THE ROLE OF ONCOGENES IN HEMATOLOGIC MALIGNANCIES

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KEY WORDS: protooncogene, leukemia, chromosomal translocations, fusion proteins, transcription factors

ABSTRACT

Oncogenes are activated forms of cellular genes involved in normal cell growth and development. Some oncogenes play a role in human malignancies. In hematologic malignancies, researchers have identified many transcription factors as oncogenes based on one of the following criteria: their association with transforming retroviruses in animals, their translocation into either the immunoglobulin or T-cell receptor loci, or the production of fusion proteins resulting from chromosomal translocations. The molecular characterization of oncogenes in hematologic malignancies has led to the discovery of new methods for diagnosis and detection of minimal residual disease. In the future, researchers probably will develop novel treatment strategies to interfere with the function of these oncogenes.

ONCOGENES IN HUMAN MALIGNANCIES

All malignancies disable normal growth-control mechanisms. The protein products of genes identified as oncogenes are involved in the pathways that control proliferation (1, 2). Oncogenes were first identified in the normal genome. These were homologous to genes present in RNA tumor viruses (retroviruses). More recently, investigators have discovered new oncogenes by examining the genes active in chromosomal translocations (3, 4). This chapter
discusses some of the oncogenes active in hematologic neoplasms, with an emphasis on the oncogenes that encode nuclear proteins.

**Protooncogenes and Retroviruses**

The study of transforming retroviruses has enabled researchers to identify several oncogenes. The retroviruses integrate into the genome of the host cell, where they incorporate genetic material from the host into their own genome. In some cases, the gene is modified, disrupting the normal regulation of its expression. This process can lead to the creation of an oncogene. Protooncogenes are precursors of oncogenes and help control cellular proliferation and differentiation. Classes of protooncogenes include nuclear proteins, growth factors, growth factor receptors, and signal transducers. Protooncogenes become activated oncogenes either by undergoing a structural change (either mutation or deletion of a part of the oncogene) or by amplification of the gene. Mutation or deletion may yield an activated protein that does not require the normal growth stimulus for activation, whereas gene amplification leads to overexpression of the protein. Each type of activation has a dominant effect and increases function. Another mechanism of oncogenesis is the loss of function or even total inactivation of a tumor-suppressor gene (5). Because tumor-suppressor genes normally restrain cell growth, their inactivation can cause uncontrolled cell proliferation. Tumor-suppressor genes are recessive: both copies of the gene must be deleted or mutated for the effect to be observed. Gain-of-function and loss-of-function genetic alterations are often found in the same tumor-cell genome.

**New Oncogenes Discovered in the Study of Chromosomal Translocations**

The majority of malignant cells have chromosomal abnormalities. In most cases, all cells of a given malignancy undergo the same chromosomal changes and thus represent a clonal population (6). Numerous nonrandom chromosomal alterations occur in hematologic malignancies. Over the past few years, investigators have identified and characterized many genes involved in these translocations. These genes most likely play a critical role in the malignant process, and they are also classified as oncogenes.

Chromosomal translocation causes oncogenic activation through one of two mechanisms. The first involves translocation into one of the immunoglobulin genes or into one of the T-cell receptor loci (7–10), which are collectively referred to as antigen receptors. These translocations probably occur during the normal process of rearrangement of the antigen receptors and culminate in the deregulated expression of the oncogene. Researchers believe that the antigen receptor locus is directly responsible for the deregulated expression, although this hypothesis has not been demonstrated conclusively.
The second mechanism involves a fusion of the coding regions of two genes, which in turn leads to a fusion or chimeric protein with altered functional properties (3). The t(9;22) chromosome translocation in chronic myelogenous leukemia (CML) combines the \textit{bcr} and \textit{abl} genes to produce a chimeric BCR-ABL protein.

**TRANSCRIPTION FACTORS**

Transcription factors are nuclear proteins that bind to specific DNA sequences in the regulatory regions of genes and control their expression or transcription by means of the enzyme RNA polymerase (11, 12). These proteins determine which genes are expressed in a given cell and also determine all life processes, including differentiation and aging. Normal cell regulation requires the precise control of gene expression. The changes seen in malignant cells are the result of altered gene expression. In these cells, transcription factors are frequently deregulated. Because transcription factors control the regulation of numerous genes, these target genes are also deregulated as a consequence of the altered expression of the transcription factor. The deregulated expression of a single transcription factor can thus disrupt the normal expression of many proteins. The frequent deregulation of transcription factors in malignant hematopoietic cells illustrates the importance of transcription factors and transcriptional control in the normal processes of proliferation, differentiation, and cell death.

