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WORKING WITH CML: KNOWING THE DISEASE.

Oncology

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ABSTRACT

Chronic myeloid leukemia (CML), also known as chronic myelogenous leukemia, is a type of cancer that starts in certain blood-forming cells of the bone marrow. In CML, a genetic change takes place in an early (immature) version of myeloid cells - the cells that make red blood cells, platelets, and most types of white blood cells (except lymphocytes). This hallmark physical change initiating CML is a reciprocal translocation involving two different genes i.e. ABL from chromosome 9 and BCR from chromosome 22 that leads to the emergence of a new abnormal fused or chimeric oncogene, BCR-ABL, on a changed chromosome 22, called the Philadelphia chromosome.

KEYWORDS

Introduction

CML is a clonal myeloproliferative disorder of the hematopoeitic stem cell (HSC). In the initial chronic phase, myeloid progenitors and mature cells accumulate in the blood and extramedullary tissues [1]. After 3–4 years, the disease transforms and terminates in a blast crisis characterized by a maturation arrest in the myeloid or lymphoid lineage [2,3,4]. In CML, ABL from chromosome 9 and BCR from chromosome mutually engage into a reciprocal translocation event causing formation of an abnormal fused or chimeric oncogene, BCR-ABL, on a changed chromosome 22, called the Philadelphia chromosome [5,6]. This abnormal fusion or hybrid gene turns the cell into a leukemic CML cell. The leukemia cells grow and divide, building up in the bone marrow and spilling over into the blood. In time, the cells can also settle in other parts of the body, including the spleen. CML is a fairly slow growing leukemia, but it can also change into a fast-growing acute leukemia that is hard to treat.

The characteristic peripheral blood and bone marrow smears of a CML patient look as in Fig.1. CML originates in a highly specialised tissue inside the long bones, which is called Bone marrow (Fig 2). This is the location of the process called hematopoiesis that leads to the formation of different blood cells. Leukemia, like CML is the direct outcome of the disorder in the process of hematopoiesis which is responsible for the production of different cell lineages constituting blood (Fig 3).

1. CML- Historical background

CML was the first type of leukemia to be described. The original case reports from Edinburgh in 1845 were entitled: 'Case of hypertrophy of the spleen and liver in which death took place from suppuration of the blood', [7] and 'Case of disease of the spleen in which death took place in consequence of the presence of purulent matter in the Blood' [8]. About the same time in Berlin, Virchow, [9] then an intern, published his classic papers entitled, 'Weisses Blut' and 'Weisses Blut and Milztumoren', in which he recognized that the 'white blood' and splenic enlargement did not represent a suppurative process, but rather represented a distinct disease entity, thereafter called leukemia. He later distinguished between a predominantly splenic form of leukemia (CML) and one in which lymphadenopathy was more prominent (chronic lymphocytic leukemia, CLL). A decade later, Friedreich [10] first described acute leukemia, but it was not until the turn of the century that further conceptual advances and development of new staining techniques permitted a definitive distinction between the acute and chronic forms of myelogenous leukemia [11,12] and between the myelogenous leukemias and the lymphocytic leukemias and lymphomas [13].

2. Milestones

The history of the hybrid BCR/ABL gene started in 1960, when an abnormal, shortened chromosome 22, termed the Philadelphia chromosome, was described in the leukemic cells of a patient affected by chronic myeloid leukemia [14]. Only 13 years later, the use of quinacrine fluorescence and Giemsa banding in chromosome studying, allowed to clarify that Ph chromosome is the result of a translocation of part of chromosome 22 to chromosome 9 [6]. The molecular characterization of this translocation was facilitated when the c-ABL gene, the human homologous of v-ABL, an oncogene originally identified in the Abelson murine leukemia virus, was localized to the long arm of the chromosome 9 [15]. Two ABL probes, which normally map to chromosome 9, were shown to hybridize on chromosome 22q-(the Ph chromosome) of somatic cell hybrids originating from human CML leukocytes and rodent cells [16], while sequences of the c-is oncogene, generally found on chromosome 22, were shown to be translocated to the chromosome 9 in CML cells [17]. These studies indicated a reciprocal translocation for the Ph chromosome, and suggested that activation of the transforming potential of the c-ABL proto-oncogene was an important consequence of the translocation.

