14. The molecular pathogenesis of the Philadelphia-positive leukemias: Implications for diagnosis and therapy

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Introduction

The Philadelphia chromosome is the cytogenetic hallmark of the myeloproliferative disease chronic myelogenous leukemia (CML), and is also found in many cases of acute lymphoblastic leukemia (ALL) and some cases of acute myelogenous leukemia (AML). In 1973, Rowley demonstrated that the Philadelphia chromosome, a small form of human chromosome 22 [1], was the product of a reciprocal translocation between the long arms of chromosomes 9 and 22: t(9;22)(q34.1;q11.21) [2]. A decade later, the molecular structure of the Philadelphia chromosome was determined, showing that the Philadelphia translocation results in the juxtaposition of the human proto-oncogene \(c-ABL\) on chromosome 9 with a gene denoted \(BCR\) on chromosome 22, resulting in the generation of a chimeric \(BCR/ABL\) fusion gene [3–8]. In the ensuing years, progress has been rapid, sometimes astonishingly so. The product of the \(BCR/ABL\) gene was identified in malignant cells from CML patients as a 210-kd fusion protein, \(P210^{BCR/ABL}\) [9]. Like other members of the \(abl\) family, the \(P210^{BCR/ABL}\) protein was shown to be a nonreceptor protein-tyrosine kinase [10]. The role of \(BCR/ABL\) as an oncogene was strengthened with the findings that this gene could transform factor-dependent lymphoid and myeloid cells in culture to growth factor-independence and tumorigenicity [11,12], and by its ability to transform immature primary lymphoid progenitor cells in long-term in vitro culture [13]. The importance of the tyrosine kinase activity for transformation and oncogenicity was confirmed with the study of mutants that were temperature-sensitive or defective for tyrosine kinase activity [14,15]. A distinct and smaller form of \(Bcr/Abl\) protein, \(P190^{BCR/ABL}\), was identified in some patients with Philadelphia-positive ALL and AML, resulting from a molecular variant of the Philadelphia chromosome translocation [16–19]. Recently, animal models of the \(BCR/ABL\) leukemias have become available, employing either transgenic strains of mice carrying the \(BCR/ABL\) gene [20,21], or retroviral transduction of \(BCR/ABL\) genes into mouse bone marrow followed by bone marrow transplantation [22–24]. Thus, this group
of human leukemias is now one of the best characterized at the clinical and molecular levels, and is the subject of several recent reviews [25,26].

This chapter summarizes the diagnostic and therapeutic impact of basic research on the human Philadelphia-positive leukemias. Where possible, the chapter will emphasize the insights into pathophysiology that molecular biology affords us, and make predictions about which areas might be advanced in the near future.

**Diagnosis and classification of the Philadelphia-positive leukemias**

Application of molecular biological techniques to the Philadelphia-positive leukemias has revolutionized the diagnosis, classification, and risk stratification of these diseases. These techniques and selected applications are briefly reviewed below.

**Molecular diagnostic techniques for BCR/ABL**

Tumor cells from patients with CML contain three unique products that distinguish them from nonmalignant hematopoietic cells: the chimeric BCR/ABL fusion gene itself, the 8.5-kb mRNA transcript of the chimeric gene, and the 210-kd fusion protein product, P210BCRABL. In principal, detection of any one of these tumor-specific products provides the same diagnostic information as the presence of the Philadelphia chromosome in a metaphase spread.

The molecular structure of the Philadelphia chromosome was originally elucidated by the observation of rearrangements of restriction endonuclease fragments of genomic DNA on Southern blots. Indeed, the name BCR is an acronym for breakpoint cluster region, since it was discovered that breakpoints on chromosome 22 in CML fell into a small 5.8-kb region, and probes from this region detected rearrangements in virtually all CML patients [5]. Application of Southern blotting as a diagnostic tool in cases of suspected CML has increased the frequency of detection of the BCR/ABL translocation in cases where the Ph\(^1\) chromosome itself has been obscured by a complex series of translocations [27,28]. However, the sensitivity of Southern blot is about the same as cytogenetics, with both able to detect a minimum of about 5% malignant cells in a population of normal cells.

The CML-specific 8.5-kb mRNA transcript [29] may also be detected by RNA blotting and hybridization techniques (so-called Northern blots), but this is a laborious and technically difficult process rarely employed outside of a research environment. In practice, this RNA is most commonly detected by the application of the polymerase chain reaction (PCR; for review, see [30]). Reverse transcriptase is initially used to provide a cDNA copy of the BCR/ABL message, then BCR- and ABL-specific oligonucleotide primers are employed to amplify the junction region of the chimeric cDNA. By