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Chronic myeloid leukemia: why does it evolve from chronic phase to blast transformation?

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1. ABSTRACT

Clinically chronic myeloid leukemia is a biphasic or triphasic disease that is usually diagnosed in the initial 'chronic', 'indolent' or 'stable' phase and then spontaneously evolves after some years into an advanced phase. This advanced phase can sometimes be subdivided into an earlier accelerated phase and a later blast phase or blast transformation – in about one-half of patients the chronic phase transforms unpredictably and abruptly to a blast phase, while in the other half of patients, the disease evolves somewhat more gradually, through an accelerated phase, which may last for months or years, before a blast phase ensues; this may have myeloblastic or lymphoblastic features. Although much is now known about the molecular biology of the disease, the molecular basis of disease progression is still obscure. The popular thinking has been that one or more probably a sequence of additional genetic events occurs in the BCR-ABL positive clone. When the critical combination of additional events is achieved, clinically definable transformation occurs. Here we review what is known of the mechanisms underlying the evolution of chronic myeloid leukemia from a chronic phase to a blast transformation.

2. INTRODUCTION

Although chronic myeloid leukemia (CML) was the first human leukemia to be recognised as a separate entity almost 160 years ago, and probably the first human malignancy in which a consistent cytogenetic abnormality, the Philadelphia (Ph) chromosome, was identified, the mechanism underlying its apparently inexorable progression from an initial, rather indolent, phase, usually termed the chronic phase (CP), to a more advanced phase or blast phase (BP) remains largely unknown (1,2). Patients with CML typically present in the CP, during which myeloid progenitor numbers are greatly increased in the bone marrow and blood. This usually rather indolent phase may continue for as little as one year or as long as 20 years, but eventually it transforms into BP (also known as blast transformation, BT), in which an increasing proportion of blast cells are found in the marrow and peripheral blood (3). In about half the patient population the CP disease transforms directly into BP while in the remainder there is an intermediate period of accelerated phase disease. In this paper we review the current understanding of the molecular pathogenesis of chronic phase CML and its evolution BT.