The cloning of the BCR/ABL breakpoint was the subsequent milestone. Heisterkamp and colleagues applied the `chromosome walking' technique to show a chromosomal breakpoint within a 14 kb sequence of chromosome 9 homologous to the v-ABL, in one of three patients with CML [18]. However, the chromosome 9 rearrangement was not evident in the remaining two patients, and this prompted Groffen et al. [17] to direct their attention to chromosome 22 breakpoints. They used the sequences complementary to chromosome 22 previously isolated from the genomic clone of the positive CML patients as probe to screen for genomic rearrangements, and found a rearrangement of chromosome 22 in a second CML patient. Noteworthy, this second rearrangement of chromosome 22 mapped very closely (1kb) to that identified in the first positive CML patient. When the same probe and restriction analysis was applied to a series of 17 leukemic patients, all but two cases (both Ph chromosome negative) showed a chromosome 22 rearrangement clustered within a 5.8 kb BglII – BglII genomic fragment, which was thus named the `bcr' or breakpoint cluster region [17]. This region was later renamed `M-bcr' (major breakpoint cluster region) to distinguish it from the `minor breakpoint region'(m-bcr) – a chromosome 22 fragment located further upstream, which appeared to harbor breakpoints of Ph-positive acute leukemias [19].

It was rapidly evident that the involvement of M-bcr was highly specific for CML, as the rearrangement of this chromosome region was consistently found only in this type of leukemia and not in other hematopoietic neoplasias. The identification of an abnormally large (8.0 kb) v-ABL related mRNA in Ph positive cell lines and in CML cells, but not in normal cells and in Ph negative leukemias [20], and the characterization of a large (210 kDa) ABL related phosphorprotein, the P210 [21], reinforced the concept that CML was related to a fusion gene. Importantly, the tyrosine kinase activity of P210 resembled that of protein v-ABL [22], which was phosphorylated, whereas the 145 kDa c-ABL protein which was not phosphorylated in vivo, in normal cells [23].

The definitive proof of the presence of a fusion gene in CML cells, came from the studies of Shtivelman et al. [24] that first cloned the full

length cDNA corresponding to the 6.0 kb cABL encoding transcript, and used the 5′ part of this cDNA to clone the CML-specific transcript from cDNA libraries prepared from both K562 and EM2 PH positive cell lines. The sequence analysis of these transcripts isolated from both cell lines revealed that they contain a novel 5′ sequence fused in frame with c-ABl exon 2, and interestingly, a genomic fragment which hybridized to these non-ABL sequences contained the M-bcr region previously identified by Groffen et al. [17]. Taken together, these data suggested that the 8 kb CML-specific mRNA contains the BCR joined to the c-ABL sequences that was transcribed from a BCR/ABL fusion gene. In addition the authors suggested that the amino terminal substitution was responsible for the increased tyrosine kinase activity of the ABL-derived sequences. These first but extremely important data open the way to a huge amount of studies on the structure of this hybrid gene and on molecular mechanisms of Ph positive leukemias.

Finally, the transforming potency of BCR-ABL fusion gene was later established by using a murine model. Bone marrow cells infected with retrovirus that encoded the BCR-ABL fusion gene were transplanted into lethally irradiated mice. The transplanted cells induced hematologic malignancies in 40-50% of the recipients, of which the most prominent one was a myeloproliferative disorder that resembled CML in humans [25,26,27]. The stimulus that led to the uncontrolled proliferation of the bone marrow cells already thought by William Dameshek [28] was therefore a fusion gene encoding a chimeric protein with increased tyrosine kinase activity.

3. Epidemiology of CML

CML accounts for about 15-20% of all cases of leukemia and 7–20% of adult leukemias. The annual incidence is 1–2 cases per 100,000 individuals and it has not changed over the last 50 years. CML affects males more often than females (ratio of 1.3–2.2). CML tends to be a disease of middle life with a slowly increasing age trend in Western countries. The median age at presentation reported from large cohort studies is 45–55 years [29,3031]. About 10% of CML cases are found in subjects between 5 and 20 years of age. CML represents about 3% of all childhood leukemias. There is no concordance of the disease between identical twins [32].

