Chronic Myelocytic Leukemia – Part I: History, Clinical Presentation, and Molecular Biology

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DATA SOURCES: Current literature.

DATA SYNTHESIS: Chronic myelocytic leukemia (CML) was initially described in 1845 and is considered one of the first leukemias to be discovered. Diagnosis of CML was dramatically improved with the discovery of the Philadelphia chromosome by Nowell and Hungerford in 1960. However, the rudiments of our understanding of the molecular cause of CML began in 1973 when Janet Rowley discovered that the Philadelphia chromosome is a reciprocal translocation between chromosomes 9 and 22. The leukemogenic mechanisms of CML were hypothesized 20 years later when it was discovered that the t(9;22) translocation produced a fusion gene involving the BCR gene from chromosome 9 and the ABL protooncogene from chromosome 22. Multiple breakpoints in BCR produce fusion genes that are translated into chimeric protein products of different lengths that are associated with different leukemic subtypes.

CONCLUSION: Although CML has a rich history of interest to hematologists, it also represents a leukemogenic paradigm to the molecular biologist. Nearly all malignancies result from a series of mutagenic events, which culminate in full malignant transformation. However, it appears that CML results from a single mutagenic event involving the t(9;22) translocation leading to the development of the BCR/ABL fusion gene and the corresponding fusion protein. The successful transcription and translation of the BCR/ABL fusion protein led researchers to carefully study its involvement in leukemogenesis. The BCR/ABL fusion protein exhibits increased and constitutive tyrosine kinase activity that differs depending on which BCR breakpoint is expressed, resulting in varying clinical presentations.

ABBREVIATIONS: ABL = Ableson oncogene found in a strain of mouse leukemia virus; ALL = acute lymphocytic leukemia; BCR = breakpoint cluster region; CML = chronic myelocytic (myelogenous) leukemia; FAB = French-American-British; FAK = focal adhesion kinase; GEF = GDP-GTP exchange factor; JAK-STAT = janus kinase-signal transducers and activators of transcription; PI-3 Kinase = phosphoinositide-3 kinase; RAC GAP = RAS-like GTPase GTP activator; WHO = World Health Organization.

INDEX TERMS: BCR/ABL; chronic myelocytic leukemia; Philadelphia chromosome; t(9;22); tyrosine kinase inhibitor.

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Focus Continuing Education Credit*: see pages 57 to 59 for learning objectives, test questions, and application form.*

LEARNING OBJECTIVES: Following careful study of this review, the reader will be able to:

1. Discuss the first scientific description of CML;
2. Discuss the history of the Philadelphia chromosome to include the discovery of the "minute" chromosome 22 and the t(9;22) reciprocal translocation;
3. Describe the clinical and laboratory features of CML;
4. Sketch the t(9;22) translocation that produces the Philadelphia chromosome;
5. Describe the molecular biology of the four primary BCR/ABL fusion genes to include the four discrete breakpoints and the resulting gene arrangements;
6. Discuss the leukemogenic mechanisms in CML involving the BCR/ABL fusion protein; and
7. Compare three different versions of the fusion protein and discuss disease associations.

Chronic myelocytic leukemia (CML) is a myeloproliferative disorder that engenders scientific interest among groups as diverse as clinical hematologists, clinical laboratory professionals, molecular biologists, and oncologists. To the clini-

cal hematologist, CML represents a common hematologic disorder requiring careful scrutiny of both clinical and diagnostic information to make informed therapeutic decisions to maintain quality patient care. To the laboratory professional, CML is known to be the first malignancy directly linked to a genetic mutation, creating a reliable diagnostic tool. Molecular biologists are intrigued by the effect of a single mutagenic event on signal transduction pathways leading to malignant transformation. Since leukemogenic transformation in CML is sufficiently accomplished by the t(9;22) translocation, the multistep mechanisms of carcinogenesis necessary in most other forms of cancer are not required in CML creating a carcinogenic paradigm to the oncologist.

LEUKEMOGENIC AND THERAPEUTIC PARADIGM

Nearly all malignancies, regardless of type, are thought to be the result of a series of mutations in a progenitor cell that causes the cell to lose control of growth, differentiation, and apoptotic mechanisms resulting in full malignant transformation. When a cell line has received sufficient mutations to alter phenotype, but insufficient mutations to produce full malignant transformation, the cell line is said to be dysplastic or pre-malignant. The list and sequence of mutations commonly identified in a given cancer type seems to vary significantly between patients diagnosed with the same malignancy. This makes diagnosis and prognosis based on genetic abnormalities difficult for most malignancies. For a particular cancer type, patients will express different lists of mutations that appear at different stages during the progression of their disease. However, there are exceptions to this model and two such exceptions are hematopoietic malignancies, namely AML:M3 (also known as acute promyelocytic leukemia) and CML. In both cases it appears that a single mutation is sufficient to produce full leukemic transformation.

In AML:M3 the mutation is the t(15:17) translocation involving the PML/RARα genes, and in CML it is the t(9;22) translocation forming the Philadelphia chromosome that produces the BCR/ABL fusion gene. The phenomenon of full leukemogenic transformation from a single mutation makes both AML:M3 and CML leukemogenic paradigms. In addition, the discovery of single mutation transformations and their impact on leukemogenesis resulted in the development of tailored, single agent therapies targeting the products of these mutations, creating a therapeutic paradigm.

HISTORICAL ORIGIN OF CML

The first scientific description of CML is credited to John Hughes Bennett in Edinburgh in 1845.[1] However, patients with vague but similar symptoms can be found in the French literature as early as 1825. It is possible that some of these earlier patients may have also suffered from CML. For example, Velpeau reported a case of a 63-year-old woman who, at autopsy, was found to have an enormous spleen and whose blood was "thick like gruel such that one might have asked if it were not rather laudable pus, than blood".[2] Later in 1839 the French microscopist Paul Donne' described a 44-year-old woman who presented at autopsy with an enlarged spleen and whose blood seemed "semipurulent under the microscope with more than half of the cells appearing to be white globules".[3] Nevertheless, Bennett's description was more complete and scientific in nature thereby earning him credit as the first description of CML. Bennett became interested in the disorder when his mentor, Dr David Craigie, observed two patients admitted to the Royal Infirmary in Edinburgh with unusual blood consistency and a splenic tumor. The first patient was observed in 1841 but was dismissed as unusual until 1844 when a 28-year-old man presented with similar symptoms. John Bennett was given permission to perform an autopsy and study the pathology of this second case. His report entitled "Case of hypertrophy of the spleen and liver in which death took place from supperation of the blood" was published in the *Edinburgh Medical and Surgical Journal* in October 1845.[1]