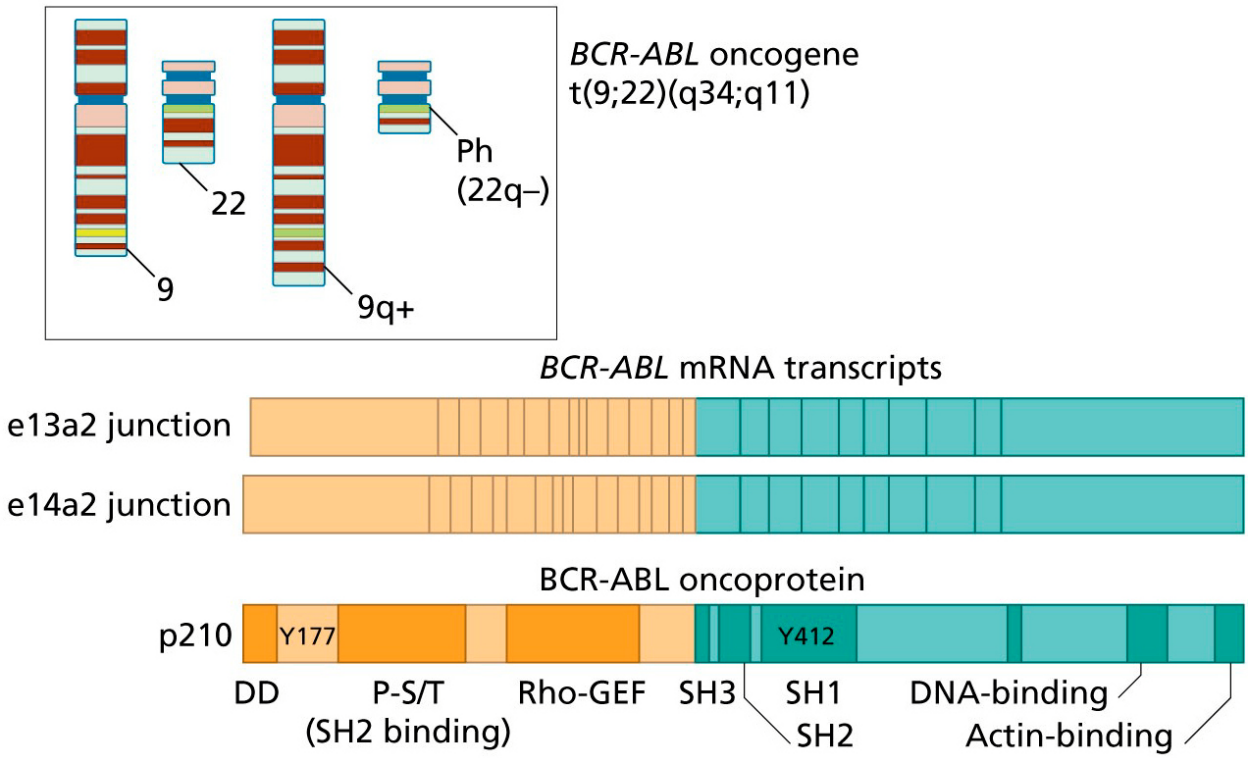

Figure 1. The t(9;22)(q34;q11) translocation and its products: the BCR-ABL oncogene on the Ph chromosome and the reciprocal ABL-BCR on the derivative 9q+ chromosome. In the classic CML, BCR-ABL is transcribed into mRNA molecules with e13a2 or e14a2 junctions, which are then translated into the $p210^{bcr/abl}$ oncoprotein. This oncoprotein is a hybrid containing functional domains from the N-terminal end of BCR [dimerization domain (DD)], SRC-homology 2 (SH2)-binding and the Rho GTP-GDP sxchange factor (GEF) domains and the C-terminal end of ABL. [Only SRC-homology regions 2,3 and 1 (SH2, SH3 and SH1 respectively). Tyrosine 177 (Y177) in the BCR portion of the fusion gene and tyrosine 412 (Y412) in the ABL portion are important for the docking of adapter proteins and for BCR-ABL autophosphorylation respectively. P-S/T denotes phosphoserine and phosphothreonine [Published, with permission from Goldman & Mughal, Chronic Myeloid Leukaemia, Ed: Hoffbrand, Tuttenham & Catovsky, Blackwell Science, Oxford, UK (2005)].

3. CML IN CHRONIC PHASE

3.1. The Philadelphia chromosome

About 90-95% of patients with CML have the Ph chromosome, which is an acquired cytogenetic abnormality found in almost all myeloid cells, including dendritic cells (4), and some B- and T-lymphocytes. The Ph chromosome results from a reciprocal translocation of genetic material between one chromosome 9 and one chromosome 22, designated t(9;22)(q34;q11) (Figure 1). The Ph chromosome carries a *BCR-ABL* fusion gene that expresses an oncoprotein, $p210^{BCR-ABL}$, which is considered to be the principal 'cause' of the CP. 5-10% of patients with hematologically acceptable CML lack a Ph chromosome and are designated Ph-negative CML. About half such patients have a *BCR-ABL* fusion gene located on a normal-appearing chromosome 22 and are referred to as Ph-negative, *BCR-ABL*-positive cases. The remainder show no evidence of a *BCR-ABL* fusion gene; in most of these cases the relevant genetic lesions are not known, although very rare cases of CML-like disease have other consistent cytogenetic translocations, notably t(5;12) and t(8;13), that associated with activation of receptor tyrosine kinases (5).

3.2. The molecular anatomy of the BCR-ABL fusion genes

The genomic events resulting in the production of the $p210^{BCR-ABL}$ oncoprotein can be summarised as follows: There are three separate zones where breakpoints occur in the BCR gene on chromosome 22 (Figure 4) (6). Breaks in the major breakpoint cluster region (M-BCR) nearly always occur in the intron between exon e13 and e14 or in the intron between exon e14 and e15 (toward the telomere). By contrast, the position of the breakpoint in the ABL gene on chromosome 9 is highly variable and may occur at almost any position upstream of exon a2. The Ph translocation results in the juxtaposition of 5' sequences from the BCR gene with 3' sequences from the ABL gene. This event generates the BCR-ABL fusion gene which is expressed as an 8.5 kb mRNA and a protein of 210 kD ($p210^{BCR-ABL}$) that has a greater tyrosine kinase activity compared to the normal ABL protein. The different breakpoints in the M-BCR result in two slightly different BCR-ABL fusion genes, expressed as either e13a2 or e14a2 transcripts. The type of BCR-ABL transcript has no important prognostic significance. The second breakpoint location in the BCR

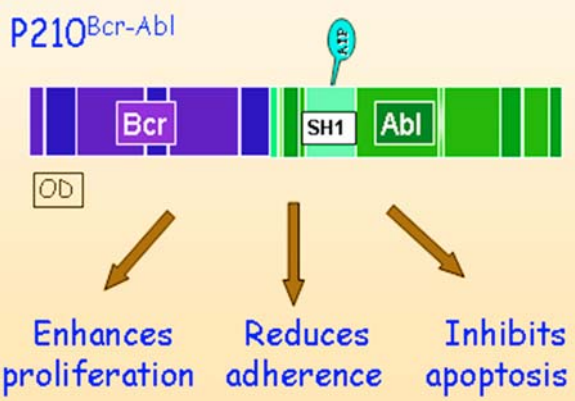

Figure 2. Schematic representation of the possible mechanisms by which the activated $p210^{BCR-ABL}$ induces the clinical phenotype of CML. Though the precise mechanisms by which BCR-ABL alters stem cell kinetics remain ill-defined, it is possible that it may act by enhancing cellular proliferation; alternatively it may reduce cellular adherence. As an activated ABL opposes cellular apoptosis, the BCR-ABL gene might act by impeding 'programmed cell death' in target stem cells.

gene occurs between exons e1 and e2 in an area designated the minor breakpoint cluster region (m-bcr) and forms a BCR-ABL transcript that is transcribed as an e1a2 mRNA encoding a $p190^{BCR-ABL}$(11). This is found in about two-thirds of patients with Ph positive acute lymphoblastic leukemia (ALL). A third breakpoint location is found in patients with the very rare Ph positive chronic neutrophilic leukemia. This is designated the micro breakpoint cluster region (μ-bcr) and results in e19a2 mRNA, which encodes a larger protein of 230kD ($p230^{BCR-ABL}$) (12).