As far as the incidence of CML in India is concerned, the up-to date figures are not available. However, according to 6 population-based cancer registries (covering <0.3% of the total population), the incidence of CML in India varies from 0.8 to 2.2 per 100000 of population for men and from 0.6 to 1.6 per 100000 population for women [33]. Hospital-based studies have reported a higher frequency of CML ranging from 40% to 82% of all cases of leukaemia among adults [34]. In India, CML is seen prevalent in third and fourth decades [35] and the median age of onset is 38–40 years [34] compared to about 50 years in the West. Here in Kashmir (North India), our institute happens to be the only referral centre for leukemias and on an average, we receive 3-4 patients with CML every month with almost even gender distribution. As per a study [36], the leukemia's rank fifth in order of occurrence among the cancers prevalent in Kashmir valleys, wherein CML figures as the second most incident leukemia after Acute Lymphoid Leukemia (ALL).

4. Etiology of CML

Leukemogenesis is a multistep phenomenon that is divided into initiation, promotion, and progression phases. The initiation phase involves acquisition of a genetic defect that confers cell survival advantage. What triggers the generation of the initiation step in CML is unknown. In experiments in which leukemic cell lines were exposed to gamma irradiation, fusion genes characteristic of different forms of leukemias were generated, although these defects were also detected at a low level in untreated cells [37]. The generation of the BCR-ABL gene is now recognized as the key molecular event leading to CML. What induces this molecular rearrangement is unknown though.

Using highly sensitive polymerase chain reaction (PCR) techniques, BCR-ABL transcripts could be detected in the bone marrow cells of 25–30% of healthy volunteers and in 5% of infants, but not in cord blood cells [38,39]. Because clinical CML is reported to develop in only 1–2 of 100,000 individuals, it follows that in most of these individuals, those cells expressing BCR-ABL do not produce overt CML disease. This observation suggests that immune regulatory processes or additional molecular events contribute to the development of CML. There is no evidence supporting hereditary or genetic factors. BCR-ABL is found only in hematopoietic cells, and there is no increased incidence of CML in monozygotic twins or in the relatives of patients with CML. No chemical or infectious exposures have been linked to CML. The incidence of CML is reported to be higher in survivors of the atomic bomb or nuclear exposures [40].

5. Signs and symptoms of CML

The symptoms of CML are often vague and are more often caused by other things as well including non-cancerous conditions. They include: Weakness, Fatigue, Night sweats, Weight loss, Fever, Bone pain, Enlarged spleen (felt as a mass under the left side of the ribcage), Pain or a sense of "fullness" in the belly, Feeling full after eating even a small amount of food. Some patients have bone pain or joint pain caused by leukemia cells spreading from the marrow cavity to the surface of the bone or into the joint. The most common sign of CML is an abnormal white blood cell count.

Many of the signs and symptoms of CML occur because the leukemia cells replace the bone marrow's normal blood-making cells. As a result, people with CML do not make enough red blood cells, properly functioning white blood cells, and blood platelets. Accordingly, they may have.

- Anemia is a shortage of red blood cells. It can cause weakness, tiredness, and shortness of breath.
- Leukopenia is a shortage of normal white blood cells. This shortage increases the risk of infections. Although patients with leukemia may have very high white blood cell counts, the leukemia cells do not protect against infection the way normal white blood cells do.
- Neutropenia means that the level of normal neutrophils is low. Neutrophils, a type of white blood cell, are very important in fighting infection from bacteria. People who are neutropenic have a high risk of getting very serious bacterial infections.
- Thrombocytopenia is a shortage of blood platelets. It can lead to excess bruising or bleeding, with frequent or severe nosebleeds and bleeding gums. Some patients with CML actually have too many platelets (thrombocytosis). But since those platelets often do not function properly, these people often have problems with bleeding and bruising as well.

6. Phases of chronic myeloid leukemia

CML is said to occur in 3 phases that help predict outlook. The phases are based mainly on the number of immature white blood cells — myeloblasts (blasts) — that are seen in the blood or bone marrow. Different groups of experts have suggested slightly different cut-offs to define the phases, but a common system (proposed by the World Health Organization) is described below [41,42]. However, not all doctors may agree with or follow these cut-off points for the different phases.

6a. Chronic phase (CP)

Patients in this phase typically have less than 10% blasts in their blood or bone marrow samples. These patients usually have fairly mild symptoms (if any) and usually respond to standard treatments. Most patients are diagnosed in the chronic phase.

