A (<5%); RAS pathway genes like KRAS (10%), NRAS (10%), and CBL (5-10%); and splicing complex genes like SF3B1 (5-10%), ZRSF2 (5-10%), and U2AF1. CMML, a type of cancer with clonal hematopoietic stem cell disorder. Patients with CMML present with sustained abnormal numbers of monocytes in the blood for more than 3 months (with a level above 950/ μ L equal to or greater than 10% monocytes) in addition to dysplastic features within the BM. CMML is usually defined as chronic leukemia with a persistent abnormal number of monocytes in the blood, and it has both proliferative and dysplastic features [32, 33]. Hence, a new category of myelodysplastic syndrome (MDS)/myeloproliferative neoplasms (MPN) has been established by the WHO so that CMML is not confused with other myeloproliferative disorders [34].

Furthermore, the WHO has subdivided CMML into CMML-1 and CMML-2 based on cell counts in BM biopsy and blood smears. CMLs with less than 5% blood cells in the blood and less than 10% in the BM cells are classified as CMML-1, and CMLs in which blast cells make up 5-20%

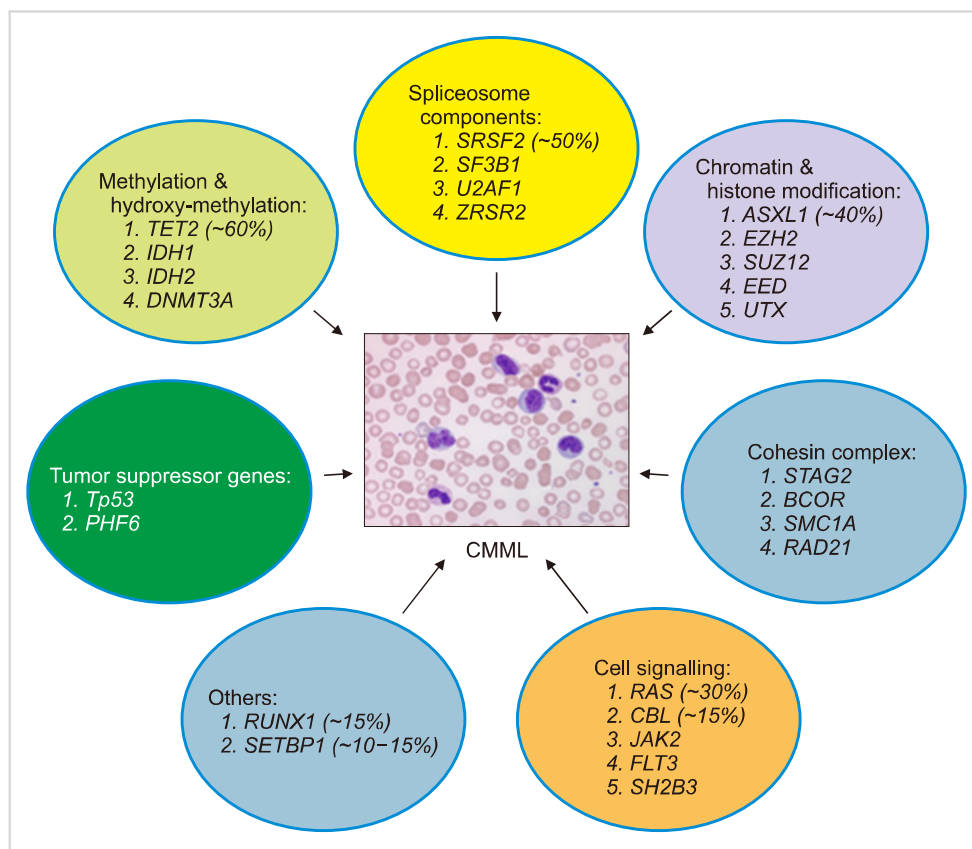


Fig. 2. Gene mutations detected in patients with chronic myelomonocytic leukemia (CMML).

of the WBC count and 10–20% of the BM cells are placed in the CMML-2 subcategory [35].

CMML is also associated with other mutations like JAK2 (5–10%), RUNX1 (10–15%), FLT3 (<5%), NPM1 (<5%), TP53 (1%) [36]. The exact genetic sequence leading to CMML is under investigation, but mutations in TET2 or ASXL1 have been seen in 40–60% of the patients. Furthermore, single-cell tracking experiments have shown that these mutations are the initial cellular changes that lead to CMML [37–39].

JUVENILE MYELOMONOCYTTIC LEUKEMIA

Juvenile myelomonocytic leukemia (JMML) is a rare, deadly form of blood cancer that affects younger children, usually below the age of 3 years [40, 41]. JMML is a bridging disorder between MDS and MPN. Although it was earlier classified as an MDS by the French-American-British scheme, the presence of both dysplastic and proliferative features raised controversies with respect to its classification. Hence, it has now been placed in a new category of MDS/MPN by the WHO to recognize the overlap between the two disorders [42–44].

A major criterion for the diagnosis of JMML is the absence of the Philadelphia chromosome (*BCR-ABL* fusion gene). Chromosomal abnormalities like monosomy 7, deletion of 7q, are seen in 25–30% of the patients, but these chromosomal translocations are non-consistent [45].

Spontaneous proliferation of granulocyte-macrophage colony stimulating factor (GM-CSF) from myeloid progenitor cells in colony forming assays (CFU-GM) can be seen in some cases of JMML; however, it is now considered a non-consistent feature for diagnosis [46] and is also noted in children with viral infections [47].

In most cases of JMML, genetic mutations in RAS/MAPK pathway genes such as PTPN11, KRAS, NRAS, and NF-1 have been noted [48]. Less commonly occurring mutations that activate intracellular signaling pathways, including RAS and JAK/STAT signaling, have also been identified, such as *RAC*, *RRAS*, and *JAK3*. The co-occurrence of JMML with RAS pathway genes has only been observed in some cases [49–51]. The relationship between RAS pathway mutations and clinical symptoms in JMML patients is under investigation. Some studies have shown spontaneous disease regression with *NRAS* mutations [52, 53]. However, cases of JMML regeneration of JMML been recorded in patients with *PTPN11* mutations [54].

CONCLUSION

According to WHO guidelines, the diagnosis of CML requires the results of morphological, immunophenotypic, and genetic testing with the examination of prior medical history and clinical details. Molecular testing is required to detect relevant chromosomal anomalies in CML. The presence of

immature megakaryocytes is a hallmark of CML and is confirmed by *BCR-ABL1* cytogenetic or molecular testing. The clinical findings in CML are insidious. The disorder is usually detected in the chronic phase when increased WBC counts are found in the blood or when splenomegaly is discovered on a general physical examination. Additionally, flow cytometry can be used to diagnose abnormal blasts and/or mature myeloid cells in the blood and BM. In a blast crisis, tracking lineage and atypical immunophenotypes can be useful. In addition, other subtypes of myeloproliferative neoplasms should be ruled out when making a diagnosis of CML. For example, ET presents with large and mature megakaryocytes on morphology, whereas PMF consists of abnormally maturing megakaryocytes with hyperchromatic and irregularly folded nuclei. Similarly, BM biopsy of PV displays hypercellularity, including marked proliferation of erythrocytes, granulocytes, and megakaryocytes. The diagnosis of PV is confirmed by the mutation of *JAK2* V617F or *JAK2* exon 12 [55].

Furthermore, recent developments in molecular genetics have shown the potential for further improvements in our understanding of CML and its treatment.

Authors' Disclosures of Potential Conflicts of Interest

No potential conflicts of interest relevant to this article were reported.

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