3.3. The molecular biology of the Ph chromosome

The mechanism by which the activated $p210^{BCR-ABL}$ induces the clinical phenotype of CML remains poorly defined (6). Possible mechanisms include constitutive activation of mitogenic signaling, reduced apoptosis, impaired adhesion of cells to the marrow stroma and extracellular matrix, and enhanced proteasome-mediated degradation of ABL inhibitory proteins (Figure 2). The deregulation of the ABL tyrosine kinase facilitates autophosphorylation, resulting in a marked increase of phosphotyrosine on BCR-ABL itself, which creates binding sites for the SH2 domains of other proteins (7,8). A variety of such substrates, which can be tyrosine phosphorylated, have now been identified. Although much is known of the abnormal interactions between the Bcr-Abl oncoprotein and other cytoplasmic molecules, the actual pathways through which the leukemogenic proliferative signal is mediated, such as the RAS-MAP kinase, JAK-STAT, and the PI-3K/Akt pathways, are ill-defined and their relative contributions to the leukemic 'phenotype' are still unknown (Figure 3) (9). Altered expression of tyrosine phosphatases may also play a role in the transformation process (10).

3.4. Cytokinetic effects of BCR-ABL proteins

At the cytokinetic level, the mechanism by which the *BCR-ABL* genes results in the preferential proliferation and differentiation of myeloid stem cells is also unclear (13). It is likely that acquisition of a *BCR-ABL* fusion gene enhances survival and differentiation but may only increase self-renewal of leukemia stem cells to a minor degree, if at all (14,15). Conversely normal myeloid progenitor cells are probably held in Go as a result of proliferation of leukemic cells but can, under certain circumstances, be induced to proliferate. This conclusion is based on the demonstration that Ph-negative colonies can be cultured *in vitro* from the blood and marrow of untreated CML patients, on the observation that Ph-negative progenitors can be identified in the blood in the recovery phase following high-dose chemotherapy, and on the ability of interferon-alfa and more recently imatinib mesylate to restore Ph-negativity in the bone marrow in a high proportion of CML patients (16-18).

3.5. The functions of the BCR and ABL proteins

Both *BCR* and *ABL* genes are expressed ubiquitously. The *BCR* product is a 160-kd cytoplasmic protein with several functional domains (19). The N-terminal 426 amino acids of Bcr, encoded by the first exon, are retained in all Bcr-Abl fusion protein isoforms. This region contains a serine-threonine kinase domain, whose only known substrates are Bcr and Bap-1, and two serine/threonine-rich regions that bind Src homology 2 (SH2) domains of other proteins, including Abl, $p190^{BCR-ABL}$, and $p210^{BCR-ABL}$ (7,20). The proximal SH2-binding domain appears to be important for the transformation of murine fibroblasts by Bcr-Abl (8). The two key motifs of the first *BCR* exon are tyrosine 177 and the coiled-coil domain contained in aminoacids 1 to 63, which appear to be important for the dimerization of Bcr-Abl, which is crucial for the Abl kinase activity and, indeed, for the Bcr-Abl to 'cause' leukemias (9,21). The Bcr regions 3' of exon 1 appear not to be important for the leukemogenic process, but do influence the specific phenotype of the leukemia. This conclusion is based on the observation that since the Abl component of the fusion gene is largely invariant, the variability in the different leukemias generated may be due to the amount of BCR upstream. There appears to be an inverse relationship between the aggressiveness of the phenotype and the amount of BCR upstream: thus the smallest Bcr-Abl oncoprotein, $p190^{BCR-ABL}$, results in the clinically 'aggressive' ALL, in contrast to the rather indolent clinical course of chronic neutrophilic leukemia which is associated with the largest of the fusion proteins, $p230^{BCR-ABL}$. Phosphorylated tyrosine 177 forms a binding site for Grb-2, which plays a critical role in linking Bcr to the Ras pathway and is crucial for the leukemogenesis of myeloid leukemias (22,23).