6b. Accelerated phase (AP)

Patients are considered to be in accelerated phase if any of the following are true:

- The bone marrow or blood samples have more than 10% but fewer than 20% blasts.
- High blood basophil count (basophils making up at least 20% of the white blood cells).
- High white blood cell counts that do not go down with treatment.
- Very high or very low platelet counts that are not caused by treatment.
- New chromosome changes in the leukemia cells.
- Patients whose CML is in accelerated phase may have symptoms such as fever, poor appetite, and weight loss. CML in the accelerated phase does not respond as well to treatment as CML in the chronic phase.

6c. Blast phase or blast crisis (BC)

Bone marrow and/or blood samples from a patient in this phase have more than 20% blasts. The blast cells often spread to tissues and organs beyond the bone marrow. These patients often have fever, poor appetite, and weight loss. In this phase, the CML acts much like an aggressive acute leukemia.

7. Prognostic factors for chronic myeloid leukemia

Along with the phase of CML, there are other factors that may help predict the outlook for survival. These factors are sometimes helpful when choosing treatment. Factors that tend to be linked with shorter survival time are called adverse prognostic factors, which include:

- Accelerated phase or blast phase.
- Enlarged spleen.
- Areas of bone damage from growth of leukemia.
- Increased number of basophils and eosinophils (certain types of granulocytes) in blood samples.
- Very high or very low platelet counts.
- Age 60 years or older.
- Multiple chromosome changes in the CML cells.
- Many of these factors are taken into account in the Sokal system, which develops a score used to help predict prognosis. This system considers the person's age, the percentage of blasts in the blood, the size of the spleen, and the number of platelets. These factors are used to divide patients into low, intermediate, or high-risk groups.
- Another system, called the Euro score, includes the above factors, as well as the number of blood basophils and eosinophils. Having more of these cells indicates a poorer outlook.
- The Sokal and Euro models were helpful in the past, before the newer, more effective drugs for CML were developed. It's not clear how helpful they are at this time in predicting a person's outlook.

8. Physiological function of the translocation partners

The ABL gene is the human homologue of the v-abl oncogene carried by the Abelson murine leukemia virus (A-MuLV) [43] and it encodes a non-receptor tyrosine kinase [44]. Human Abl is a ubiquitously expressed 145-kd protein with 2 isoforms arising from alternative splicing of the first exon [44]. Several structural domains can be defined within the protein (Fig. 4). Three SRC homology domains (SH1-SH3) are located toward the NH2 terminus. The SH1 domain carries the tyrosine kinase function, whereas the SH2 and SH3 domains allow for interaction with other proteins [45]. Proline-rich sequences in the center of the molecule can, in turn, interact with SH3 domains of other proteins, such as Crk [46]. Toward the 39 end, nuclear localization signals [47] and the DNA-binding [48] and actin-binding motifs [49] are found.

Several fairly diverse functions have been attributed to Abl, and the emerging picture is complex. Thus, the normal Abl protein is involved in the regulation of the cell cycle, [50,51] in the cellular response to genotoxic stress, [52] and in the transmission of information about the cellular environment through integrin signalling [53]. Overall, it appears that the Abl protein serves a complex role as a cellular module [54] that integrates signals from various extracellular and intracellular sources and that influences decisions in regard to cell cycle and apoptosis. It must be stressed, however, that many of the data are based solely on in vitro studies in fibroblasts, not hematopoietic cells, and are still controversial. Unfortunately, the generation of ABL knockout mice failed to resolve most of the outstanding issues [55,56].

The 160-kd Bcr protein, like Abl, is ubiquitously expressed [44]. Several structural motifs can be delineated (Fig. 5). The first N-terminal exon encodes a serine–threonine kinase. The only substrates of this kinase identified so far are Bap-1, a member of the 14-3-3 family of proteins, [57] and possibly Bcr itself [44]. A coiled–coil domain at the N-terminus of Bcr allows dimer formation in vivo [49]. The center of the molecule contains a region with dbl-like and pleckstrin-homology (PH) domains that stimulate the exchange of guanidine triphosphate (GTP) for guanidine diphosphate (GDP) on Rho guanidine exchange factors, [58] which in turn may activate transcription factors such as NF-kB [59]. The C-terminus has GTPase activity for Rac, [60] a small GTPase of the Ras superfamily that regulates actin polymerization and the activity of an NADPH oxidase in phagocytic cells [60]. In addition, Bcr can be phosphorylated on several tyrosine residues, [61] especially tyrosine 177, which binds Grb-2, an important adapter molecule involved in the activation of the Ras pathway [62]. Interestingly, Abl has been shown to phosphorylate Bcr in COS1 cells, resulting in a reduction of Bcr kinase activity [62,63].

