Holy BCR-ABL Fusion Protein! Is It Really Chronic Myelogenous Leukemia?

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Chronic myelogenous leukemia (CML) is a multilineage hematologic malignancy that progresses in three distinct stages: a chronic phase, a poorly defined accelerated phase, and a blast phase often resulting in death. CML is a pluripotent hematopoietic stem cell disorder characterized by the Philadelphia chromosome translocation and resultant production of the constitutively activated BCR-ABL tyrosine kinase. The Philadelphia chromosome is created by the translocation between a gene of unknown function on chromosome 22, denoted BCR, with the coding sequence for the c-ABL gene on chromosome 9 and appears in myeloid, erythroid, megakaryocytic and lymphoid cell types of CML patients. The exact structure and function of the BCR and ABL genes are still unclear. Additionally, it is unclear as to how these genes play a role in CML eventually leading to its characteristic increased cell proliferation and decreased differentiation and apoptosis. Numerous experimental models have established that BCR-ABL is an oncogene sufficient to produce CML-like disease in mice. Further research has shown the crystallized structure of the oligomerization domain of the BCR gene, which is needed to activate the ABL tyrosine kinase. The use of molecularly targeted drugs has offered new hope for treatment of CML and other cancers while proving that cancer may eventually become a manageable chronic disease analogous to diabetes and arthritis.

Basic Training

Chronic myelogenous leukemia (CML) is a myeloproliferative disorder resulting from the clonal expansion of a transformed hematopoietic stem cell (1). One of four characterized myeloproliferative disorders, CML accounts for 20% of all cases of leukemia, with an annual incidence of 1-1.5 cases per 100,000 (1). Additionally, CML accounts for 7 to 15% of all adult leukemias and occurs slightly more often in men (2-4). Although CML affects all age groups, especially those with increasing age, peak incidence occurs between the fourth and fifth decades of life, with the median age of death being 70.0 years (1).

The Signs of CML

The course of CML is divided into three phases. Following an initial chronic phase lasting 4-5 years, CML progresses through a poorly defined accelerated phase to terminal acute leukemia, or blast phase, often resulting in death. In up to 40% of patients, however, CML progresses directly from the chronic to the blast phase (2, 3, 5).

Most patients are already in the chronic phase at diagnosis, yet they exhibit very few outward symptoms. In fact, 40% of patients show no signs of CML at all (6). The other 60% of patients experience various forms of fatigue, weight loss, and splenomegaly with abdominal discomfort and early satiety (1). During the chronic phase, leukemic cells retain the ability to differentiate normally into mature granulocytes.

As CML progresses into the accelerated phase, patients experience symptoms of fever, fatigue, bone pain, progressive splenomegaly, and chloroma, marked by deeper internal collections of myeloid cells (6). The majority of patients do not experience a significant change in symptoms; however, onset of the accelerated phase is distinguished by hematologic progression (elevating blood counts) or

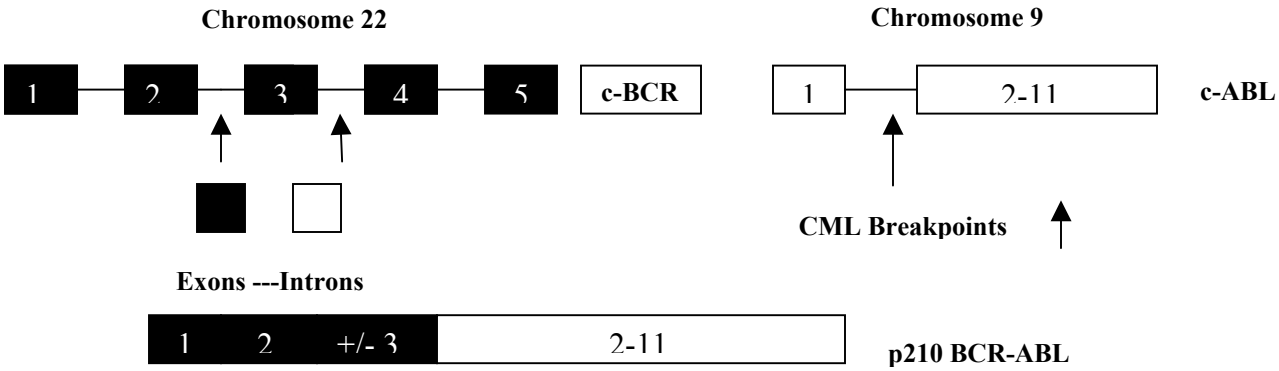

Figure 1 **The Philadelphia Chromosome.** The majority of CML cases involve a chromosome abnormality found only in leukemic white blood cells. In CML, a break occurs between the first and second exon of c-ABL. When this break occurs, nearly all of the c-ABL is translocated to chromosome 22, into the BCR gene. The breakpoint in BCR at the second or third exon results in the p210 BCR-ABL fusion protein that exhibits constitutive tyrosine kinase activity.

cytogenetic evolution (increased development of chromosomal abnormalities) in the peripheral blood or bone marrow (1,2,6). As CML progresses further, there is increasing myeloid immaturity as cells lose the ability to terminally differentiate.