In the same year, Robert Virchow, a 24-year-old graduate of the Berlin Army Medical School, observed his first case of CML while studying the pathology of phlebitis. A 50-year-old woman was admitted to the Charite' Hospital in Berlin complaining of fatigue, nosebleeds, swelling of the legs and abdomen, and died within four months. Virchow noted the enlarged spleen and liver, but also described blood vessels full of material resembling pus. In 1847 Virchow suggested the term "leukamie" for the disorder, meaning white blood, but it did not achieve universal approval because many physicians agreed that "blood was never white".[3] In 1852, Bennett recommended the term "leucocythemia", meaning increased white blood cells, which was better accepted, especially in light of the 37 cases Bennett had described to date.[4] In 1856, Virchow was credited with concluding that the disorder was not the result of an infectious process but rather was caused by the tissue that produced the white cells. He also categorized two types of chronic leukemia, splenic and lymphatic, which we now know as leukemia and lymphoma, respectively.[1]

Today we understand CML as a malignant clonal disorder of the pleuripotential hematopoietic stem cell, resulting in proliferation of predominantly immature myeloid cells. However, CML also affects cells of the monocytoid, eryth-

roid, megakaryocytic, B-lymphoid and occasionally T-lymphoid lineages. The malignant transformation results from a reciprocal translocation between chromosomes 9 and 22, producing a short chromosome known as the Philadelphia chromosome. This translocation sets into motion a sequence of events that results in uncontrolled proliferation predominantly involving the myeloid hematopoietic element, incomplete differentiation and reduced apoptosis.

CLINICAL PRESENTATION

The clinical presentation of CML most commonly involves middle-aged men who complain of fatigue, anorexia, and weight loss. However, individuals of any age or gender can develop CML. Other symptoms include abdominal fullness associated with splenomegaly, bleeding and purpura resulting from abnormal platelet counts and function, leukocytosis from the increased proliferation and impaired differentiation of the WBCs, and anemia from bone marrow suppression. In the not so distant past the five-year survival was less than 30% but, as we will see in this discussion, that statistic is rapidly changing.

CML typically follows a triphasic clinical course that begins in the chronic phase and progresses through the accelerated phase to the terminal blast crisis phase. The chronic phase is a slow, smoldering period in which patients experience mild symptoms. In the accelerated phase, the disease is shifting from a chronic disorder featuring immature myeloid cells that are intermediate in the differentiation pathway, to a more acute picture characterized by an increase in the number of blasts. The accelerated phase progresses quickly into blast crisis phase, where the leukemia has fully converted from a chronic to an acute form.

The diagnosis of CML primarily occurs in the laboratory. The documentation of a very high WBC count (often greater than 100 x 10^9/L), a left shift, occasional blasts, low leukocyte alkaline phosphatase (LAP), mild anemia, thrombocytosis, and the presence of the t(9;22) Philadelphia chromosome, establishes the diagnosis. Although most patients will not exhibit all these characteristics, the presence of a high WBC count, left shift, and Philadelphia chromosome is sufficient to confirm the diagnosis. Molecular assays involving Southern blot, PCR, or fluorescence in-situ hybridization (FISH), are usually considered for suspicious cases that are Philadelphia chromosome negative by karyotype analysis.

Uncontrolled proliferation, poor differentiation, and diminished apoptosis resulting from the 9;22 translocation, is exemplified in the high WBC, left shift, and occasional blast. The stem cell origin of the disorder is reflected in the concomitant thrombocytosis and the eventual production of blasts that are frequently not of the myeloid line. The low LAP confirms abnormal myeloid function suggesting a malignant origin. A bone marrow analysis is often unnecessary to establish the diagnosis, but when performed will generally reflect the peripheral findings. The bone marrow will be hypercellular with an elevated M:E ratio corresponding to the elevated WBC count and the left shift. An increase in megakaryocytes is responsible for the thrombocytosis, and the combination of bone marrow suppression by the leukemic myeloid cells and the production of fibrotic tissue produce the anemia.

PHILADELPHIA CHROMOSOME (Ph1)

The discovery of the Philadelphia chromosome had a major impact on CML diagnosis. The Philadelphia chromosome was discovered by Nowell and Hungerford in 1960 at the University of Pennsylvania in Philadelphia.[5] Although experimentation in chromosomal analysis began in the 1940s, it wasn't until the 1950s that the technology was applied to neoplastic disorders and leukemias. Nowell and Hungerford described a minute acrocentric chromosome, termed the Philadelphia chromosome, that was initially observed in two male patients, followed by seven other patients all with CML. Blood cells from the CML patients were cultured on a microscope slide tilted in a culture bottle to provide a gradient of cell numbers, oxygen tension, and oxidation/reduction potential. As the cells matured they fell off the slide and settled in the bottom of the jar. Colchicine was added to stop cell division in metaphase and the cells were swollen in a hypotonic medium. The chromosomes were then photographed and observed for deviation from normal.

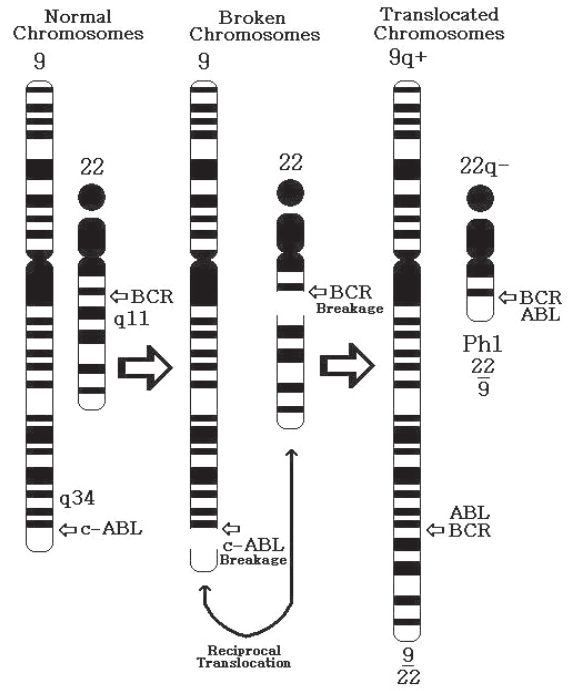
The Philadelphia chromosome was identified as a "minute" chromosome and was subsequently observed in about 90% of patients with CML. The name was later shortened to Ph1 to indicate the first chromosome in what researchers expected to be a string of associations between consistent chromosomal abnormalities and malignancies. However, for more than a decade the Philadelphia chromosome remained the only chromosomal lesion consistently associated with a specific neoplastic disease.