Although these data argue for a role of Bcr in signal transduction, their true biological relevance remains to be determined. The fact that BCR knockout mice are viable and the fact that an increased oxidative burst in neutrophils is thus far the only recognized defect [64] probably reflect the redundancy of signalling pathways. If there is a role for Bcr in the pathogenesis of Ph-positive leukemias, it is not clearly discernible because the incidence and biology of P190BCR-ABL-induced leukemia are the same in BCR2/2 mice as they are in wild-type mice s[65].

9. Molecular anatomy of the BCR-ABL translocation

The classic BCR-ABL gene of CML results from the fusion of parts of two normal genes: the ABL gene on chromosome 9 and the BCR gene on chromosome 22. Both genes are ubiquitously expressed in normal tissues, but their precise functions are not well defined. In the translocation that forms the fusion gene, a break occurs in ABL somewhere upstream of exon a2, and simultaneously a break occurs in the major breakpoint cluster region of the BCR gene. As a result, a 5' portion of BCR and a 3' portion of ABL are juxtaposed on a shortened chromosome 22- the derivative 22q, or Ph, chromosome (Fig. 6).

The messenger RNA (mRNA) molecules transcribed from this hybrid gene usually contain one of two BCR-ABL junctions, designated e13a2 (formerly b2a2) and e14a2 (or b3a2). There is no evidence that the type of junction has any prognostic significance. Both BCR-ABL mRNA molecules are translated into an 210-kD onco-protein, usually referred to as p210BCR-ABL. Other variant breakpoints and fusions can give rise to full-length, functionally oncogenic BCR-ABL proteins, notably p190BCR-ABL (associated with an e1a2 mRNA junction) and p230BCR-ABL (associated with an e19a2 mRNA junction), [66] but they are rather rare in classic CML [67,68].

The leukemogenic potential of p210 BCR-ABL resides in the fact that the normally regulated tyrosine kinase activity of the ABL protein is constitutively activated by the juxtaposition of alien BCR sequences (Fig. 7).

BCR acts by promoting dimerization of the oncoprotein, such that the two adjacent BCR-ABL molecules phosphorylate each other on tyrosine residues in their kinase-activation loops [49,69]. The uncontrolled kinase activity of BCR-ABL then usurps the physiologic functions of the normal ABL enzyme by interacting with a variety of effector proteins, the net result of which is deregulated cellular proliferation, decreased adherence of leukemia cells to the bone marrow stroma, and a reduced apoptotic response to mutagenic stimuli. Unfortunately, the relative contributions of these effects to the phenotype of chronic phase-CML is still poorly understood [70].

The structure of the BCR-ABL protein and the biochemical pathways in which it is involved have been extensively studied [71]. Knowledge of the roles of several functional domains derived from the parental BCR and ABL proteins allows one to test for certain properties of the fusion product. Thus the tyrosine kinase encoded by the SRC homology 1 (SH1) domain of the ABL component of BCR-ABL is undoubtedly the most crucial for oncogenic transformation. Other important motifs in the ABL portion are the protein-interaction SRC homology 2 (SH2) and the C-terminal actin binding domains. On the BCR moiety the coiled-coil motif encoded by the first BCR exon is responsible for the dimerization of the oncoprotein; a 'tyrosine' at position 177 is crucial for the binding of adaptor proteins such as growth factor receptor-bound protein 2; and N-terminal 'phosphoserine' and 'phosphothreonine' residues are required for interaction with SH2-containing proteins, including ABL itself.

