MINIREVIEW

The Molecular Genetics of Hematologic Malignancies

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INTRODUCTION

Malignancies, including those developing in cells of the hematologic system, have a molecular genetic basis in that they evolve as a consequence of the expression of aberrant genes and/or the aberrant expression of normal genes. The vast majority of these malignancies occur because of acquired genetic events and are clonal, making them particularly suitable for molecular (DNA and/or RNA) diagnostic studies. In addition to being ideally suited for an initial diagnostic evaluation, molecular studies can provide useful prognostic information, helping to define prognostically relevant molecular subtypes of diseases that appear homogeneous by conventional criteria. Furthermore, given the exquisite sensitivity of some molecular assays (in particular, PCR-based assays), such studies are useful in minimal disease evaluation, be it for initial disease staging or for the evaluation of response to therapy. These acquired somatic abnormalities generally affect genes that are physiologically involved in cell growth, proliferation, differentiation, and survival (the proto-oncogenes and tumor suppressor genes), and the molecular abnormalities are recapitulated in the clonal progeny of the neoplastic cells. Indeed, the molecular dissection of genetic events in tumors has led to the discovery of a variety of novel genes, which has enhanced our understanding of both normal and neoplastic cell biology. Traditionally, only fresh, unfixed tissue was believed to be amenable to nucleic acid-based analysis. However, it is now clear that no source of material is immune to study, including formalin- and B5-fixed tissues, smears on glass slides, and even single cells.

This minireview will endeavor to provide an overview of how an evaluation of the molecular genetics of hematologic malignancies may be useful diagnostically, prognostically, in assessing minimal residual disease, and in providing insights into neoplastic and normal cell biology.

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DIAGNOSIS

The precise and accurate diagnosis and classification of tumors of hematopoietic cells can be facilitated through a variety of molecular strategies, including those examining antigen receptor gene rearrangements and chromosomal abnormalities as well as other clonality studies.

Antigen receptor gene rearrangements. The unraveling of the mechanisms of antigen receptor gene rearrangements (immunoglobulin genes in B cells and T-cell receptor genes in T cells) and the cloning of probes of these regions have been pivotal to both the documentation of clonality and the assignment of lineage in hematologic malignancies (50). In T-cell proliferations, and in B-cell proliferations that lack surface antibody (which would have allowed an analysis of the ratio of kappa-light-chain-bearing-lambda-light-chain-bearing cells), an analysis of the configuration of these genes is essential for the documentation of clonality and, hence, neoplasia. Consequently, such analyses are critical to the distinction of monoclonal (malignant) from polyclonal (reactive) lymphoid proliferations. A variety of immune cell malignancies, whose lineage was unclear on the basis of morphology and immunophenotypic studies, have had lineages assigned on the basis of the discovery of clonal antigen receptor gene rearrangements. For example, the finding of immunoglobulin heavy-chain gene rearrangements in 100% of cases of common acute lymphoblastic leukemia (ALL) (35) and hairy-cell leukemia (34) was used to firmly establish these leukemias as being of B-cell lineage, while an evaluation of T-cell receptor gene rearrangements is useful in classifying clonal diseases of large granular lymphocytes (45).

However, antigen receptor gene rearrangements, in isolation, can be misleading if used for lineage assignment (76). It is now well recognized that cross-lineage rearrangements are not uncommon: immunoglobulin gene rearrangements are found in immature T-cell malignancies, and T-cell receptor gene rearrangements are seen in precursor B-cell malignancies (51, 76). Both crossover types of rearrangement occur in a small but significant proportion of cases of acute nonlymphoblastic leukemia (ANLL). Hence, antigen receptor gene rearrangements should always be evaluated, when used as a diagnostic tool, in the context of conventional morphology, cytochemistry, and immunophenotyping. While immunoglobulin heavy-chain gene rearrangements occur not uncommonly in non-B-cell malignancies, with very few exceptions, immunoglobulin light-chain gene rearrangements are essentially specific for documenting B-cell lineage. Antigen receptor gene rearrangements traditionally have been evaluated by using standard Southern blot methodology with its attendant disadvantages of a requirement for the presence of fresh, unfixed tissue and high-molecular-weight DNA, use of radioactive materials, and lengthy turn-around time. These disadvantages have been overcome, to some degree, by the advent of PCR-based assays for documentation of the rearrangement of these genes (72).

Molecular cytogenetics. Many hematologic malignancies carry chromosomal abnormalities, which may be qualitative (for example, translocations) or quantitative (for example, gains and losses). Some specific disease types have specific, nonrandom cytogenetic abnormalities, including chromosomal translocations, deletions, and inversions (23, 68). In lymphoid malignancies, one of the antigen receptor genes is involved in approximately 60% of all translocations. These translocations...
probably result from errors in the normal DNA recombination, which physiologically occur in the antigen receptor gene rearrangements in early lymphoid cells. Using these known, and characterized, antigen receptor genes as a handle, molecular techniques such as chromosomal walking and breakpoint cloning have led to the discovery of the reciprocal gene(s) involved in the translocation event (see “Biology,” below). The determination of the genes affected by these karyotypic abnormalities has allowed for the development of molecular methods, using probes and/or primers, to detect their involvement. Broadly, molecular cytogenetic evaluation may be achieved in one of three ways: (i) Southern analysis (for example, c-myc and bcl-2 rearrangements in Burkitt’s and follicular non-Hodgkin’s lymphomas, respectively), (ii) Northern (RNA) analysis (aberrant abl and pml transcripts in chronic myeloid leukemia [CML] and acute promyelocytic leukemia [APL], respectively), and (iii) PCR (in situations where chromosomal breakpoints are clustered and sequences flanking both the breakpoints are known [Tables 1, 2, and 3]).