The Philadelphia chromosome was first thought to be chromosome 21 but when banding techniques were introduced it was discovered that the Philadelphia chromosome was actually a deletion of chromosome 22. It wasn't until 1973 when Janet Rowley, from University of Chicago, was able to show that the Philadelphia chromosome was not simply a deletion of the long arm of chromosome 22, but rather a reciprocal translocation between chromosomes 9 and 22.[6] Rowley separated the red blood cells

from the white blood cells in CML patients and cultured the WBCs both with and without phytohemagglutinin (PHA), a lymphocyte mitogen. Cells in PHA were used as the normal control because PHA stimulates cell division. In contrast, only leukemic cells could proliferate in the absence of PHA. The t(9;22) translocation was resolvable using standard staining and fluorescent staining techniques. As can be seen in Figure 1, chromosome 22 is broken just below the centromere while chromosome 9 is broken near the distal end of the long arm. The small piece from chromosome 9 is translocated to chromosome 22 creating a significantly shortened chromosome 22, previously described as a "minute" by Nowell and Hungerford. In contrast, chromosome 9 is lengthened due to the removal of a small segment from the distal end and the addition of a large segment from chromosome 22. The Philadelphia chromosome translocation is now known to occur as t(9;22)(q34;q11).

The value of the Philadelphia chromosome as a diagnostic tool became immediately obvious when it was observed in the karyotypes of approximately 90% to 95% of the CML cases tested. When molecular hybridization techniques were introduced and applied to Philadelphia negative CML cases, it was found that many of these exhibited the translocation at the molecular level.[7] Apparently, the original translocation may be followed by another translocation, either between chromosomes 9 and 22 or other chromosomes, that redistributes sufficient genetic material to restore the chromosomes to nearly the original length. The slight difference in genetic material between karyotype negative and karyotype positive Philadelphia chromosome samples in this scenario is not resolvable by karyotype analysis. Therefore, taken together, nearly all patients with CML were found to have the translocation producing the Philadelphia chromosome identified either at the chromosomal or molecular level.

Figure 1. Philadelpha chromosome t(9:22)



ABL = Ableson oncogene, BCR = Breakpoint Cluster region, 9q+ = Lengthened chromosome 9, 22q− = Shortened chromosome 22, Ph1 = Philadelphia Chromosome

In addition, the Philadelphia chromosome is found in 5% of children with ALL, 15% to 30% of adults with ALL and 2% of patients with newly diagnosed AML.[8,9] The most obvious explanation for these findings is that many of these patients may represent the blast crisis phase of a previously undiagnosed CML. However, increasing numbers of investigators were finding cases of apparently de novo acute leukemia that are Philadelphia chromosome positive. This phenomenon can best be explained by the discovery that the site of breakage on chromosome 9 occurs at different places, which alters the product of the translocation and thus the leukemogenic mechanism.

PATHOPHYSIOLOGY OF CML

The pathophysiology of CML is a very interesting story that has become clearer in recent years. The origin of the malignant transformation in CML is understood to be the Philadelphia chromosome. This connection is supported by three pieces of evidence. First, nearly all patients with CML have the 9;22 translocation. Second, in most cases of CML, Philadelphia chromosome is the only genetic lesion detected. Last, Philadelphia chromosome is rarely detected in malignancies that do not have a CML origin. The cause of the 9;22 translocation is not known, but an increased incidence has been associated with radiation and benzene exposure. The link to radiation was made when the number of cases of CML increased 100 fold in survivors of the nuclear bombing of Hiroshima and Nagasaki during World War II. A significant increase was also noted among radiologists prior to lead shielding and in workers exposed to benzene. There are about two new cases of CML each year per 100,000 people, accounting for about 15% of leukemias in adults. CML affects middle-aged men more frequently, with a male to female ratio of 3:2 and a median age at diagnosis of 53 years. About 40% of CML diagnoses are made in asymptomatic patients solely from laboratory observations of abnormal blood counts and differentials.

As stated earlier, CML follows a triphasic clinical course with the chronic phase lasting approximately four years, the accelerated phase between six to eighteen months and the blast crisis phase terminating in death in less than eight months. The chronic phase is characterized by a high WBC count, a left shift, thrombocytosis, and a mild to moderate normocytic/normochromic anemia. The WBC is usually greater than 100 x 10^9/L with a typical range of 200-500 x 10^9/L. WBC counts as high as 1.0 x 10^{12}/L have been reported. The left shift reveals all stages of myeloid differentiation with a noticeable increase in promyelocytes, metamyelocytes, and myelocytes. Basophilia and eosinophilia are also common.

Approximately half of the patients will present with thrombocytosis and a mild to moderate anemia producing a hemoglobin value of between 9 and 13 g/dL. A bone marrow analysis is usually not necessary for diagnosis but when performed will exhibit hypercellularity, an M:E ratio of between 10:1 to 50:1, a notable left shift, and occasional presence of fibrotic tissue. Blasts may be increased but must be less than 30% in the bone marrow to be classified as chronic leukemia by FAB criteria and less than 20% by WHO criteria. Most patients present with minimal symptoms but usually exhibit hepatosplenomegaly, resulting from extramedullary hematopoiesis in the liver and spleen.

The accelerated phase of CML is marked by an increasing WBC and basophil count, a decreasing platelet and RBC count and, most notably, an increase in circulating blasts. Increasing blasts in the presence of immature myeloid cells indicate the transformation from chronic leukemia to acute leukemia. The bone marrow shows an increased number of blasts with suppression of erythroid and megakaryocytic proliferation, which is responsible for the presence of peripheral blasts, anemia, and thrombocytopenia in the blood. The promyelocytes, myelocytes, and metamyelocytes observed in the chronic phase are more likely to be released into circulation as compared to the blasts that accumulate in the accelerated phase.