The Abl gene, of which there are two principal isoforms, Ia and Ib, codes for a 145-kd non-receptor tyrosine kinase (24). The Ib isoform contains a unique myristoylation site, which allows it to be anchored to the plasma membrane. Although Abl is expressed largely in the nucleus, it appears to transit between the nucleus and cytoplasm. Its functions are complex, and include cell cycle inhibition and signal transduction from growth factor receptors and integrins. The three domains located towards the N-terminus of Abl appear to play different roles: the

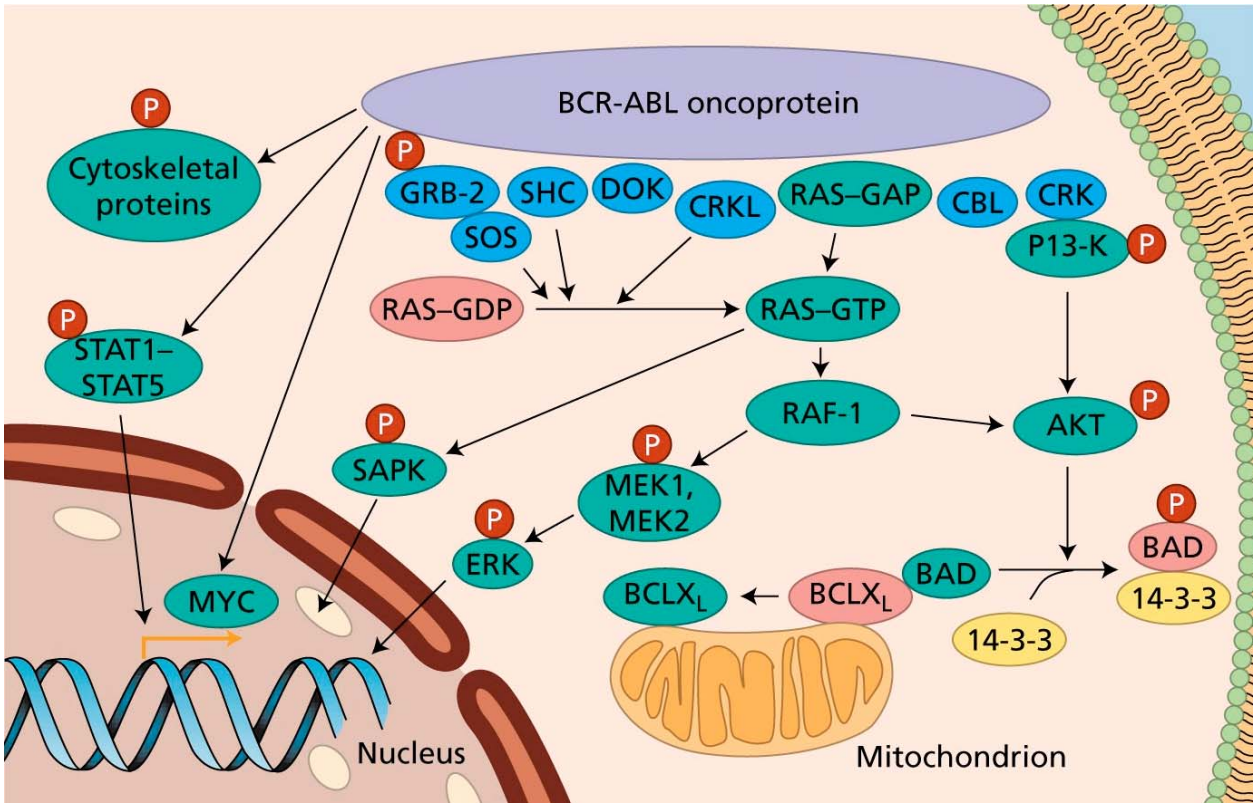

Figure 3. Signal transduction pathways involved in CML. Schematic representation of pathways that may be involved in transmitting the BCR-ABL signal to the cell nucleus in the clinical setting. Note the STAT pathway to the left, the RAS/RAF/1-MEK1 pathway centrally and the PI3 kinase/AKT pathways to the right. Molecules known to be phosphorylated by activated BCR-ABL are marked with the letter 'P'[published, with permission from Goldman & Mughal, Chronic Myeloid Leukaemia, Ed: Hoffbrand, Tuttenham & Catovsky, Blackwell Science, Oxford, UK (2005)].

SH1 domain is the tyrosine kinase, the SH2 domain binds phosphotyrosine-containing consensus sites, and the SH3 domain binds to proline-rich consensus sequences in proteins such as Crk and Crkl (25,26). The C-terminal region of Abl is long (90 kd) and contains both actin- and DNA-binding domains, in addition to three nuclear localization signals, and one nuclear export signal (27-29). The N-terminal 'cap' region of Abl also appears to be important for the regulation of kinase activity (30,31).

3.6. The effects of the BCR-ABL fusion transcripts on myeloid progenitors

In cells with a ***BCR-ABL*** fusion gene, the $p210^{BCR-ABL}$ oncoprotein, in contrast to the normal $p145^{ABL}$, has enhanced tyrosine kinase activity which is essential for cellular transformation. The exclusive cytoplasmic localization of this protein facilitates the assembly of a number of key phosphorylated substrates, discussed above, which activate anti-apoptotic and mitogenic signals. The central role of the $p210^{BCR-ABL}$ in generating the chronic phase of CML was elegantly demonstrated in murine model systems, in which the direct introduction of the fusion gene in a retroviral vector into murine stem cells in experimental animals induced a disease closely resembling CML in man (32,33).