The ability to subject DNA (or cDNA) to PCR is dependent on the clustering of, and a knowledge of the sequences of, the genes that flank the breakpoints of a chromosomal translocation, so that primers can be designed to amplify only the translocation, if present, and not any nontranslocated gene(s). Thus, in addition affording enhanced sensitivity, PCR assays are specific for specific translocations. The specificity can be exploited for diagnostic purposes, while the sensitivity can be used in a number of different scenarios (see “Minimal residual disease,” below). While all the translocations noted in Table 3 are amenable to PCR (invariably RNA-based RT-PCR), not all of the translocations noted in Tables 1 and 2 are. For example, the breakpoints in and around the myc gene are extremely widely dispersed, precluding the generation of useful primers for the performance of PCR assays. Consequently, a translocation event involving the myc gene is determined by Southern blot analysis, with probes of the appropriate region.

Mechanistically, some translocations result in the generation of novel fusion genes, while others lead to the dysregulated expression of a proto-oncogene. A few representative examples, illustrating the contribution of molecular cytogenetics to the diagnosis of hematologic malignancies, are presented below.

CML is characterized by the proliferation of granulocytic cells, in all phases of maturation, and the classic Philadelphia chromosome as a consequence of the t(9;22) translocation. The abl proto-oncogene on 9q34 is juxtaposed with the bcr gene on 22q11, and portions of the two genes are then transcribed into a fusion mRNA of ~8.5 kb and subsequently translated into a chimeric p210 protein (56). Although cytogenetically identical to that seen in CML, the t(9;22) typically seen in adult ALL is molecularly distinct in that the break in the bcr gene occurs further upstream (5′) than it does in CML. Consequently, a smaller portion of the bcr gene is fused to abl, resulting in a smaller fusion mRNA (~7.0 kb) and protein (~185 kDa). Southern and Northern analysis, with appropriate bcr probes, can be used to detect and distinguish between the two forms of the translocation. An RT-PCR assay can also be used to specifically and sensitively (down to 10 pg of RNA, equivalent to that in a single cell, when a nested reaction is employed) detect this translocation event, with different bcr primers used to distinguish the CML from the ALL translocation (32).

Pre-B-cell ALL is characterized by a maturation arrest of immature B cells at the stage when cytoplasmic (but not surface) μ immunoglobulin heavy chains are expressed. A t(1;19) translocation, which results in the fusion of the E2A and PDX1 genes, is seen in approximately 30% of these leukemias, and it can be analyzed in a manner analogous to that for the t(9;22) translocation. Similarly, it can be detected in cases with a grossly normal karyotype, as discerned cytogenetically (27). APL is associated with an accumulation of hypergranulated (in the classic form) CD33⁺, CD13⁺, CD34⁺, HLA-DR⁺, and CD15⁺ promyelocytes, due to a maturation arrest at this stage of granulocytic development. In virtually 100% of cases, a t(15;17) translocation is present. As in the preceding two examples, this translocation results in the fusion of two previously distinct genes, RARα and PML, which can be detected by Southern, Northern, and RT-PCR analysis (18). The presence of this genetic fusion, even if not detected cytogenetically, is clinically relevant in that it predicts which patients will respond (albeit transiently) to therapy with retinoic acid (52). Also, the region where the breakage occurs in the PML gene appears to be associated with the morphologic phenotype of the APL, namely, either the typical, hypergranulated form or the variant, hypogranular or microgranular form (9).

The t(14;18) translocation is characteristic of follicular non-Hodgkin’s lymphoma (NHL), which is the most common form of NHL occurring in North America. The translocation may be detected by a PCR assay similar to those noted in the three examples given above, but this case is different in that the amplification can be, and indeed usually is, performed with DNA rather than RNA, since the most frequent break is in an exon (albeit in a nontranslated region) of the bcl-2 gene on chromosome 18 (5). The detection of this translocation is con-

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### TABLE 1. Dysregulated expression of oncogenes* by translocation in B-cell malignancies

<table>
<thead>
<tr>
<th>Oncogene</th>
<th>Locus</th>
<th>Disease</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>myc</td>
<td>8q24</td>
<td>Burkitt’s NHL</td>
<td>Transcription factor</td>
</tr>
<tr>
<td>bcl-1</td>
<td>11q13</td>
<td>Mantle cell NHL</td>
<td>Cell cycle regulation</td>
</tr>
<tr>
<td>bcl-2</td>
<td>18q21</td>
<td>Follicular NHL</td>
<td>Apoptosis inhibition</td>
</tr>
<tr>
<td>bcl-3</td>
<td>19q13</td>
<td>Chronic lymphocytic leukemia (minority)</td>
<td>Transcriptional regulation</td>
</tr>
<tr>
<td>bcl-6</td>
<td>3q27</td>
<td>Diffuse large-cell NHL</td>
<td>Transcription factor</td>
</tr>
</tbody>
</table>

* Most of these translocations, which involve an immunoglobulin gene as a partner, are detectable by standard Southern analysis. PCR assays are available for some of the translocations, where there is breakpoint clustering.