One of the most striking differences between the normal ABL protein and BCR-ABL is in their contrasting subcellular locations. The ABL protein is found in both the nucleus and the cytoplasm and can shuttle between these two compartments under the influence of its nuclear-localization signal and nuclear-export signal domains, whereas BCR-ABL is exclusively cytoplasmic. Nuclear ABL is an essentially proapoptotic protein, playing a key part in the cellular response to genotoxic stress. BCR-ABL, in contrast, is largely antiapoptotic and, although it retains the ABL nuclear-localization and nuclear-export sequences, seems unable to enter the nucleus. The main reason the BCR-ABL protein is retained in the cytoplasm is its constitutively activated tyrosine kinase [72]. When the kinase is inhibited in vitro with imatinib and its nuclear export simultaneously blocked with leptomycin B, the oncoprotein enters the nucleus and is trapped there. Interestingly, when imatinib is removed and the now nuclear BCR-ABL is allowed to reactivate its tyrosine kinase, it is converted from an antiapoptotic to a proapoptotic protein and induces, rather than prevents, cell death. It has recently been shown that BCR-ABL also

translocates to the nucleus in leukemia cells subjected to genotoxic stress and there slows DNA repair by interaction with effectors of the ataxia telangiectasia–related (ATR) protein, a phenomenon that may underlie the genomic instability of the CML clone [73].

10. Relevance of the breakpoint in Translocation

The breakpoints within the ABL gene at 9q34 can occur anywhere over a large (greater than 300 kb) area at its 5′ end, either upstream of the first alternative exon Ib, downstream of the second alternative exon Ia, or, more frequently, between the two [67] (Fig 8). Regardless of the exact location of the breakpoint, splicing of the primary hybrid transcript yields an mRNA molecule in which BCR sequences are fused to ABL exon a2. In contrast to ABL, breakpoints within BCR localize to 1 of 3 so-called breakpoint cluster regions (bcr). In most patients with CML and in approximately one third of patients with Ph-positive acute lymphoblastic leukemia (ALL), the break occurs within a 5.8-kb area spanning BCR exons 12-16 (originally referred to as exons b1-b5), defined as the major breakpoint cluster region (M-bcr). Because of alternative splicing, fusion transcripts with either b2a2 or b3a2 junctions can be formed. A 210-kd chimeric protein (P210 BCR-ABL) is derived from this mRNA. In the remaining patients with ALL and rarely in patients with CML, characterized clinically by prominent monocytosis [68,74], the breakpoints are further upstream in the 54.4-kb region between the alternative BCR exons e2′ and e2, termed the minor breakpoint cluster region (m-bcr). The resultant e1a2 mRNA is translated into a 190-kd protein (P190BCR-ABL).

A third breakpoint cluster region (m-bcr) was identified downstream of exon 19, giving rise to a 230-kd fusion protein (P230 BCR-ABL) associated with the rare Ph-positive chronic neutrophilic leukemia, [66] though not in all cases [75]. If sensitive techniques such as nested reverse transcription–polymerase chain reaction are used, transcripts with the e1a2 fusion are detectable in many patients with classical P210BCR-ABL CML [76].

The low level of expression of these P190-type transcripts compared to P210 indicates that they are most likely the result of alternative splicing of the primary mRNA. Occasional cases with other junctions, such as b2a3, b3a3, e1a3, e6a2, [77] or e2a2, [78] have been reported in patients with ALL and CML. These “experiments of nature” provide important information as to the function of the various parts of BCR and ABL in the oncogenic fusion protein. Interestingly, ABL exon 1, even if retained in the genomic fusion, is never part of the chimeric mRNA. Thus, it must be spliced out during processing of the primary mRNA; the mechanism underlying this apparent peculiarity is unknown.

Based on the observation that the Abl part in the chimeric protein is almost invariably constant while the Bcr portion varies greatly, one may deduce that Abl is likely to carry the transforming principle whereas the different sizes of the Bcr sequence may dictate the phenotype of the disease. In support of this notion, rare cases of ALL express a TEL-ABL fusion gene [79.80], indicating that the BCR moiety can in principle be replaced by other sequences and still cause leukemia. Interestingly, a fusion between TEL(ETV6) and the ABL-related gene ARG has recently been described in a patient with AML [81]. Although all 3 major Bcr-Abl fusion proteins induce a CML-like disease in mice, they differ in their ability to induce lymphoid leukemia, [82] and, in contrast to P190 and P210, transformation to growth factor independence by P230BCR-ABL is incomplete, [83] which is consistent with the relatively benign clinical course of P230 positive chronic neutrophilic leukemia [66].