The blast phase of CML brings about symptoms of fever, weight loss, night sweats, bone pain, a more progressive form of splenomegaly, chloroma, and constitutional symptoms related to anemia or infection. These symptoms include skin lesions (leukemia cutis), central nervous system disease, and bleeding secondary to progressive thrombocytopenia (2,6). Additionally, the blast phase involves aggressive proliferation of immature hematopoietic cells arrested at an early stage of differentiation (7).

Pathology

The majority of cases of CML are associated with the presence a chromosomal translocation of genetic material between chromosomes 9 and 22 (1, 6). This translocation produces a fusion gene, BCR-ABL, created by the amalgamation of the Abelson murine leukemia (ABL) proto-oncogene on chromosome 9 and a portion of the breakpoint cluster region (BCR) gene on chromosome 22. This process results in a shortened chromosome 22 known as the Philadelphia chromosome (Ph) (1). The BCR-ABL gene product is a fusion protein that varies in size, depending on the breakpoint site in BCR (1, Figure 1). The most common BCR-ABL fusion protein, p210, is found in approximately 95% of patients with CML, while the p185 protein seen in about 10% of adult patients with acute lymphocytic leukemia (ALL) (1). The p210 and p185 BCR-ABL fusion proteins have constitutive tyrosine kinase activity essential to their transforming ability. Several studies show the protein's role in leukemogenesis by demonstrating that introduction of BCR-ABL into murine hematopoietic stem cells followed by transplantation of these stem cells into mice produces a CML-like syndrome (1).

Despite the idea that the unregulated tyrosine kinase activity of BCR-ABL is considered the initial disease-causing event in CML, the acquisition of other molecular and cytogenetic abnormalities is likely to be responsible for CML progression (1). BCR-ABL activity causes activation of numerous intracellular signaling pathways, ultimately leading to alterations in the proliferative, adhesive, and survival properties of CML cells (1, 8).

CML Treatments

Currently, there are many treatments available for CML, each one specific to the respective phase of the disease. Treatment outcome yields the most promising results with early therapeutic intervention (1,6). Therapies for CML and other cancers do not necessarily cure the cancer; they increase the potential for improving a patient's quality of life until a cure is found.

Standard treatment options for patients in the chronic phase of CML include hydroxyurea, busulfan, allogeneic stem cell

transplantation, or interferon-α based regimens (1,6,9). Hydroxyurea, a ribonucleotide reductase inhibitor, is effective at controlling blood counts and generally well tolerated by patients (1). Unfortunately, although hydroxyurea controls symptoms of disease in 70% of patients, reduction in the percentage of Ph-positive bone marrow is rare and the inhibitor only delays onset of blast phase and prolongs survival by about fifty months (1,6). Busulfan is a form of chemotherapy similar to hydroxyurea. Given the observed leukemogenic effects of the two, hydroxyurea has largely replaced busulfan due to its better toleration by CML patients (6).

Allogeneic stem cell transplantation is the only proven curative therapy for CML. It produces long-term survival in 50-80% of patients and disease-free survival in 30-70% (10). Outcome form transplant yields much more successful results during earlier-stage CML—within the first year after diagnosis—and in younger patients (1). Despite being the only proven curative therapy, few patients are eligible for this treatment because of donor availability and age restriction (1, 10).

Interferon-α is a member of a family of glycoproteins that exhibits antiviral properties, immunomodulating effects, and antiproliferative activity on normal and transformed cells (11, 12). Since its discovery in the early 1980's as an active agent against CML, it has become the non-transplant treatment of choice for chronic phase patients (1,13). In several, large, randomized trials done by scientists, interferon-α increased survival by 57% when administered in chronic phase as compared to a 42% survival rate with hydroxyurea and busulfan (1,14).