### TABLE 2. Dysregulated expression of oncogenes by translocation in T-cell malignancies*

<table>
<thead>
<tr>
<th>Oncogene</th>
<th>Locus</th>
</tr>
</thead>
<tbody>
<tr>
<td>tal-1</td>
<td>1p15</td>
</tr>
<tr>
<td>tal-2</td>
<td>1p13</td>
</tr>
<tr>
<td>tal-3</td>
<td>10q24</td>
</tr>
<tr>
<td>tal-5</td>
<td>1p32</td>
</tr>
<tr>
<td>tal-6</td>
<td>9q32</td>
</tr>
<tr>
<td>tal-7</td>
<td>9q34</td>
</tr>
<tr>
<td>tal-8</td>
<td>19p13</td>
</tr>
<tr>
<td>tal-9</td>
<td>1p32</td>
</tr>
</tbody>
</table>

* Most of these translocations (or deletions, as for sil), which involve a T-cell receptor gene as a partner, are detectable by standard Southern analysis. PCR assays are available for some of the translocations, where there is breakpoint clustering. Essentially all of the malignancies which harbor these translocations are ALL. Functionally, all oncogenes (where known), except for sil (which has topoisomerase I homology), appear to play a role in gene transcription, either directly or indirectly.

sil does not involve a T-cell receptor gene translocation. Rather, in a deletional event, it is juxtaposed with tcl-5 (tal-1, scl), resulting in the dysregulation of the latter. This event is perhaps the most common known molecular abnormality in T-cell ALL, occurring in approximately 30% of cases.
TABLE 3. Creation of chimeric genes and proteins by chromosomal translocations

<table>
<thead>
<tr>
<th>Disease</th>
<th>Freq</th>
<th>Translocation</th>
<th>Genes involved</th>
</tr>
</thead>
<tbody>
<tr>
<td>CML</td>
<td>−100</td>
<td>(t(9;22)(q34;q11)</td>
<td>ABL-BCR</td>
</tr>
<tr>
<td>ALL</td>
<td>−5</td>
<td>(t(9;22)(q34;q11)</td>
<td>ABL-BCR</td>
</tr>
<tr>
<td>Acute nonlymphoblastic leukemia with maturation (FAB M2)</td>
<td>−40</td>
<td>(t(8;21)(q22;q22)</td>
<td>ETO-AML1</td>
</tr>
<tr>
<td>APL (FAB M3)</td>
<td>−100</td>
<td>(t(15;17)(q24;q21)</td>
<td>PML-RARx</td>
</tr>
<tr>
<td>Acute mixed-lineage leukemia in infants</td>
<td>&gt;50</td>
<td>(t(11;19)(q23;p13)</td>
<td>AF4-MLL</td>
</tr>
<tr>
<td>ANLL (with basophilia)</td>
<td>&lt;2</td>
<td>(t(6;9)(p23;q34)</td>
<td>CAN-DEK</td>
</tr>
<tr>
<td>Anaplastic large-cell lymphoma</td>
<td>−30</td>
<td>(t(2;5)(p23;q35)</td>
<td>NPM-ALK</td>
</tr>
<tr>
<td>Acute myelomonoblastic leukemia with eosinophilia (FAB M4Eo)</td>
<td>?</td>
<td>(t(16;16)(p13;q22)</td>
<td>CBFB-MYH11</td>
</tr>
<tr>
<td>Chronic myelomonocytic leukemia</td>
<td>?</td>
<td>(t(5;12)(q33;p13)</td>
<td>PDGFRα-TEL</td>
</tr>
<tr>
<td>Myelodysplastic syndrome (especially t-MDS)</td>
<td>?</td>
<td>(t(3;21)(q26;q22)</td>
<td>EAP-AML1</td>
</tr>
<tr>
<td>Pre-B-cell ALL</td>
<td>−30</td>
<td>(t(1;19)(q23;p13)</td>
<td>PBX1-E2A</td>
</tr>
</tbody>
</table>

* Approximate frequency (percent) within the specific disease entity. The reported frequency of these genetic abnormalities will probably be redefined, given that molecular positivity is sometimes seen with grossly normal karyotypes. ?, frequency unknown. See text for references.
* Since the breakpoints are intronic, most translocations can be detected by RT-PCR assays, as well as by Southern analysis (detection of nongerm line band, with appropriate probes) or Northern analysis (to detect abnormally sized transcript).
* More common (~20%) in adults.
* The three translocations (t(9;22), t(4;11), and t(1;19), which are seen in ~12% of childhood cases of ALL, are associated with a relatively poor prognosis.
* Site of break in BCR gene in ALL is usually different from that in CML (see text).
* The three cytogenetic abnormalities t(8;21), t(15;17), and inv(16), which are seen in ~35% of cases of de novo adult ANLL, are associated with a relatively good prognosis.
* There are numerous other translocation partners of 11q23. Translocations involving 11q23 are also seen in secondary leukemias, particularly following therapy with topoisomerase II inhibitors.
* More common is inv(16).