Blasts possess the necessary homing receptors to a greater degree than do their more mature counterparts, which facilitate retention in the bone marrow resulting in cellular accumulation. The increasing number of blasts and the proliferation of fibrotic tissue contribute to bone marrow suppression that produces the anemia and thrombocytopenia characteristic of the accelerated phase of CML. Symptoms of fever, night sweats, weight loss, and splenomegaly are exacerbated in the accelerated phase. Additional chromosomal mutations are observed in this stage of CML and are largely responsible for the transformation from the chronic to the acute clinical picture. The accelerated phase of CML lasts approximately six to eighteen months with 30% of patients dying prior to entering blast crisis.[10]

Prior to tyrosine kinase inhibitors, once a patient enters blast crisis, interventions were futile and death imminent. Both the symptoms and the peripheral blood abnormalities intensify. However, in about one fourth of CML patients, the blast crisis phase occurs without the typical transition through the accelerated phase.[10] The differential reflects the increasing number of blasts and the worsening anemia and thrombocytopenia. Patients develop bleeding symptoms from the thrombocytopenia, bone tenderness from the expanding bone marrow and gouty arthritis from uric acid build-up, as cell turnover increases. The bone marrow reflects the increasing blasts, and the stage is marked by a bone marrow blast count of >30% by FAB criteria. About 25% to 35% of patients that enter blast crisis produce ALL, while about 65% to 75% result in AML. Less than 10% of cases result in acute leukemias of other lineages. Historically, death would occur in less than eight months after entering blast crisis, generally from bleeding, infection, or bone marrow aplasia. Patients with ALL blast crisis have a higher complete remission rate (60%) as compared to patients with AML blast crisis (20-30%), but the duration of remission is less than one year.[11] The hopelessness associated with blast crisis is changing with the advent of targeted molecular therapy involving tyrosine kinase inhibitors.

An atypical form of CML has been observed in children. This atypical form is termed juvenile CML by the FAB group and is omitted in the WHO classification system. It accounts for between 1% and 5% of childhood leukemias and generally affects children under five years of age. The WBC count is usually between 15 x 10^9/L and 100 x 10^9/L at diagnosis with a mean of 30 x 10^9/L. There is a slow increase in blasts and the children develop skin rashes and infections. Juvenile CML progresses faster than adult CML producing death in about two years.

MOLECULAR BIOLOGY OF t(9;22) IN CML

Our understanding of the molecular biology of the t(9;22) translocation has contributed to the current theories of leukemogenesis in CML. Once the ABL oncogene was mapped to the long arm of chromosome 9 it was quickly confirmed that the t(9;22) translocation brought together the ABL oncogene to an unknown area of chromosome 22. It was later discovered that the breakpoints that occurred on chromosome 22 clustered within a limited region on the long arm that was subsequently termed breakpoint cluster region (BCR).[12] There-

fore, the t(9;22) translocation creates a BCR/ABL fusion gene that is transcribed into a chimeric BCR/ABL mRNA and translated into a hybrid protein.[8] As can be seen in figure 1, the 9;22 translocation interrupts the ABL oncogene on chromosome 9 and the BCR gene on chromosome 22. The ABL gene is a murine viral oncogene that is 230 kilobases in length and contains 11 exons with two splice sites (Figure 2a). The ABL gene normally codes for a 145 kilodalton nuclear protein, called p145, that possesses tyrosine kinase activity. In contrast, the BCR gene complex is composed of at least four separate genes termed BCR1, BCR2, BCR3, and BCR4. BCR1 is the most common BCR gene involved in the 9;22 translocation and is illustrated in the upper panel of Figure 2a. BCR1 is approximately 100 kilobases in length and divided into 20 exons with two splice sites. The gene normally codes for a 160 kilodalton protein (p160) that is constitutively expressed in many cell types, but strongly expressed in hematopoietic cells.

NORMAL PROTEIN PRODUCT OF THE ABL GENE (p145)

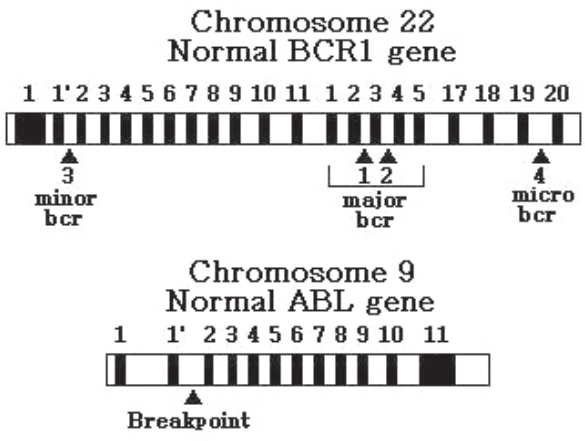
P145 is the gene product of the normal c-abl protooncogene, and is a nuclear protein with non-receptor, tyrosine kinase activity. The p145 protein has been shown to migrate between the nucleus and cytoplasm.[13,14] This activity has been linked to cellular growth control by being associated with several growth factor receptors like epidermal growth factor (EGF), platelet derived growth factor (PDGF), and colony stimulating factor (CSF). The protein p145 is found in Drosophila and functions in the regulation of normal cell proliferation. It is also highly conserved in vertebrates and strongly expressed in hematopoietic cells. Based on this information, it is generally accepted that p145 is involved in signal transduction pathways through the phosphorylation and subsequent activation of nuclear proteins.

Signal transduction pathways are biochemical pathways that, through a series of molecular reactions, transmit a signal through the cytoplasm, to the nucleus. The signal is usually initiated by a ligand/receptor interaction at the cell surface that sets the pathway in motion. Nuclear proteins will be stimulated to bind DNA and facilitate the activation of select genes. The protein products of these genes will serve a specific function in the cell, growth control in the case of CML. The functional domains of p145 are illustrated in Figure 3a. The myristoylation site near the amino terminus functions to localize the protein to the nucleus. The SH3 domain suppresses tyrosine kinase activity and therefore functions to down regulate cell proliferation. SH2 domain interacts with tyrosine-phosphorylated proteins, essentially holding them in place so the SH1 domain can perform the actual phosphorylation function. In most signal transduction pathways the phosphorylation of target messenger proteins activates the proteins to turn on the pathway. The C-terminal domains are poorly defined but seem to function to bind nuclear proteins, DNA, and actin.