The BCR-ABL oncoprotein perturbs numerous signal transduction pathways important for the normal cellular growth of myeloid progenitors, as mentioned above, and depicted in Figure 2; this allows for an autonomous cellular growth which somehow circumvents the apoptosis of these cells. *Pari passu*, perturbation of other important pathways, such as those involved in DNA repair, result in the acquisition of secondary genetic abnormalities which appear to be *sine qua non* for the progression of CML to BT (34,35).

3.7. RAS and other BCR-ABL protein-activated pathways

The Bcr-Abl oncoprotein binds directly to a number of proteins which lead to the GTP-bound active form of Ras. Autophosphorylation of tyrosine 177 results in a binding site for Grb-2, which links with the Sos protein and activates Ras (7,36). Two other substrates of Bcr-Abl, Src and Crkl, can also activate Ras; Grb-2 also recruits the protein Gab2, which is critical for the activation of PI-3K/Akt and Ras/Erk (37,38). Once activated, Ras binds to the serine-threonine kinase Raf-1, activates it by tyrosine phosphorylation and initiates the signaling cascade led by the mitogen-activated protein kinase (MAPK) pathway (39,40).

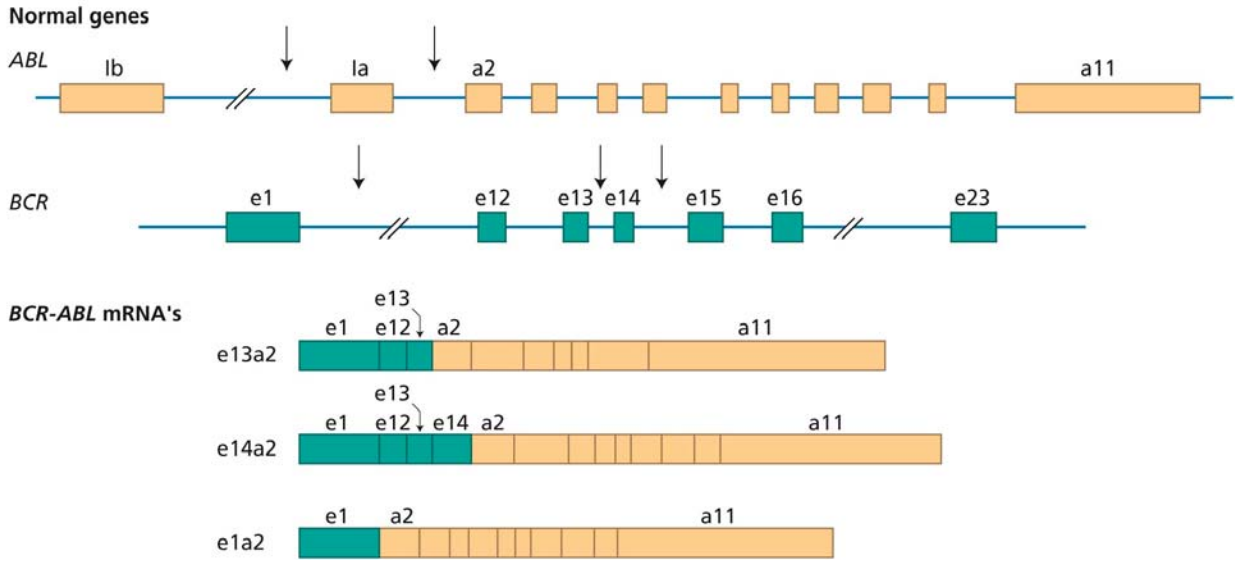

Figure 4. Schematic representation of the various breakpoints in the ABL and BCR genes and the encoded proteins in the BCR-ABL positive leukemias. The genes are shown at top and the RNA transcripts and corresponding proteins below. The arrow show the possible sites on breakage in the ABL gene (above) and the possible sites of the two alternative breaks in M-BCR (below) that are characteristic of CML (namely $p210^{BCR-ABL}$ with an e13a2 junction or $210^{BCR-ABL}$ with an e14a2 junction. Breaks m-bcr and μ-bcr are characteristic of Ph-positive acute lymphoblastic leukemia and Ph-positive chronic neutrophilic leukemia respectively [published, with permission from Goldman & Mughal, Chronic Myeloid Leukaemia, Ed: Hoffbrand, Tuttenham & Catovsky, Blackwell Science, Oxford, UK (2005)].

3.8. Phosphatidylinositol 3 kinase pathway

The PI-3K/Akt pathway appears to be important for the stability of the cyclin-dependent kinase (CDK) inhibitor p27, though the precise mechanisms involved remain unclear (41,42). The disruption of p27 activity enhances the proliferation of BCR-ABL containing cells (43,44). It triggers an Akt-dependent cascade which facilitates the BCR/ABL transformation by regulating the activity of proteins such as Bad (a proapoptotic protein), Mdm2 and the forkhead transcription factors (45).