Considered indicative of an NHL originating from a follicular center cell and is useful in distinguishing histologically equivocal cases from atypical, benign, reactive follicular hyperplasias. However, a significant diagnostic caveat is the detection of t(14;18)-bearing cells in healthy individuals (40), a phenomenon which increases with age (42).

**Other clonality studies.** For hematologic malignancies without an antigen receptor gene rearrangement or molecularly characterized translocation, there are other molecular methods to document clonality. These techniques include X-linked studies, fluorescent in situ hybridization (FISH), and comparative genomic hybridization.

The X-linked studies exploit the presence of polymorphisms of regions of the X chromosome and are analogous to the original glucose 6-phosphate dehydrogenase (G6PD) isoenzyme studies which were historically used to document clonality. As with these original isoenzyme studies, the limitation of the X-linked studies is that they are only informative if the patient is both female and polymorphic. However, some are particularly useful, such as those wherein numerous alleles are present (studies using the M27 [16] and androgen receptor [2] probes, analyzed by Southern blot and PCR, respectively), which result in a very high heterozygosity rate.

FISH can be construed as a bridge between conventional (karyotypic) and molecular cytogenetics, allowing for, amongst other applications, the detection of chromosomal abnormalities in metaphase cells (3). While this technique can be easily applied to the detection of translocations, it is perhaps better suited, in the context of hematologic malignancies, for the detection of numeric (additions or deletions) abnormalities that are not easily documented by Southern, Northern, or PCR analysis. A requirement for FISH is the availability of appropriate probes or chromosomal paints, directed against various regions of a chromosome. By contrast, comparative genomic hybridization (26) has the ability to detect numeric abnormalities without specific probing and can be thought of as reverse FISH. By this technique, normal metaphase spreads are simultaneously hybridized with fluorescent-label-tagged DNA extracted from the malignancy and fluorescent-label (a fluorochrome different from that used to label the tumor DNA)-tagged normal control DNA. Digital image analysis is then employed to detect amplifications or deletions by determining the ratio of the different fluorochromes (for example, green-fluorescein isothiocyanate versus red-rhodamine) hybridizing to the normal metaphase chromosomes.

In Hodgkin's disease, it has generally been believed that the neoplastic cell is the Reed-Sternberg (RS) cell, or a variant thereof. However, it had been a frustrating task to prove this because of the rarity of these cells (usually 0.1%) in affected tissues. Only recently have molecular methods been able to definitively show that the RS cell is indeed the malignant cell in Hodgkin's disease. The documentation of consistent, clonal genetic aberrations in these cells has been achieved through the detection of p53 mutations in single RS cells (75), image analysis of the DNA profiles and FISH (29), and an analysis of clonal immunoglobulin heavy-chain gene rearrangements (36).

**PROGNOSIS**

In hematologic malignancies, a number of cytogenetic abnormalities, including those that can be documented by using the aforementioned molecular techniques, carry prognostic information. On occasion, these cytogenetic abnormalities are detected only by molecular techniques, since they may not be apparent on conventional karyotyping.

There are three specific, nonrandom cytogenetic abnormalities which, in the context of de novo ANLL, are associated with a relatively favorable outcome (73). Together, they account for approximately one-third of cases of de novo adult ANLL. These are the translocations t(15;17) in APL or ANLL type M3 (ANLL-M3) and t(8;21) in ANLL-M2 and the inversion, inv(16), or translocation, t(16;16), associated with ANLL-M4Eo (acute myelomonoblastic leukemia with eosinophilia). The biologic explanation for the association of these molecular abnormalities with a favorable prognosis remains unclear. However, preliminary data suggest a possible reason for the correlation between inv(16) and a good outcome: this molecular inversion appears to be associated with the deletion of the MRP gene, which encodes the multidrug resistance-associated protein (37).
By contrast with the three genetic abnormalities associated with a good prognosis described above, the detection of the t(9;22), t(4;11), and t(1;19) translocations in childhood ALL portend a relatively poor prognosis (12, 64, 66). These three translocations are collectively seen in ~12% of cases of ALL in the pediatric context. The gene located at 11q23, and involved in the t(4;11) translocation, is rather promiscuous and may be involved with many other translocation partners. Poor prognostic insights in ANLL appears to be associated with the increased expression of the multidrug resistance gene MDR1, as measured at the RNA level (19). Through mechanisms independent of increased MDR1 expression, other ANLLs that overexpress bcl-2 may also have a poor response to chemotherapy (13). In the t(9;22) translocation in CML, initial data suggesting that the specific site of the break in the bcr gene in CML may be prognostically relevant have not been borne out in subsequent studies (53).