Structure and Function of the ABL Gene

The proto-oncogene ABL, located on chromosome 9, encodes a tyrosine kinase (15). Scientists still do not know the exact function of ABL, but research suggests the gene creates a protein that regulates cell cycle pathways, arresting the cell when genotoxic damage occurs (16). Normal ABL gene activity is strictly regulated, including the phosphorylation of tyrosine kinases. The c-ABL protein, produced from the ABL gene, is formed from 1,097 amino acids and contains six domains that serve important roles for the function of the protein. The six domains are SH3 and SH2 (Src-homology domains), a nuclear translocation signal, a DNA-binding domain, an actin-binding domain, and a tyrosine kinase domain (17). The c-ABL protein may be able to bind to DNA, actin, or other proteins (17).

Early cancer treatments and research focused on specifically inhibiting the ABL kinase domain. Without the activation of the ABL protein, other proteins that are phosphorylated by ABL in CML cannot become active. If the other proteins are not activated, the Ras signaling pathway is not triggered and there is no increase in cell proliferation (18).

When the Ras protein is activated, it triggers the phosphorylation of three kinases. (16). The final kinase in the cascade phosphorylates other protein kinases and gene regulatory genes. Changes in these regulatory genes and kinase proteins produce changes in cell proliferation and differentiation (16).

Structure and Function of the BCR gene

Until recent years, much of the research for CML had focused on the function of the ABL portion of the protein. Today scientists are also looking at the structure and function of the BCR gene. This gene is located on chromosome 9 and produces a protein that contains 1,271 amino acids with multiple domains (17). The domains include an oligomerization domain, a serine/threonine kinase domain, and a domain with GTPase-activating activity for Rac (RacGAP) (17). BCR phosphorylates serine and threonine kinases, unlike ABL, which phosphorylates tyrosine kinases.

The oligomerization domain has recently become the focus for research on CML, because it is required for the activation of ABL kinase (17). A study done by Zhao *et al.* used x-ray crystallography to discover the structure of the oligomerization domain of the BCR part of the fusion protein. Their results showed that a tetramer structure of the oligomerization domain was necessary for the activation of ABL in the fusion protein (17).

Road to Cancer

A cell may take one of three major paths at any given time: proliferation, differentiation, or death. For most cells these actions are strictly controlled through inhibition and activation. To become cancerous, a cell first must be mutated, preventing it from being regulated by the signals that normally control them. Once this is completed, cancer cells must find other growth-signaling pathways, and develop a way to avoid proliferation inhibition (19). Cancer does not form until daughter cells

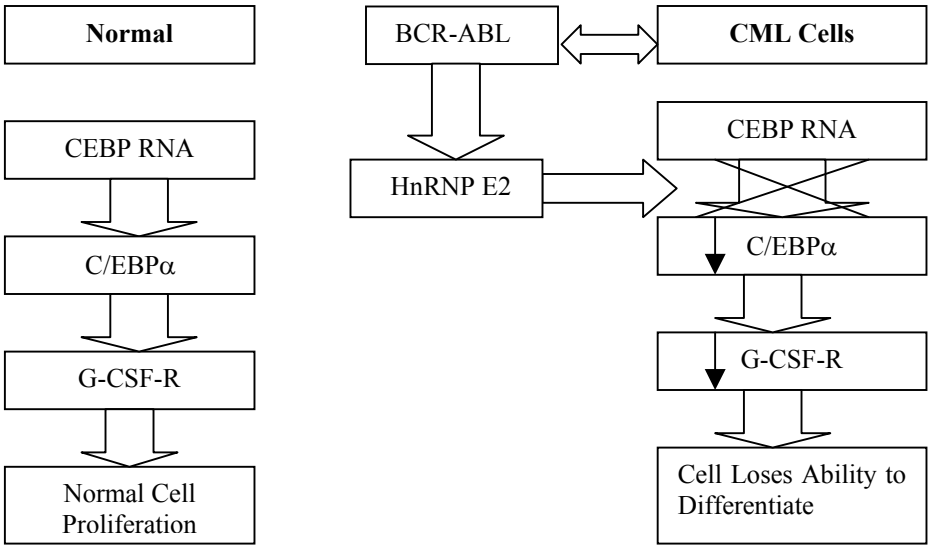

Figure 2 **Loss of Differentiation in CML cells.** In normal cells, the production of C/EBPα is not inhibited and normal cell proliferation occurs. The BCR-ABL protein in CML patients correlates to the concentration of HnRNP E2 protein, which inhibits the formation of the C/EBPα protein. Without the correct concentration of C/EBPα, the cell loses the ability to differentiate correctly.

fail to differentiate normally, at which point they proliferate at an uncontrollable rate. The last step in the transformation of cells is the ability to live indefinitely, without the possibility of apoptosis (19).