3.9. STAT5 and the Janus kinase pathway

This antiapoptotic pathway requires the activation of STAT5 by Bcr-Abl via the Src family kinase, Hck. The SH2 and SH3 domains of the BCR-ABL appear to activate this kinase, resulting in the phosphorylation of tyrosine 699 by STAT5B, which activates the antiapoptotic Bcl-X_L gene and possibly (at least in murine models) other gene targets, such as A1 and Pim 1, which also have antiapoptotic potentials (46-51). It is of interest that under physiological conditions, STAT5, and indeed all STATs, are phosphorylated by Janus kinases (Jak) that are downstream of growth factor receptors. Despite the recent observation that the great majority of patients with polycythemia have a consistent valine-to-phenylalanine mutation at position 617 in the Jak2 protein (52,53), this mutation has not been identified in patients with Ph-positive CML.

3.10. Myc pathway

The *MYC* gene encodes a transcription factor which converts mitogenic signals to altered gene expression in various human malignancies, particularly with regards to cell cycle and apoptosis (54). In CML cells it enhances the transformation from CP to BT (55). Recently workers have established that the SH2 domain and the C-terminus are essential for 'activation' of the Myc protein; in contrast, Jak2 has a negative effect by virtue of 'stabilizing' the Myc RNA (56,57).

3.11. Effects of BCR-ABL proteins on apoptosis

Although there is debate about the precise effect of the Bcr-Abl oncoprotein on CML cells, some (but not all) reports suggest that CML cells exhibit an increased resistance to apoptosis, compared to 'normal' cells (58,59). There is evidence that BCR-ABL blocks the release of cytochrome C from the mitochondria, which is necessary for the activation of caspase-3 (60). It also up-regulates the anti-apoptotic effects of the Bcl-2 protein, either via the Ras or the PI-3K pathways, and the Bclx protein, via the STAT5 pathway. It appears that the localization of Bcr-Abl within the CML cell is also important for its apoptotic effect: its presence in the nucleus is associated with an anti-apoptotic effect, while its presence in the cytoplasm is considered pro-apoptotic (61-63). These functions may or may not depend on the tyrosine kinase activity. Current evidence suggests that there are kinase-independent survival signals, which might explain the persistence of residual disease in patients who have achieved complete cytogenetic remission with imatinib; in contrast the majority of patients achieving complete cytogenetic remission following an allogeneic stem cell transplant appear not to have residual disease (64,65).

3.12. Effects of BCR-ABL on proteasomes

Bcr-Abl tyrosine kinase activity is associated with the proteasome-mediated degradation of the Abl-

interactor proteins Abi-1 and Abi-2 (66). It has been observed that Abi proteins are not present in the marrow from patients with CML in myeloid BT, in contrast to those with acute myeloid leukemia, suggesting that proteasomal degradation of the Abi proteins by Bcr-Abl might have a role in the progression of CP to BT. This speculation has led to pilot studies assessing the potential role of proteasome inhibitors in the management of patients with CML refractory to imatinib.

3.13. Is the presence of BCR-ABL fusion transcripts always pathological?

A decade ago, Biernaux and her colleagues demonstrated the (then) rather provocative finding of the BCR-ABL transcript at very low level in leukocytes from healthy individuals (albeit working in a CML laboratory), using a highly sensitive two-step reverse transcription and polymerase chain reaction (PCR) assay (67); such transcripts were detected in about 30% of all leukocytes from 'normal' healthy adults. In 1998 Melo and her colleagues confirmed these findings amongst 27% of healthy adults (68). Using primers specific for the e1a2 junction they noted the presence of BCR-ABL transcripts in 69% of healthy individuals. Both studies provide direct evidence that the generation of BCR-ABL transcripts at very low levels is either not sufficient by itself to generate the CML, perhaps because these transcripts arose in hematopoietic progenitors which by themselves were not capable of clonal expansion. It is also plausible that such transcripts arise in potentially multipotent myeloid cells, but are 'recognized' and eliminated by a healthy immune system before they can generate leukemia.

3.14. Possible mechanisms underlying progression from CP to BT

It is likely that the acquisition of a *BCR-ABL* fusion gene by the hematopoietic stem cell and the ensuing expansion of the Ph-positive clone sets the scene for acquisition and expansion of one or more subclones that are genetically more aggressive than their progenitors. The increased propensity of the Ph-positive clone to acquire such additional genetic changes has been termed 'genomic' or 'genetic' instability, but the molecular mechanism underlying this instability is poorly defined. The observation that patients in chronic phase have a tendency to acquire additional cytogenetic changes in the Ph-positive population may be one manifestation of this genomic instability. Another may be the observation that patients who developed resistance to imatinib frequent have expanded clones of cells bearing a mutation in the DNA encoding the Abl kinase domain of the Bcr-Abl oncoprotein. The target cell for disease progression is also ill-defined. Although one might simplistically assume that the critical additional genetic events occur in a cell strictly analogous to the Ph-positive pluripotential stem cell, this may not necessarily be true. The clinical observation that a significant minority of transformations involve predominantly cells of the B-lymphoid series suggest that the target cell for generation of chronic phase disease and the target cell for transformation may at least in some cases be different. The recent observation that myeloid transformation may in some cases be due to immortalization of committed myeloid progenitors further supports the non-identity of the target cell for chronic phase and transformed phases of the disease (14).