The clinical behavior and response to therapy of patients with diffuse large-cell NHL are somewhat unpredictable, with some patients entering sustained remission and others failing therapy. The reason for this heterogeneity was unclear until recently, when it was shown that molecularly detected rearrangements of the bcl-6 gene identify patients for whom the clinical outcome is favorable while rearrangements of bcl-2 in this disease are associated with a poorer prognosis (60). Mutations of the p53 tumor suppressor gene (see “Biology,” below) in ANLL, myelodysplastic syndromes, and chronic lymphocytic leukemia may be prognostic indicators of a poor response to chemotherapy, as well as shortened survival (77). Similarly, p53 mutations in blast transformation of CML (57) and in small, noncleaved NHL (20) are predictive of a shortened survival. By contrast, low levels of expression of another tumor suppressor gene, WT1, as measured by RT-PCR, were associated with a significantly better prognosis (30).

In myelodysplastic syndromes, a variety of factors in addition to the p53 mutations noted above, including a number of genetic variables, may be evaluated to determine survival and evolution into acute leukemia. Monosomy 7 and del(7q), both detectable by FISH, are such predictors of a poor prognosis (8). The presence of clonal chromosomal abnormalities, or N-ras mutations, may also be used to predict evolution into acute leukemia (74). However, some mutations in N-ras in de novo ANLL (affecting codon 13 but not codon 12) are associated with a relatively good prognosis, predicting durable remissions (14). Telomere shortening, which can be determined by Southern blot hybridization, may also be useful at the initial diagnosis of myelodysplastic syndromes, to identify those patients with a poor prognosis (61).

Accordingly, it can be seen that in addition to being useful in diagnosis, the molecular detection of the aforementioned genetic abnormalities in hematologic malignancies can be employed to define prognostically relevant subgroups. These may be used to identify and stratify disease entities that appear to be homogeneous when conventional diagnostic tools are used.

MINIMAL RESIDUAL DISEASE

On the basis of their sensitivity, many of the molecular assays can be applied to the detection of extremely small numbers of tumor cells (not discerned by morphology or immuno-phenotyping). Southern blot analysis generally can detect, at best, approximately 1 to 5% neoplastic cells within a background of normal cells. By contrast, PCR dramatically enhances sensitivity, often being able to detect tumor cells diluted 1:10^7 (0.001% or even 1:10^6 (0.0001%). PCR may be used to (i) discover occult disease for staging purposes, (ii) determine the efficacy of ex vivo purging of involved bone marrow in patients treated with autologous bone marrow transplantation, and (iii) monitor for minimal residual disease and detect early relapse following therapy (including chemotherapy and bone marrow transplantation). While a negative PCR result is, in most instances, predictive of the attainment of remission, a positive PCR result is less easy to interpret. Indeed, the finding of a positive signal by PCR does not provide any information regarding the viability or proliferative capacity of the cell (indeed, a positive signal may be obtained from extracellular DNA) and hence needs to be viewed with some caution. Generally, a positive result following therapy (bone marrow transplantation or chemotherapy) is, in itself, not indicative of relapse. Rather, the stage of therapy at which a positive result is found or the presence of sustained (for greater than 6 months, or on two or more occasions) or quantitatively increasing positivity may be predictive of recurrence.

For example, most pediatric ALL patients have a PCR-detectable antigen receptor gene rearrangement at the end of remission induction therapy (58), the detection of which decreases to approximately 30% during maintenance therapy. However, most have no PCR-detectable disease at the completion of maintenance therapy and those that do appear to be at greater risk of relapse. Similar results have been obtained by using immunoglobulin heavy-chain-gene PCR for patients with multiple myeloma treated with bone marrow transplantation. PCR positivity in the first year following bone marrow transplantation is common and not predictive of relapse, while sustained negativity may be associated with cure of the disease (11).

The CDRIII region of the immunoglobulin heavy-chain gene is usually the area amplified by PCR in these assays. The ability to exploit this to identify minimal residual disease may be problematic in some B-lineage malignancies. For example, ongoing gene rearrangements that disrupt the CDRIII region of the neoplastic clone and high rates of somatic mutation in this region occur in ALL (10) and follicular NHL (39), respectively. These phenomena would then affect primer annealing, and those primers which were useful at diagnostic presentation may no longer be useful subsequently, compromising the ability to monitor minimal residual disease.