NORMAL PROTEIN PRODUCT OF THE BCR GENE (p160)

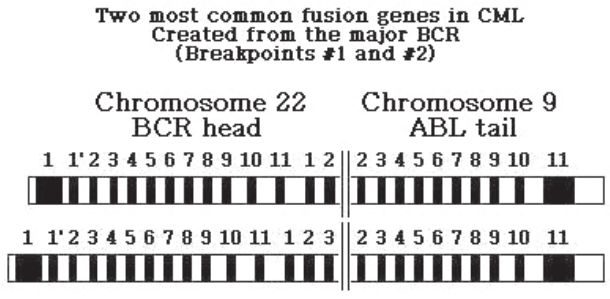
The normal function of p160 protein, transcribed from the BCR gene, is less well understood. It is constitutively expressed in many cell lines and strongly expressed in hematopoietic cells. The important functional domains are illustrated in Figure 3a. The coiled-coil motif is essential for polymerization with other proteins. The dimerization domain (DD) facilitates the formation of protein dimers. The tyrosine residue at position 177 is

Figure 2a. Normal BCR and ABL genes



ABL = Ableson oncogene; BCR = breakpoint cluster region

Figure 2b. BCR-ABL fusion gene (p210)



ABL = Ableson oncogene; BCR = breakpoint cluster region.

of particular importance in that it is an essential contact point for the binding of signal transduction proteins. The next functional domain to the right is the serine/threonine kinase domain that serves as the catalytic domain for phosphorylation activity. The GEF domain stands for GDP/GTP exchange factor and functions to compete with other GTP binding proteins for GTP, which is used as the phosphate donor for phosphorylation reactions. The RAC GAP domain controls the rate of GTP hydrolysis and is sometimes called RAS-like GTPase. The RAS GAP domain functions by converting active RAS proteins to their inactive form, when bound to GDP. So both proteins appear to function in the phosphorylation of other proteins involved in signal transduction pathways that serve to stimulate and regulate cell growth.

BCR/ABL FUSION GENES

As can be seen in Figure 2b, the 9;22 translocation brings together the 5' portion of the BCR gene with the 3' end of the ABL gene. It has been shown that there exists at least four primary versions of the BCR/ABL translocation resulting from four distinct breakpoints in the BCR gene. These four breakpoints create three different protein products, p210 (Figure 3b), p190 (Figure 3c), and p230 (Figure 3d), which may account for some of the differences in leukemogenesis between CML, Philadelphia chromosome positive de novo ALL, and chronic neutrophilic leukemia (CNL), respectively.[15,16] The four known breakpoints on the BCR1 gene are illustrated in Figure 2a by the upward arrowheads. The most common breakpoint region in the BCR 1 gene is called major BCR (M-BCR) and is located in the middle of the BCR 1 gene. Major BCR contains five exons (labeled 1-5) and two of the four known breakpoints. These five exons within M-BCR correspond to exons 12-16 of the BCR1 gene.

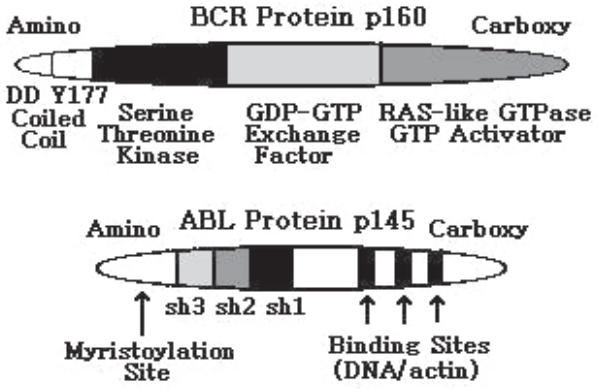
The majority of breakpoints that occur in the BCR gene are between exons 2 and 3 (arrowhead #1) or between 3 and 4 (arrowhead #2). There are only two known breakpoints in the ABL gene occurring either between exons 1 and 1' or between exons 1' and 2. The fact that there are two breakpoint options in the ABL gene is irrelevant because RNA splicing always results in the lead exon being number 2 (Figure 2a). Therefore, the genetic contribution of the ABL gene remains constant. The two common breakpoint possibilities in M-BCR, coupled with the only possibility in ABL, results in the two transcripts illustrated in Figure 2b. The upper fusion gene represents the first 13 exons of the BCR and the last 10 exons of ABL, while the lower fusion gene represents the first 14 exons of BCR and the same 10 exons from the ABL gene. In both cases the protein product is a 210 kilodalton protein that is either 902 or 927 amino acids in length. This is the protein product associated with classical CML.

An alternative breakpoint region on the BCR1 gene is termed minor BCR (m-BCR), and is located 5' of the major BCR.[15] This breakpoint is associated with the majority of cases of Philadelphia positive ALL and in rare cases of CML that tend to produce a monocytosis.[17] In this case, only exon 1 is joined to the same 10 exons of the ABL gene, translating into a smaller 185/190 kilodalton protein shown in the upper panel of Figure 2c. The last breakpoint region on BCR occurs between exon 19 and 20 creating a longer fusion protein, 230 kilodaltons in size, illustrated in the lower panel of Figure 2c.[18] This version is rarely observed in CML, but when identified seems to produce a version of CML called chronic neutrophilic leukemia (CNL), that is characterized by an abundance of more mature neutrophils and thrombocytosis. Other fusion products of the t(9;22) translocation have been described but rarely occur.[19]

BCR/ABL FUSION PROTEIN

The understanding of the composition of the BCR/ABL fusion protein and the functions of the corresponding wild-type BCR and ABL proteins, allows us to predict the function of the fusion protein and its ultimate role in leukemogenesis. The ABL moiety of the fusion protein contributes to the transforming capability of the protein in at least three ways. First, and most important, the ABL moiety of the fusion protein exhibits alterations in the normal function of the SH2 and SH3 domains that control the tyrosine kinase activity of the SH1 domain (Figure 3b). It is well

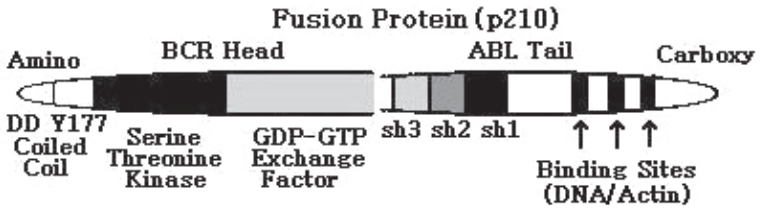
Figure 3a. Normal BCR and ABL genes



ABL = Ableson oncogene; BCR = breakpoint cluster region; GDP = guanosine diphosphate; GTP = guanosine triphosphate; RAS = reticular activating system; sh1-2-3 = SRC homology protein.