Understanding what goes wrong in each of these processes can possibly lead to better treatments and even a cure for CML and other cancers. The deregulation of tyrosine kinase activity is necessary for altering several pathways that activate signals, leading to increased proliferation, inhibition of apoptosis, and altered differentiation in CML (20).

BCR-ABL and the Ras Signaling Pathway

A study done by Puil *et al.* determined the missing gaps between the BCR-ABL protein and the Ras signaling pathway. The Ras protein is activated by tyrosine kinases when these kinases give up a phosphoro group to make GTP of GDP (16). Puil *et al.* analyzed BCR-ABL oncoproteins for interactions with other protein found in the cytoplasm (18). Polymerase chain reaction (PCR) and antibodies were used to isolate cytoplasmic proteins that might mediate Ras activation.

Three proteins were used in this study, mSos1, GRB2, and Shc. The mSos1 protein is used as a conversion factor for Ras; it changes the inactive GDP-bound Ras to an active GTP-bound Ras, through phosphorylation (18). Grb2 is known for its property of phosphorylating other proteins through its SH2 domain (18). Both the Grb2 and mSos1 proteins bind together in BCR-ABL transformed cells, to create a bridge between the BCR-ABL protein and the Ras protein (18). This was not the only pathway the scientists found to link BRC-ABL protein to the Ras protein. Three Shc proteins (proteins 66, 52, and 46) were shown to bind to both Shc and Ras (18). The Shc proteins did not just phosphorylate the Ras protein, but also phosphorylated the BCR-ABL protein (18).

The knowledge gained from this experiment helps explain how the BCR-ABL protein activates the Ras pathway, which eventually leads to increased gene transcription and cell proliferation. This allows scientists to focus on a specific area for designing new CML drug treatments.

The Loss of Differentiation

Loss of cell differentiation is one of the main characteristics of CML and other cancers. CML loses its ability to correctly differentiate correctly between the chronic and blast phases. Perrotti *et al.* showed how CML leads to the loss of differentiation through the down-modification of C/EBPα. C/EBPα is a transcription regulator protein that is essential for correct differentiation in granulocytic cells by stimulating G-CSF-R, the stimulating factor receptor that is the primary regulator of granulocytic differentiation (20,

Figure 2). As the concentration of C/EBPα decreases, the amount of activation of G-CSF-R also decreases (20). The C/EBPα protein is faintly detected in patients suffering from CML, even though the mRNA produced from the C/EBPα is clearly present (20). The decline of C/EBPα is attributed to increased amounts of HnRNP E2, which is involved in the suppression of translation of the CEBPA RNA (20). HnRNP E2 is an m-RNA binding protein that regulates transcription, including the transcription of CEBPA RNA (14). In CML amount of HnRNP E2 produced increases as the amount of BCR-ABL increases (20).

Perrotti *et al.* concluded that the suppression of C/EBPα in blast crisis is related to the concentration of BCR-ABL (20). As the concentration of BCR-ABL increases the expression of C/EBPα decreases, thus leading to the loss of differentiation. With this knowledge, scientists can design a drug that inhibits the binding of HnRNP E2, allowing for normal cell differentiation and hence, CML treatment.

CML and Apoptosis

A number of genes, some identified as oncogenes, have been implicated in the control of apoptosis (21). These genes can be classified into two categories: those that drive apoptosis, such as c-myc and p53, and those that inhibit the process, such as bcl-2 and Ras. A study related to c-myc done by McGahon *et al.* has shown that cells treated with an antisense oligonucleotide to suppress synthesis of myc protein are considerably more resistant to apoptosis when compared to their counterparts that express the myc protein (21). Conversely, Bcl-2-expressing cells show resistance to apoptosis normally induced by a variety of agents, including many chemotherapeutic compounds (21). This research allows understanding as to whether the aberrant expression of the ABL oncogene seen in a cell line derived from a CML patient contributed to the observed resistance to apoptosis (21). McGahon *et al.* showed by using laser scan microscopy that pretreating cells with antisense oligonucleotides leads to a rapid loss of the BCR-ABL protein from the cytoplasm of the cell. To confirm these results, immunoblotting was used and it was determined that there was a reduction in the levels of p210 BCR-ABL fusion protein in the cells (21). This result indicates that the inhibition of BCR-ABL, making CML cells susceptible to apoptosis, can be combined with therapeutic drugs capable of inducing apoptosis to provide an effective strategy for elimination of cancerous cells (21).