In patients with CML treated with bone marrow transplantation, the finding of a chimeric bcr-abl gene by RT-PCR appears to be more predictive of relapse for those patients with a positive result which is sustained for greater than 12 months or for those with a demonstrable increase in the intensity of expression, as discerned by quantitative analysis (41). A recent meta-analysis of studies of qualitative RT-PCR following bone marrow transplantation for CML revealed that RT-PCR positivity, in general, was not predictive of a higher relapse rate for patients receiving an unmanipulated bone marrow transplantation (54). Rather, RT-PCR positivity was a more reliable predictor for those patients whose transplants had been depleted of T cells or who had undergone transplantation in the accelerated or blastic phase of CML. bcr-abl fusions may also persist for many years in some patients in apparent long-term remission (63), and so may the AML-ETO1 chimeric genes generated by the t(8;21) translocation in ANLL-M2 (59). By contrast, in patients with APL, the finding of a fusion PML-RARA gene almost always appears to be associated with impending relapse (43). These apparent differences in the predictive value in the scenarios of CML and ANLL-M2, compared with APL, may be due to the notion that the genetic lesions detected in the former two are not, per se, sufficient for the expression of these diseases, while in APL the PML-
RARα fusion may be able, by itself, to neoplastically transform cells. PCR detection of the t(14;18) translocation, most applicable in patients with follicular NHL, may be used in a number of different clinical scenarios (17). At initial presentation, most patients with advanced-stage disease but without morphologic evidence of bone marrow involvement do indeed have PCR-detectable bone marrow involvement. Also, some patients with apparent localized disease may be “upstaged” by these analyses. It still remains to be determined whether these findings will impact the therapy of these patients. The PCR detection of this translocation does, however, appear to be useful in determining the efficacy of the immunologic purging of bone marrow prior to autologous bone marrow transplantation. Similar to the outcome with other PCR-detectable abnormalities noted previously, disease-free survival is increased for patients with negative results, while PCR positivity following therapy may not be predictive of relapse.

A potential concern regarding the utility of some of the described RT-PCR-evaluable chromosomal translocations is that the RT-PCR products are mostly identical from patient to patient within a disease group, making the exclusion of the possibility of false positivity (due to contamination) difficult. The identical nature of the RT-PCR products is related to the fact that the assays are RNA based, with heterogeneous DNA intronic breaks being spliced out when the RNA is processed. This contrasts with the unique nature of the antigen receptor gene rearrangements, which differ in each patient because of the random insertion of N nucleotides. Similarly, the PCR product sizes of the t(14;18) translocation also vary from patient to patient, primarily because of the heterogeneity in the breakpoints in the bcl-2 gene.

Through the examples given above, it has been illustrated that the ability to detect extremely small numbers of tumor cells by PCR has changed the definition of the attainment of complete remission. Accordingly, these molecular techniques should become an integral component of the evaluation of patients following therapy, with negative results generally bearing more predictive value than positive results.

**BIOLOGY**

The dissection of the molecular genetic abnormalities which occur in hematologic malignancies has led, directly or indirectly, to the identification of a variety of proto-oncogenes and tumor suppressor genes. Proto-oncogenes, when dysregulated through acquired genetic events (in particular, via translocation and mutation), are converted into activated oncogenes. The protein products of these genes are usually growth factors, growth factor receptors, intracellular signal transducers, and DNA-binding proteins. The consequence of the dysregulation is invariably to increase function in a dominant fashion. By contrast, tumor suppressor genes play a role in oncogenesis through loss of function, which requires that both alleles be disabled (through deletion or mutation) and hence act in a recessive fashion.

**Chromosomal translocations.** Functionally, the consequences of chromosomal translocation may be categorized as either (i) quantitative, with the dysregulated expression of an oncogene (Tables 1 and 2), or (ii) qualitative, through the creation of a chimeric gene and protein (Table 3). Examples of these two functional consequences are described briefly below. Burkitt’s lymphoma has been a paradigm for the former category, as well as for the dissection of the multistep pathway and molecular mechanisms of neoplasia (48). In the endemic form of this disease, as well as in Epstein-Barr virus-positive (EBV⁺) NHL in human immunodeficiency virus-positive (HIV⁺) patients (38), the molecular consequences of the classic t(8;14) translocation are similar: there is a head-to-head juxtaposition of the myc proto-oncogene with the immunoglobulin heavy-chain gene. This results in an increased transcription of myc. However, in addition to the quantitatively increased MYC levels, there are qualitative changes (point mutations) in this gene, affecting both its function and its transcriptional regulation (80). The MYC protein, which is further positively regulated through heterodimerization with MAX (which in turn may also heterodimerize with MAD, to negatively regulate function [4]), functions as a transcriptional regulator, acting to eventually promote cell proliferation.

In CML, the proto-oncogene abl is translocated into the bcr gene. Biologically, the protein product of the normal, or wild-type, abl gene functions as a weak tyrosine kinase, located in the nucleus. Recent data suggest that it may actually function as a negative regulator of cell growth (69). By contrast, the mutant BCR-ABL protein has much greater phosphorylating (kinase) activity and is subcellularly located in the cytoplasm. Provocatively, p185 of ALL has greater phosphorylating activity than does p210 of CML, which somewhat parallels the clinical behavior of these two rather different leukemias. Furthermore, the p210 BCR-ABL chimeric protein in CML also appears to function, through complexing with an adapter protein, to transduce signals along the RAS pathway (62), and it also appears to protect cells from programmed cell death (apoptosis) (7). Thus, as more studies are performed, insights into the biologic consequences of this genetic abnormality are growing.