One of the most intriguing questions relates to the events responsible for the chromosomal translocation in the first place. From epidemiologic studies it is well known that exposure to ionizing radiation (IR) is a risk factor for CML [84,85]. Moreover, BCR-ABL fusion transcripts can be induced in hematopoietic cells by exposure to IR in vitro [37]; such IR-induced translocations may not be random events but may depend on the cellular background and on the particular genes involved. Two recent reports showed that the physical distance between the BCR and the ABL genes in human lymphocytes [86] and CD34+ cells [87] is shorter than might be expected by chance; such physical proximity could favor translocation events involving the 2 genes. However, the presence of the BCR-ABL translocation in a hematopoietic cell is not in itself sufficient to cause leukemia because BCR-ABL fusion transcripts of M-bcr and m-bcr type are detectable at low frequency in the blood of many healthy individuals [88,89].

It is unclear why Ph-positive leukemia develops in a tiny minority of these persons. It may be that the translocation occurs in cells committed to terminal differentiation that are thus eliminated or that an immune response suppresses or eliminates Bcr-Abl–expressing cells. Indirect evidence that such a mechanism may be relevant comes from the observation that certain HLA types protect against CML [90]. Another possibility is that BCR-ABL is not the only genetic lesion required to induce chronic-phase CML. Indeed, a skewed pattern of G-6PD isoenzymes has been detected in Ph-negative Epstein-Barr virus-transformed B-cell lines derived from patients with CML, suggesting that a Ph-negative pathologic state may precede the emergence of the Ph chromosome [91].

Conclusion

CML though manageable is incurable and its treatment remains a challenge. The BCR-ABL enzyme is a molecular switch that keeps the stimulation of myeloid cell lineage always on resulting in uncontrolled proliferation of blood cells. This disturbs the overall hematologic homeostasis leading to the typical hematopatholgic profile manifested as a form of Leukemia.

Figures

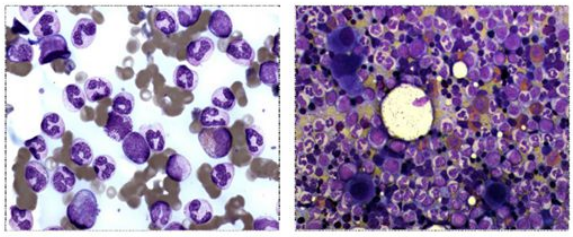

Fig.1: Peripheral blood (left) and bone marrow (right) smears of a CML patient in chronic phase, showing leukocytosis in peripheral blood and hypercellularity in bone marrow due mainly to neutrophils in different stages of maturation. Courtesy: *Dr. Satu Mustjoki.*

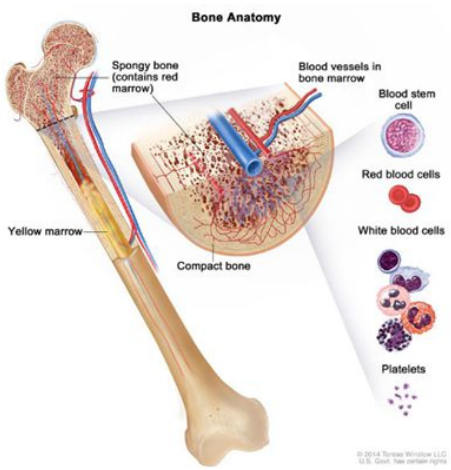

Fig. 2 : Anatomy of the long bone- The site of Hematopoiesis. *Courtesy: Terese Winslow LLC.*