3.15. Cytogenetic and molecular events preceding or coinciding with progression to BT

At present it is unclear how the additional cytogenetic events which herald the progression of CP to BT are pathogenetically important. The commonest non-random chromosomal changes are trisomy 8, trisomy 19, double Ph chromosome and isochromosome i(17q) (69). Some workers have suggested that trisomy 8 and i(17q) might represent early changes heralding BT, whilst trisomy 19 often occurs late (70,71). There has been speculation that the amplification and over-expression of Myc noted in some patients with CML in BT may be linked to the trisomy 8, though no definite correlation has been observed (72).The precise impact of the additional Ph chromosome on the progression of CML from CP to BT is also unclear. It might simply represent an increased expression of the *BCR-ABL* gene. The acquisition other less common chromosomal abnormalities such as t(3;21) and t(7;11) is also noted in myeloid BT. Additional molecular changes have been observed in a significant majority of patients with CML as they evolve from a CP to a BT. It has been speculated that the $p210^{BCR-ABL}$ might confer genomic instability and as the disease progresses from CP to BT, additional genetic abnormalities are acquired secondarily. It is plausible that the BCR-ABL gene acquires new mutations which allow an already genetically unstable phenotype to acquire further changes. Many efforts have shown that both the p53 and RB genes, which play a crucial role in cell proliferation and survival, and interestingly, not cell differentiation, are important in the genesis of BT (73-75). Other possible hypotheses include an unrestrained activity of BCR-ABL in the progenitor cell permitting the transition of the CP to BT and the probability that an increased BCR-ABL expression promotes secondary molecular changes and chromosomal changes.

Many efforts have been directed to generating a murine model system in which the $p210^{BCR-ABL}$ oncoprotein induces diseases resembling human CML in BT. Models based on work carried out by Rabbitts and colleagues, based on the $p190^{BCR-ABL}$ coding sequences, have met some success, confirming the importance of point mutations, insertions and deletions with increased frequency of 'advanced' disease, but provide little information with regards to the $p210^{BCR-ABL}$ oncoprotein (76). A group in Boston has recently described a transgenic mouse model in which the expression of Bcr-Abl protein can be controlled by incorporating a tetracycline responsive element into the expression cassette. Some of these transduced animals had an initial hematologic picture resembling chronic phase CML but subsequently progressed to a B-cell lymphoblastic disease reminiscent of lymphoid blastic transformation in man (77).

Work carried out by Green and colleagues on the derivative chromosome 9 has shed some light on the importance of primary molecular abnormalities in influencing the rate progression to CML in BT (34). They

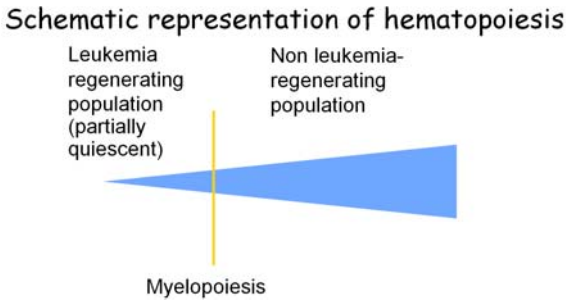

Figure 5. Schematic representation of hematopoiesis: Hematopoiesis can arbitrarily be divided into a leukemia regenerating population, which is partially quiescent, but capable of generating leukemia and responsible for relapse, and a non-leukemia-regenerating population.

noted that the 10-15% of CML patients in CP presented ***de novo*** with deletions of the derivative chromosome 9, which makes these patients progress to BT much more rapidly than those patients who lack these deletions. They suggested that this is a unique genetic mechanism which allows the Bcr-Abl oncoprotein to induce a relatively early transition from a CP to a BT.

3.16. Cytokinetic basis for progression

It is generally assumed that tissue-specific stem cells, in contrast to committed progenitor cells, have the ability to divide and form new stem cells (self-renewal), some of which have the potential to differentiate into mature cells (78). It is assumed also that the stem cell characteristic of CML in chronic phase may be analogous to the normal stem cell but has an increased propensity to self-renew and thereby to generate excessive number of myeloid cells. Differentiation however towards normal progeny is not impaired. One might assume also that the additional molecular events that underlie transformation occur predominantly at the level of the leukemic stem cell. The recent evidence from Jamieson *et al* however challenges this dogma. They were able to show that the numbers of committed myeloid progenitor cells (CFU-GM) were increased to a greater extent in blastic transformation of CML than were the numbers of putative stem cells and that the levels of ß-catenin, a component of the Wnt signalling pathway believed to be involved in stem cell renewal, were also increased in CFU-GM (14). They concluded that at least in some cases the additional molecular events underlying BT transformation occurred not in the pluripotential stem cell but at the level of the committed myeloid progenitor cell.

In practice the data reported by Jamieson *et al* do not preclude the notion that the cell 'targeted' for progression to transformation may be different in different patients. It may in some cases target a relatively undifferentiated stem cell, thereby increasing its rate of self-renewal and inducing a differentiation block; in other cases it may induce the capacity to self-renew in a cell that would not normally have this capacity (see Figure 5). Such a hypothesis would explain the paradox whereby imatinib appears to prolong chronic phase disease without necessarily reducing the number of 'quiescent' leukemic stem cells in a given patient.