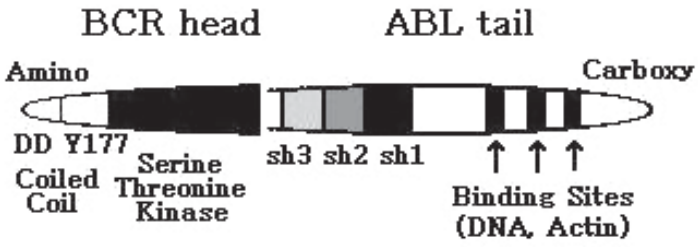
established that tyrosine kinase functions to add phosphate groups to other proteins. In signal transduction pathways designed to control cell proliferation, increased phosphorylation promotes proliferation, and dephosphorylation inhibits proliferation. In the wild-type ABL protein, the SH2 domain normally up regulates tyrosine kinase activity, and the SH3 domain down regulates tyrosine kinase activity. The t(9;22) translocation event has created a fusion gene and a resultant protein product that has lost the amino terminus of the ABL gene designed to regulate tyrosine kinase activity and has gained BCR genes that will affect the tyrosine kinase activity of the SH2 and SH3 domains. The result is constitutive tyrosine kinase activity.

Figure 3b. BCR-ABL fusion protein (p210)



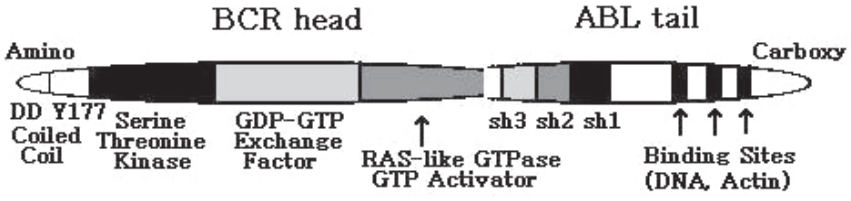
DD = dimerization domain; GDP = guanosine diphosphate; GTP = guanosine triphosphate; sh1-2-3 = SRC homology protein; Y177 = tyrosine 177.

Figure 3c. BCR-ABL fusion protein (p190)



DD = dimerization domain; GDP = guanosine diphosphate; GTP = guanosine triphosphate; sh1-2-3 = SRC homology protein; Y177 = tyrosine 177

Figure 3d. BCR-ABL fusion protein (p230)



DD = dimerization domain; Y177 = tyrosine 177; GDP = guanosine diphosphate; GTP = guanosine triphosphate; RAS = reticular activating system; sh1-2-3 = SRC homology protein.

Second, the breakpoint in the 5' end of the ABL gene occurs in the myristoylation domain. The loss of genetic material in the myristoylation domain of ABL results in an altered binding affinity for F-actin. This may contribute to a reduction in adherence of CML cells to bone marrow stromal elements resulting in a reduction of contact inhibition and the premature release of immature myeloid cells into circulation.[20] In vitro studies have confirmed that primary CML cells adhere poorly to bone marrow stroma.[21,22] Antisense oligonucleotides to BCR-ABL and interferon-alpha have both been shown to reverse the loss of adhesion of CML progenitor cells to bone marrow stroma and fibronectin resulting in a reduction in proliferation.[23-25]

Third, loss of the myristoylation domain may also interfere with apoptotic mechanisms. The myristoylation domain normally confers nuclear localization properties to the wild-type ABL protein allowing it to shuttle between the nucleus and cytoplasm of the cell. The loss of this domain may be responsible, at least in part, for the lack of nuclear localization observed for the fusion protein. The BCR/ABL fusion protein is restricted to the cytoplasm due mainly to the constitutive activation of the tyrosine kinase. Wild-type ABL located in the nucleus has apoptotic properties, while BCR/ABL localized to the cytoplasm has anti-apoptotic functions.[26]

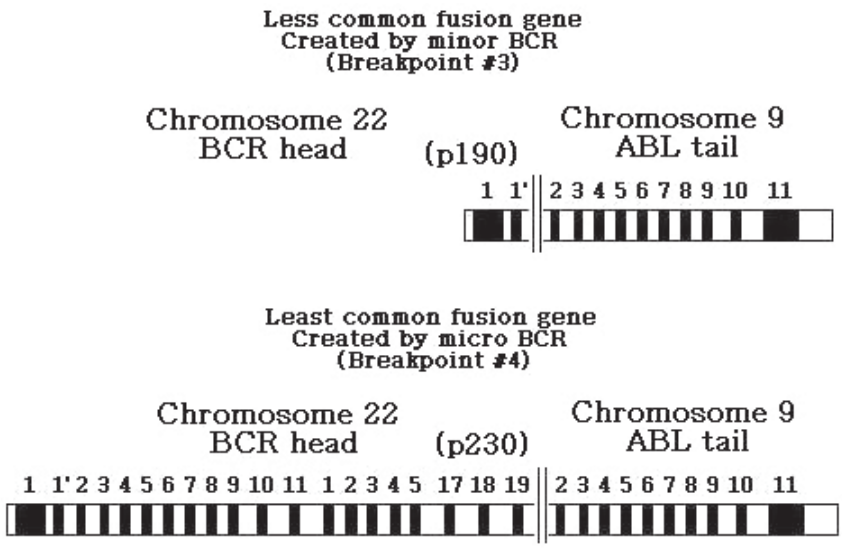
The leukemogenic contribution of BCR to the fusion protein centers on the coiled-coil motif and the serine/

threonine domain. The t(9;22) translocation preserves these two domains and transposes them to the 5' end (head) of the fusion gene. The coiled-coil motif of BCR stimulates SH2 and inhibits SH3 resulting in continuous tyrosine kinase activity. The tyrosine at position 177 (Y177) in the coiled coil motif is crucial to the binding of adaptor proteins like Grb-2 that serve to initiate signal transduction pathways. The serine/threonine kinase domain of BCR participates in leukemic transformation by retaining kinase activity, and by the activation of several signal transduction pathways involving SH2 proteins, the most important of which is the RAS pathway.[20]

The two remaining BCR domains, GEF (GDP-GTP exchange factor) and RAC GAP (RAS-like GTPase), may also contribute to the transforming function of the fusion protein. GEF domain binds GTP to facilitate phosphorylation, the primary function of the kinases. The RAC GAP domain normally functions to help regulate kinase activity. Therefore, the loss of this domain results in a loss of phosphorylation control within the serine/threonine domain.

Taken together, these aberrant cellular functions within both the BCR and ABL domains of the fusion protein collaborate to produce the clinical picture associated with the chronic phase of CML. The continuous activation of tyrosine kinase, together with the altered binding affinity of the fusion protein for membranes and DNA, and the activation of signal transduction pathways involving oncogenes like RAS, are sufficient to produce full transforming capabilities. It is thought that the BCR-ABL fusion protein activates the same signal transduction pathways normally activated by cytokines that control growth and differentiation. Therefore, the cells bearing the BCR-ABL fusion protein are behaving as though they are receiving constant cytokine signals stimulating proliferation at the expense of differentiation.[27] In contrast, one potential explanation for the acute leukemia phenotype associated with the p190 fusion protein (Figure 3c)and the chronic presentation associated with the p210 fusion protein involves the increased tyrosine kinase activity produced by the p190 fusion protein.[28,29]

Figure 2c. BCR-ABL fusion genes (p190 and p230)



ABL = Ableson oncogene; BCR = breakpoint cluster region.