In the t(1;19) translocation, intronic breaks of the E2A gene (which codes for an immunoglobulin enhancer binding protein) on chromosome 19 and the PBX1 gene (whose protein product contains a putative homeodomain) on chromosome 1 result in the generation of a chimeric transcription factor. Paradoxically, this fusion protein appears to have the capacity to induce both proliferation and apoptosis (15). The involvement of one of the retinoic acid receptor genes in the t(15;17) of APL is tantalizing in that this disease had previously been shown to respond in vivo, albeit transiently, to the use of the differentiating agent retinoic acid. The translocation generates a number of different mRNA transcripts, one of which results in the increased expression of a mutant, chimeric PML-RARα protein. Although previously thought to be abnormally located in the cytoplasm, it is now believed that the chimeric PML-RARα has an aberrant nuclear localization (78). It has been suggested that to return it to a normal subnuclear location requires pharmacological doses of retinoic acid, and this may explain the response of this disease to this form of therapy.

In addition to considering the quantitative and qualitative consequences of oncogene dysregulation, which has provided interesting biologic information, it is perhaps appropriate to discuss in somewhat more detail insights into other biologic processes which have been gleaned only recently. Processes recently dissected include those involved in the regulation of programmed cell death and the cell cycle, as well as in the role of viruses in the development of some hematologic malignancies.

**Apoptosis.** Simplified, traditional notions, perceiving the development of neoplasia as being primarily related to the increased proliferation of cells (for example, as induced by myc overexpression in Burkitt’s NHL) or the failure to differentiate (for example, as mediated by PML-RARα in APL), have had to be reevaluated recently, with the explosion of knowledge regarding the regulation of cell death. The molecular dissection of the t(14;18) translocation, most commonly associated
with follicular NHL, led to the detection of the \( bcl-2 \) gene (5). When translocated, the \( bcl-2 \) gene falls under the regulation of the transcriptionally active immunoglobulin gene, resulting in a quantitative increase in \( bcl-2 \) mRNA and protein, analogous to that described for \( myc \). The protein product of the \( bcl-2 \) gene is somewhat novel amongst proto-oncogenes in that it functions to prevent apoptosis. Hence, the translocation results in increased protection from programmed cell death, allowing the tumor cells to accumulate through lack of attrition, rather than increased proliferation or differentiation block, and may account for the relatively slow growth rate of follicular NHL. In addition to having a somewhat novel mechanism of action, \( BCL-2 \) also appears unique with regard to its subcellular localization. It is located primarily in the outer mitochondrial membrane, nuclear envelope, and parts of the endoplasmic reticulum, and it may function in an antioxidant pathway (25).

Not only is \( BCL-2 \) mechanistic in the pathogenesis of neoplasia, but also its overexpression may affect therapeutic decisions by rendering malignant cells resistant to conventional chemotherapy. This may account for the previously noted observation that \( bcl-2 \) translocation, and increased protein expression, in some patients with large-cell NHL is associated with a poor response to therapy (60).

Many other factors appear to be operative in the regulation of apoptosis (6), and perhaps then in other neoplasms, although not necessarily in follicular NHL. These include other protectors (such as the \( BCL-2 \)-related protein \( BCL-X_{L} \), \( BCR-ABL \) [in CML], PML-RAR\( \alpha \) [in APL], and MCL-1) and inducers (such as BAX, \( BCL-X_{S} \), p53 [see below], MYC, ICE, PRCE, and FAS) of apoptosis, which, when dysregulated, may be mechanistic in malignant transformation. BAX and/or \( BCL-X_{S} \) are able to heterodimerize with \( BCL-2 \) to neutralize its ability to prevent apoptosis. Furthermore, p53 may be able to induce \( bax \) expression (55). When p53 is mutated, as occurs with the histologic transformation of follicular NHL (44), this effect may be lost, allowing for unchecked \( BCL-2 \) overexpression, preventing tumor cells from undergoing apoptosis.

**Cell cycle.** The cell cycle is another area of cell biology in which our understanding has increased dramatically through the dissection of the genes involved in neoplasia (49). It is now becoming increasingly clear that dysregulation of the machinery that directly regulates progression through the cell cycle is an important component of malignant cell transformation. These phenomena are in addition to the derangements in growth factor-mediated signal transduction, which indirectly affect cell cycling. The cancer-associated genes involved in the direct control of the cell cycle are mostly tumor suppressor genes, although there are now examples of dominantly acting protooncogenes which are directly involved. Some of these will be briefly reviewed.

The protein p53 appears to be the major conductor of the orchestra involved in the regulation of the cell cycle. In response to DNA damage, wild-type p53 protein accumulates. If the levels of DNA damage are relatively low, p53 is able to mediate arrest of the cell cycle in \( G_{1} \), allowing DNA repair to occur prior to reentry into the cell cycle. If the degree of DNA damage is high, p53 protects the cell (or rather the host) somewhat more dramatically, by inducing apoptosis. The p53 gene is perhaps the single most commonly affected gene in cancer overall (21). When it is mutated and its function is lost, it is no longer able to protect the cell from the consequences of DNA damage, allowing unchecked (and possibly error-containing) proliferation through the cell cycle to occur, which leads to the formation of a neoplastically transformed cell. p53 is mutated, to a greater or lesser degree, in most hematologic malignancies (28) and is often incriminated in disease progression. p53 appears to mediate its function through the regulation of expression of a variety of other genes, including WAF1 (p21), which does not appear to be directly altered in hematologic malignancies (71). Other candidate tumor suppressor genes, MTS1 and MTS2 (p16 and p15, respectively), also appear to function by controlling progression through the \( G_{1} \) phase of the cell cycle. In contrast to WAF1, they appear to be directly involved in the neoplastic transformation of some hematologic malignancies, being homozygously deleted in T-cell, but not B-cell, ALLs (22).