3.17. Effects of BCR-ABL on DNA repair

Efforts directed towards the understanding of the molecular mechanisms underlying the genetic instability which appears to be *sine qua non* for the progression of CP to BT, have suggested a potential relationship between the *BCR-ABL* expression and a number of proteins involved in DNA repair, in particular the double-strand breaks (DSB) proteins (79-81). In CML cells, levels of a co-factor for DSB, DNA-PKcs, are down-regulated (82). In-vitro studies have confirmed that the down regulation of DNA-PKcs was associated with a higher frequency of chromosomal abnormalities when CML cells were exposed to ionizing radiation (83). An inverse link between BCR-ABL expression in CML cells and the down-regulation of the BRCA-1 gene, which appears to play a major role in the maintenance of genomic stability, has also been observed in CML patients (84,85). A further DNA repair protein, RAD51, a protein which participates in homologous recombination repair (HRR), has also been noted to have an enhanced activity/expression (86). Expression of BCR-ABL increases the efficiency of HRR in RAD51 and often results in a higher frequency of chromosomal abnormalities.

3.18. Molecular evolution from a chronic phase to blast crisis

The most common gene mutations in the evolution of CP to BT involve the p53 gene; it is mutated in up to 30% of patients with CML in myeloid BT (73). The mutated p53 gene loses its function and probably allows an 'unchecked' BCR-ABL to promote the evolution of CP to BT, typically myeloid. Murine models have confirmed that the loss of p53's function is associated with cells which are more resistant to apoptosis and exhibit a high clongenic potential (74). The next most common mutations involve the loss of p16 and the INK41/ARF exon 2, which is homozygously deleted in about half of all patients with lymphoid BT (87,88). The loss of p16 INK 4A results in mutations or deletion in the RB gene; in about 20% of patients with BT, the RB gene is inactivated spontaneously, particularly in those with lymphoid BT (75). These observations have suggested that there may well be specific molecular determinants of myeloid and lymphoid BT.

It was recently shown that the Lyn kinase, like Abl a member of the Src family of kinases, is over-expressed in chronic phase CML cells resistant to imatinib (89). This suggests the possibility that one mechanism of imatinib resistance involves the capacity of some clones to switch from reliance on Bcr-Abl to reliance on an activated Lyn. *LYN* is also over-expressed in the cells from patients in lymphoid blastic transformation of CML, and short interfering RNA targeting the Lyn kinase induces apoptosis in BCR-ABL positive blast cells while leaving normal cells relatively intact (90). Lyn could thus be one example of the link between imatinib resistance and a predisposition to disease progression.

3.19. Kinase domain mutations

The advent of imatinib mesylate has transformed the management of CML in chronic phase. Between 70 and

80% of previously untreated chronic phase patients achieve complete cytogenetic remissions and these seem in general to be durable (91). Results for patients who start treatment in advanced phases of the CML are less impressive. A high proportion of those who respond and then lose their response to imatinib show evidence of expansion of sub-clones with point mutations in the ABL kinase domain which by implication impede binding of imatinib but do not prevent the phosphorylation of downstream substrates that mediate the leukemia signal. The precise position of the mutation appears to dictate the degree of resistance to imatinib; some mutations are associated with minor degrees of resistance while others, notably the T315I mutation, are associated with near total non-responsiveness to imatinib and other tyrosine kinase inhibitors (92,93). The acquisition and expansion of clones with mutations in the ATP phosphate-binding loop (P-loop) of the kinase domain may be associated with increased risk of disease progression and relatively early mortality (94). The mechanism by which such kinase mutations increase the aggressiveness of the leukemia are unclear but they of course merely be a marker for a high level of genetic instability that predisposes to other non-BCR-ABL linked mechanisms of progression.

4. CONCLUSIONS

Though the precise pathogenesis of the evolution of CML in CP to BT remains undefined at present, a number of important abnormalities have been identified. Most patients in the CP acquire secondary changes involving genes encoding nucleus-localized proteins. These changes affect, directly or indirectly, gene mutations/loss of function of tumor suppressor genes. The p53 gene is genetically or functionally inactivated in many patients with CML in BT, particularly those in myeloid BT. A significant impediment to the study of CML in BT, so far, has been the lack of a suitable animal model. This has particularly frustrated attempts to identify the critical molecular pathways affected. It will be also useful to correlate the levels of Bcr-Abl and disease progression. A recent study has suggested that BT cells express higher levels of Bcr-Abl in comparison to CP cells (95). From a therapeutic perspective, an important goal of imatinib is to prevent the development of BT by a complete eradication of the disease, a target that is currently difficult or impossible to achieve. Most patients do achieve a complete cytogenetic remission, but continue to have identifiable disease. The identification of a self-renewing population of progenitors of BT with possible involvement of the ß-catenin pathway suggests new treatment strategies for patients in BT, for whom currently no treatment offers the chance of durable remissions.

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