Progression of CML from the chronic phase to the accelerated and blast crisis phases generally involves additional genetic mutations. Cells dividing more rapidly than normal and containing genetic lesions, as do the myeloid cells in the chronic phase of CML, are more prone to additional genetic mutations as compared to normal cells. In addition, chemotherapy increases the rate of genetic mutations. Therefore, given the mutagenic predisposition of the 9;22 translocation and chemotherapeutic interventions, the additional mutations needed to progress from the chronic phase to the accelerated and blast crisis phases will usually occur. Some of the additional mutations responsible for progression to the accelerated and blast crisis phases of CML can be identified by karyotype analysis, while others require molecular techniques for identification. Monosomy of chromosomes 7, 17, or Y, trisomy 8, 17, 19, and 21, an additional Philadelphia chromosome, and the 3;21 translocation that is sometimes encountered in acute leukemias, are examples of compounding chromosomal lesions. Additional genetic mutations, resolvable at the molecular level include p53, RB1, c-MYC, RAS, and AML-EVI-1.[20] Mutations in these genes have produced proteins associated with malignant transformation in many other cancer systems.

PROPOSED LEUKEMOGENIC MECHANISM

The most widely accepted and significant leukemogenic mechanism attrib-

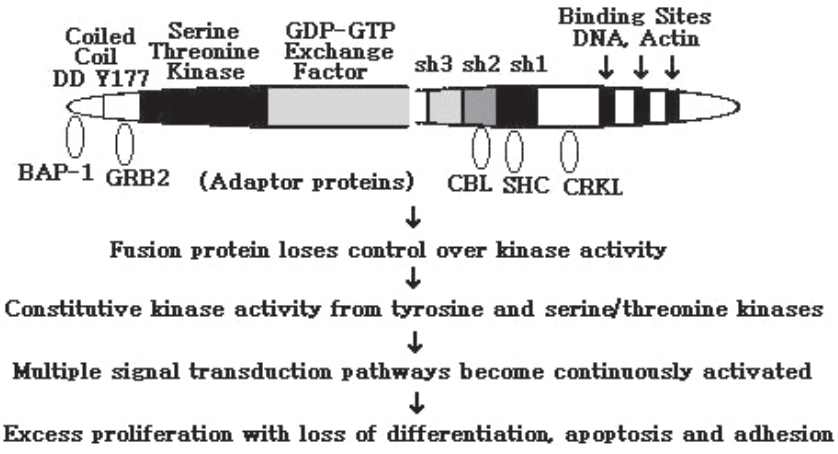
uted to the fusion protein involves the constitutive stimulation of tyrosine kinase. The tyrosine kinase activity affects a variety of signal transduction pathways. Various regions of the fusion protein bind and activate several adapter proteins, five of which are illustrated in Figure 4 as, from left to right, BAP-1, GRB2, CBL, SHC and CRKL. These adaptor proteins normally bind the same regions of the wild-type ABL and BCR proteins as occurs with the fusion protein. However, binding of the adaptor proteins to the fusion protein dramatically alters their normal activation cycle. As stated earlier, the coiled coil motif and the serine/threonine kinase domain at the amino terminus of BCR, up regulates the SH2 domain and inhibit SH3 domain of the ABL moiety, occupying the carboxy terminus of the fusion protein. This participates in constitutive stimulation of the tyrosine kinase activity of the fusion protein. The serine/threonine kinase domain of the BCR moiety may also contribute additional kinase activity to increase the overall rate of phosphorylation.

Increased phosphorylation of adaptor proteins stimulates several signal transduction pathways, maintaining them in the "on" position. The most important of these signal transduction pathways is the RAS pathway. The constitutive tyrosine kinase activity produced by the fusion protein results in an increased activation of RAS, a known oncogene, creating proliferation that is independent of cytokine control. It has been shown in vitro that leukemogenic transformation can be prevented in cells expressing BCR-ABL tyrosine kinase activity by inhibiting RAS pathways.[30] This aberrant activation also appears to protect against the pathway of natural cell death called apoptosis. The result is malignant transformation of myeloid cells that are prone to accelerated division, reduction in apoptosis, and failure to fully differentiate.

In a similar way, the constitutive tyrosine kinase activity phosphorylates adaptor proteins that up regulate other signal transduction pathways like JAK-STAT (Janus kinase-signal transducers and activators of transcription) and PI-3 kinase (phosphoinositide-3 kinase). These pathways are thought to induce cell proliferation and, when inhibited, prevent the growth of cells expressing the BCR-ABL fusion gene.[31] Therefore, a larger number of myeloid precursors are engaged in cell division and fewer succumb to apoptosis. The combination of reducing apoptosis, increasing proliferation, and incomplete differentiation, results in an increase in immature WBCs in the bone marrow.

The phosphorylation activity of the fusion protein also activates the FAK (Focal Adhesion Kinase) pathway. The FAK pathway seems to decrease cellular adhesion to bone marrow stroma in vitro.[21,22] CML cells that are unbound, or free in suspension, tend to divide more readily than cells that are bound to another cell or to a molecular matrix normally found in the bone marrow. The normal process that produces a reduction in proliferation from cells binding to each other or to matrices is called contact inhibition. The expression of integrins on the cell surface facilitates adhesion. Therefore, a reduction in integrins minimizes adhesion thus reducing contact inhibition. A reduction in inhibition is effectively stimulating division. A lack of adhesion to molecular matrices in the bone marrow also facilitates the premature release of CML cells from the bone marrow into circulation. This process allows the immature myeloid cells that are accumulating in the bone marrow to be released into circulation, increasing the peripheral WBC count and producing the typical left shift associated with CML. In addition, the premature release of these myeloid cells reduces the crowding and choking of the normal bone marrow element effectively minimizing

Figure 4. Leukemogenic mechanisms in CML



DD = dimerization domain; GDP = guanosine diphosphate; GTP = guanosine triphosphate; sh1-2-3 = SRC homology protein; Y177 = tyrosine 177

the anemia and thrombocytopenia that is typically associated with acute leukemias.