Another critical regulator of the cell cycle is yet another tumor suppressor, the Rb protein (79). Rb is one of the targets of the cell cycle kinases, with the degree of phosphorylation varying during the cell cycle. Rb appears to be a weaker tumor suppressor than p53, but, analogous to the situation with p53, its inactivation leads to unchecked cell division. Its function may be lost in some hematologic malignancies, albeit with a frequency generally less common than seen with p53 (65). However, it appears that when Rb is found to be structurally abnormal in leukemias, there is an association with those showing monocytic features (1).

In contrast to the aforementioned recessively acting tumor suppressor genes, which play an important role in the regulation of the cell cycle, \( bcl-1 \) is a dominantly acting proto-oncogene involved therein. It was originally identified as the locus involved in the \( t(11;14) \) translocation, now most commonly associated with mantle cell NHL. The protein product of this gene now appears to be cyclin D1, which functions in an important phase of the cell cycle. Overexpression of cyclin D1 leads to a subversion of \( G_{1} \) phase control (47). Furthermore, cyclin D1 may act in concert with a \( myc \) gene to neoplastically transform cells (46).

**Oncogenic viruses.** At least two viruses, EBV and human T-cell leukemia virus type 1 (HTLV-1), have been shown to be causally associated with some specific types of hematologic malignancies.

EBV is known to induce the virtually indefinite proliferation of B lymphocytes. In the context of an effective cell-mediated immune response, the EBV infection usually becomes self-limited. However, when a robust infection with EBV occurs in the setting of an impaired immune response, neoplasia may ensue. Amongst other neoplasms, EBV is implicated in the pathogenesis of endemic Burkitt’s lymphoma and a subgroup of HIV-associated lymphomas, as well as other B-cell lymphomas arising in immunocompromised (both congenitally, as in X-linked lymphoproliferative syndrome, and by acquisition, as in the transplantation scenario) individuals. The evolution to neoplasia in these situations appears to be a multistep process, with chromosomal translocations dysregulating the \( myc \) protooncogene occurring in the setting of polyclonal B-cell activation and with oligoclonal of B cells having been allowed to grow out because of the immunosuppression (33). The EBNA1 protein is almost consistently expressed in tumors associated with EBV. This may be crucial to the development of these malignancies as the protein is not recognized by cytotoxic T cells, thus allowing for the evasion of cell-mediated tumor surveillance. Furthermore, EBNA1 may also enhance \( myc \) gene expression (31), while another EBV protein, LMP1, may also be involved in neoplastic transformation through the induction of \( bcl-2 \) expression (24). An evaluation of the fused terminal fragments of the episomal form of EBV may also be used as a marker of clonality in EBV-associated malignancies (67).

The retrovirus HTLV-1 is incriminated in the development of adult T-cell leukemia (ATL), a malignancy with distinct clinicopathologic features. In vivo, HTLV-1 almost exclusively infects CD4\(^{+}\) T-cells, which is the phenotype of the neoplastically transformed cell. In vitro, HTLV-1 can immortalize T
cells. The leukemic cells from ATL patients reveal monoclonal integration of the proviral DNA, indicating that these cells originated from a single HTLV-1-infected cell, strongly implying a causative role for the virus in leukemogenesis (81). It is believed that HTLV-1 does not directly mediate neoplastic transformation but rather that trans-acting factors, especially related to the tax gene, are involved in leukemogenesis. The product of this tax gene has been shown to induce the transcription of a number of genes, including the proto-oncogenes fos and sis. Although ATL cells contain clonally integrated provirus, no common site of integration has been seen, suggesting that HTLV-1's role in leukemogenesis is not dependent on its site of integration. However, it has been proposed that certain integration patterns may be clinically significant, which explains the heterogeneous behavior of ATL (70). Unlike the situation with EBV-associated Burkitt's lymphoma, in which the myc dysregulation has been characterized, specific secondary cytogenetic events have yet to be characterized in ATL.

CONCLUSION

The cloning of the antigen receptor genes, and of the genes involved in nonrandom cytogenetic abnormalities that occur in malignancies of cells of the hematologic system, has had a dramatic impact on our understanding of many facets of the molecular mechanisms integral to these diseases. This brief review, with a few representative examples, has attempted to summarize some of these concepts and has hopefully illustrated how an appreciation thereof can be of tremendous utility at a variety of levels: (i) in the diagnosis of these malignancies, (ii) in the identification of prognostic markers, (iii) in the enhancement of our ability to monitor minimal disease, and (iv) in providing insights into the biology of both neoplastic and normal cells.

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