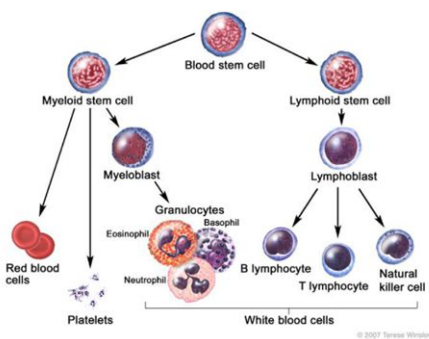

Fig. 3: Haematopoietic cascade. *Courtesy: Terese Winslow.*

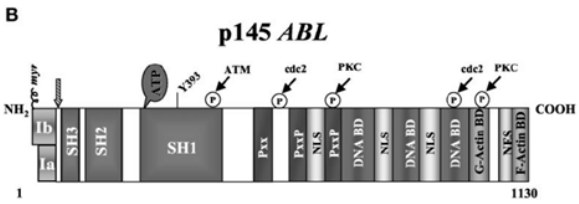

Fig. 4: Structure of the Abl protein. Type Ia isoform is slightly shorter than type Ib, which contains a myristoylation (myr) site for attachment to the plasma membrane. Note the 3 SRC-homology (SH) domains situated toward the NH2 terminus. Y393 is the major site of autophosphorylation within the kinase domain, and phenylalanine 401 (F401) is highly conserved in PTKs containing SH3 domains. The middle of each protein is dominated by proline-rich regions (PxxP) capable of binding to SH3 domains, and it harbors 1 of 3 nuclear localization signals (NLS). The carboxy terminus contains DNA as well as G-and F-actin–binding domains. Phosphorylation sites by Atm, cdc2, and PKC are shown. The arrowhead indicates the position of the breakpoint in the Bcr-Abl fusion protein. Reprinted from *Review-Molecular biology of CML by Michael WN Deininger, 2000.*

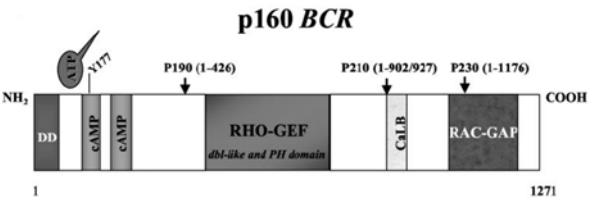

Fig. 5: Structure of the Bcr protein. Note the dimerization domain (DD) and the 2 cyclic adenosine monophosphate kinase homologous domains at the N terminus. Y177 is the autophosphorylation site crucial for binding to Grb-2. The center of the molecule contains a region homologous to Rho guanidine nucleotide exchange factors (Rho-GEF) as well as dbl-like and pleckstrin homology (PH) domains. Toward the C-terminus a putative site for calcium-dependent lipid binding (CaLB) and a domain with activating function for Rac-GTPase (Rac-GAP) are found. Arrowheads indicate the position of the breakpoints in the BCR-ABL fusion proteins. *Reprinted from Review-Molecular biology of CML by Michael WN Deininger, 2000.*

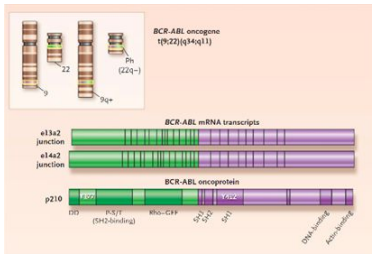

Fig. 6: The t(9;22) translocation and its products: The BCR-ABL fusion oncogene on the Philadelphia chromosome and the reciprocal ABL-BCR fusion gene on the derivative 9q+ chromosome. *Adopted from Review on CML by John M Goldman, 2003.*

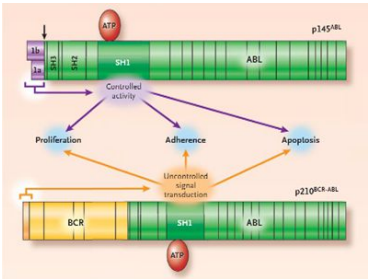

Fig. 7: Physiologic regulation by the normal ABL protein and de-regulation by BCR-ABL oncoprotein of key cellular processes such as Proliferation, Adhesion and Apoptosis. *Adopted from Review on CML by John M Goldman, 2003.*

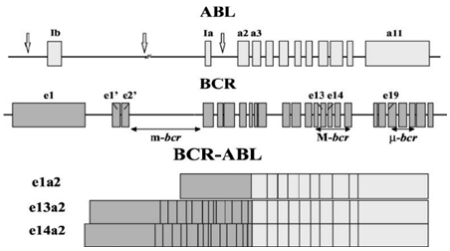

Fig. 8: Locations of the breakpoints in the ABL and BCR genes and structure of the chimeric/fusion mRNAs derived from the various breaks. *Reprinted from Review-Molecular biology of CML by Michael WN Deininger, 2000.*

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