An understanding of the role of the BCR-ABL gene product in the leukemogenesis of CML has led researchers to develop designer drugs to specifically target the fusion protein. These tyrosine kinase inhibitors are replacing the conventional chemotherapeutic approach to the treatment of CML. Although patient responses are very encouraging, these designer drugs are not without side effects. Some patients have experienced drug resistance and adverse events. In addition, molecular techniques used to monitor patient responses to tyrosine kinase inhibitor therapy, appear to predict outcome and guide therapeutic decisions. Therapeutic approaches involving tyrosine kinase inhibitors and the prognostic value of molecular monitoring will be discussed in Part II of this review.

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REFERENCES

1. Bennett JH. Case of hypertrophy of the spleen and liver in which death took place from supperation of the blood. Edinburgh Med Surg J 1845;64:413-23.
2. Hayhoe FJG. Leukaemia: research and clinical practice. London: Churchhill; 1960. p 2-5.
3. Geary CG. The story of chronic myeloid leukaemia. Br J Haematol 2000;110(1):2-11.
4. Bennett JH. Leucocythaemia, or white cell blood in relation to the physiology and pathology of the lymphatic glandular system. Sutherland and Knox, Edinburg, 1852.
5. Nowell P, Hungerford DA. A minute chromosome in human granulocytic leukemia. Science 1960;132:1497-9.
6. Rowley J. A new consistent chromosomal abnormality in chronic myeloid leukemia identified by quinadine fluorescence and Giemsa staining. Nature 1973;243:290-3.
7. Toth FD, Kiss J, Kiss A, and others. Co-expression of c/abl and c-myb oncogenes in Philadelphia chromosome-negative, bcr-negative chronic myeloid leukemia. Leuk Res 1994;18:373-80.
8. Kurzrock R, Gutterman JU, Talpaz M. The molecular genetics of Philadelphia chromosome-positive leukemias. N Engl J Med 1988;319:990-8.
9. Specchia G, Mininni D, Guerrasio A, and others. Ph positive acute lymphoblastic leukemia in adults: molecular and clinical studies. Leuk Lymphoma 1995;18 Suppl:37-42.
10. Kantarjian HM, Dixon D, Keating MJ, and others. Characteristics of accelerated disease in chronic myelogenous leukemia. Cancer 1988;61:1441-6.
11. Derderian PM, Kantarjian HM, Talpaz M, and others. Chronic myelogenous leukemia in the lymphoid blastic phase: characteristics, treatment responses, and prognosis. Am J Med 1993;94:69-74.
12. Groffen J, Stephenson JR, Heisterkamp N, and others. Philadelphia chromosome breakpoints are clustered within a limited region, bcr on chromosome 22. Cell 1984;36:93-9.
13. Van Etten RA, Jackson P, Baltimore D. The mouse type IV c-abl gene product is a nuclear protein, and activation of transforming ability is associated with cytoplasmic localization. Cell 1989;58:669-78
14. Lewis JM, Baskaran R, Taagepera S, and others. Integrin regulation of c-abl tyrosine kinase activity and cytoplasmic-nuclear transport. Proc Natl Acad Sci USA 1996;93:15174-9.
15. Kurzrock R, Shtalrid M, Romero P, and others. A novel c-abl protein product in Philadelphia-positive acute lymphoblastic leukemia. Nature 1987;325:631-5.
16. Denny CT, Shah NP, Ogden S, and others. Localization of preferential sites of rearrangement within the bcr gene in Philadelphia chromosome-positive acute lymphoblastic leukemia. Proc Natl Acad Sci 1989;86:4254-8.
17. Ravandi-Kashani F, Cortes J, Talpaz M, and others. Chronic myelogenous leukemia (CML) with the p185 BCR/ABL hybrid protein (Abstract). Blood 1998;92 S1:94a.
18. Pane F, Frigeri F, Sindona M, and others. Neutrophilic-chronic myeloid leukemia: a distinct disease with a specific molecular marker (BCR/ABL with C3/A2 junction). Blood 1996;88:2410-4.
19. Melo JV. The diversity of BCR/ABL fusion proteins and their relationship to leukemia phenotype. Blood 1996;88:2375-84.
20. Faderl S, Talpaz M, Estrov Z, and others. Mechanisms of disease: the biology of chronic myeloid leukemia. N Engl J Med 1999;341(3):164-72.
21. Gordon MY, Dowding CR, Riley GP, and others. Altered adhesive interactions with marrow stroma of haematopoietic progenitor cells in chronic myeloid leukaemia. Nature 1987;328:342-4.
22. Bhatia R, McCarthy JB, Verfaillie CM. Interferon-alpha restores normal beta 1 integrin-mediated inhibition of hematopoietic progenitor proliferation by the marrow microenvironment in chronic myelogenous leukemia. Blood 1996;87:3883-91.
23. Bhatia R, Verfaillie CM. Inhibition of BCR-ABL expression with antisense oligodeoxynucleotides restores beta-1 integrin-mediated adhesion and proliferation inhibition in chronic myelogenous leukemia hematopoietic progenitors. Blood 1998;91:3414-22.
24. Bhatia R, Verfaillie CM. The effect of interferon-alpha on beta-1 integrin mediated adhesion and growth regulation in chronic myelogenous leukemia. Leuk Lymphoma 1998;28:241-54.
25. Kimura A, Kawaishi K, Sasaki A, and others. L-selectin expression in CD34 positive cells in chronic myeloid leukemia. Leuk Lymphoma 1998;28:399-404.
26. Vigneri P, Wang JY. Induction of apoptosis in chronic myelogenous leukemia cells through nuclear entrapment of BCR-ABL tyrosine kinase. Nat Med 2001;7:228-34.
27. Sawyers CL. Medical Progress: chronic myeloid leukemia. N Engl J Med 1999;340(17):1330-40.
28. Lugo TG, Pendergast AM, Muller AJ, and others. Tyrosine kinase activity and transformation potential of BCR-ABL oncogene products. Science 1990;247:1079-82.
29. Voncken JW, Kaartinen V, Pattengale PG, and others. BCR/ABL p210 and p190 cause distinct leukemia in transgeneic mice. Blood 1995;86:4603-11.
30. Sawyers CL, McLaughlin J, Witte ON. Genetic requirement for Ras in the transformation of fibroblasts and hematopoietic cells by the BCR-ABL oncogene. J Exp Med 1995;181:307-13.
31. Skorski T, Kanakaraj P, Nieborowska-Skorska M, and others. Phosphatidylinositol-3 kinase activity is regulated by BCR-ABL and is required for the growth of Philadelphia chromosome-positive cells. Blood 1995;86:726-36.