A study done by Cortez *et al.* was done in hopes of identifying structural motifs within the BCR-ABL protein that activate specific signaling pathways required for transducing antiapoptotic and transforming activities in hematopoietic cells (22). To determine whether BCR-ABL uses multiple pathways for activating signaling pathways such as Ras, the scientists examined biological effects of the GRB2 binding site, Src homology 2 domain, and an autophosphorylation site in the tyrosine kinase domain. In so doing, the researchers determined that these BCR-ABL mutants do not diminish the antiapoptotic and transforming properties of BCR-ABL in hematopoietic cells. However, when the three mutations were combined, a severe decrease in transforming and antiapoptotic activities was exhibited, in addition to the activation of the Ras pathway, the phosphorylation of the SHC adapter protein and binding of GRB2 (22). The scientists' results showed that BCR-ABL requires additional signaling components to obtain tumorigenic growth, which are distinct from those required to block apoptosis.

CML Mouse Models

Murine model systems are often used in cancer research because they are a powerful tool for growing human hematopoietic cells *in vivo* (23). To demonstrate suppression of leukemic cell growth using antisense oligonucleotides and oncogene inactivation by creation of a designer ribozyme, scientists used such a system.

Research performed by Tanabe *et al.* using ribozyme-based therapy demonstrated promising results for future CML treatment therapies. The scientists created a designer ribozyme—a maxizyme—that is dimeric and specifically cleaves BCR-ABL mRNA, inducing apoptosis in CML cells. A CML cell line, BV173, was transduced with a control vector (maxizyme sequence deleted) or with the maxizyme-encoding vector. The cells were then injected into mice. Results indicated that all mice injected with the control BV173 cells died of diffuse leukemia 6-13 weeks later, while those injected with maxizyme-transduced BV173 cells remained healthy. Essentially, use of this maxizyme may be useful for purging bone marrow of leukemic cells in cases of CML treated by autologous transplantation due to its ability to reduce the incidence of relapse by

decreasing the tumorigenicity of contaminating CML cells in the transplant (23, 24).

In a study previously mentioned McGahon *et al.* the use of antisense oligonucleotides led to a rapid loss of the BCR-ABL protein from the cytoplasm of the cell. In hopes of producing the same result *in vivo*, Skorski *et al.* injected leukemic mice with a 26-mer BCR-ABL antisense oligodeoxynucleotide (1 mg/day for 9 days) (25). Results showed that there was an induced disappearance of leukemic cells and a marked decrease in BCR-ABL mRNA in mouse tissues (25). Mice that were untreated or treated with either a sense oligodeoxynucleotide or a mismatched antisense oligodeoxynucleotide were dead 8-13 weeks after injection of leukemic cells. Conversely, mice treated with the antisense oligodeoxynucleotide died 18-23 weeks after injection. These findings provide evidence for the *in vivo* effectiveness of anticancer therapies based on antisense oligodeoxynucleotides that target a tumor-specific gene and can be directly related to the study results of McGahon *et al* (21, 25).

Future Therapies

Recent studies show the finding of a molecular targeted drug therapy known as Gleevec, or STI-571, that functions by blocking the binding of ATP to the BCR-ABL tyrosine kinase. By doing this, the kinase activity is inhibited thus preventing cancerous cells from being activated (1). From experience with chronic and blast phase patients, STI-571 clearly works best when used in the early disease phases. Although patients in the accelerated or blast phases initially respond to STI-571, 80% relapse in less than a year (1). Patients relapse due to point mutation or gene amplification, which allows the BCR-ABL to overexpress itself so that STI-571 is no longer able to inhibit the BCR-ABL tyrosine kinase activity (1). Research as of 4 April 2002, however, has shown that STI-571 is the proof of principle that enables the blocking of signaling pathways that cancer depends on. By combining STI-571 with other angiogenesis inhibitors and repeating numerous "cocktails" of drugs tailored to specific tumor types, kinase and angiogenesis inhibitors that block critical signaling pathways hold great potential for the future treatment of CML (19). In the future, scientists predict that cell biologists will have derived a complete integrated circuit of the cell's signaling pathways, which will allow these biologists to model how specific genetic perturbations cause cancer, thus predicting how to correct the problem using drugs acting on key points in the circuit (19).

Conclusion

Chronic Myelogenous Leukemia is one of the best-studied malignant conditions in humans (10). Progress in the understanding of the molecular pathways has led to a very promising future for effective treatment strategies of CML. With new breakthroughs of targeted therapies like STI-571, safer and much more effective therapies lie waiting to be discovered. Determining the structure and function of the BCR and ABL genes, in addition to further understanding the signaling components of the BCR-ABL fusion protein will allow for the characteristic increased cell proliferation and decreased differentiation and apoptosis of CML to be better illustrated.

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