Transcription factors are grouped into classes according to motifs that correspond to DNA binding regions or to dimerization (protein-protein interaction) regions. Researchers have identified several hundred transcription factors. Some are expressed in all tissues, whereas others are expressed only in a single cell type.

**THE USE OF MOLECULAR TECHNIQUES TO DETECT CHROMOSOMAL TRANSLOCATIONS**

Southern blotting can be used to determine the presence of a chromosomal translocation (13). In this procedure, agarose gel electrophoresis separates DNA fragments by size. The DNA is then transferred to a filter, and a labeled DNA probe encompassing the region of interest is hybridized to the filter. If a chromosomal translocation is present, the detected DNA fragment will be of a different size than the fragment derived from the normal gene. If the DNA fragment spans the chromosomal breakpoint, probes derived from each of the genes involved in the translocation will hybridize to the same DNA fragment. Southern blotting usually takes 10–14 days to complete and is not widely used in clinical situations.
The polymerase chain reaction (PCR) is a powerful technique that allows amplification of a specific DNA sequence if the sequence of the flanking regions is known (13). PCR is more sensitive than Southern blotting and requires less time. This procedure amplifies a single-copy gene so that it becomes the major DNA species present. Investigators can then label a specific DNA probe and hybridize it to the amplified DNA to determine whether the sequence of interest is present. PCR can assay chromosomal translocations across the breakpoint because a signal will only be obtained if the translocation is present. In normal cells, the two genes would not be covalently linked and hence would not be amplified by PCR. If the breakpoints at the DNA level are heterogeneous, the RNA transcript can be amplified by a similar process.

NUCLEAR PROTOONCOGENES

Several protooncogenes identified by their presence in transforming retroviruses are transcription factors. These include the members of the myb, myc, jun, fos, and rel families. The deregulated expression of transcription factors that regulate genes active in cell growth and proliferation could provide a growth advantage for malignant cells.

Two members of the rel family, bcl-3 and lyt-10, have been found in translocations into the immunoglobulin locus (Table 1), as has c-myc (7).

Table 1  Translocations of transcription factor genes into an antigen receptor locus

<table>
<thead>
<tr>
<th>Gene</th>
<th>Translocation</th>
<th>Disease</th>
</tr>
</thead>
<tbody>
<tr>
<td>c-myc</td>
<td>t(8;14)(q24;q32)</td>
<td>Burkitt’s lymphoma and</td>
</tr>
<tr>
<td></td>
<td>t(2;8)(p12;q24)</td>
<td>B-cell ALL</td>
</tr>
<tr>
<td></td>
<td>t(8;22)(q24;q11)</td>
<td>T-cell ALL</td>
</tr>
<tr>
<td></td>
<td>t(8;14)(q24;q11)</td>
<td>T-cell ALL</td>
</tr>
<tr>
<td>bfl-1</td>
<td>t(7;19)(q35;p13)</td>
<td>T-cell ALL</td>
</tr>
<tr>
<td>tal-1/hsc</td>
<td>t(1;14)(p32;q11)</td>
<td>T-cell ALL</td>
</tr>
<tr>
<td>tal-2</td>
<td>t(7;9)(q35;q34)</td>
<td>T-cell ALL</td>
</tr>
<tr>
<td>hox-11</td>
<td>t(10;14)(q24;q11)</td>
<td>T-cell ALL</td>
</tr>
<tr>
<td></td>
<td>t(7;10)(q35;q24)</td>
<td>T-cell ALL</td>
</tr>
<tr>
<td>ttg-1/rhom-1</td>
<td>t(11;14)(p15;q11)</td>
<td>T-cell ALL</td>
</tr>
<tr>
<td>ttg-2/rhom-2</td>
<td>t(11;14)(p13;q11)</td>
<td>T-cell ALL</td>
</tr>
<tr>
<td></td>
<td>t(7;11)(q35;p13)</td>
<td>T-cell ALL</td>
</tr>
<tr>
<td>bcl-3</td>
<td>t(14;19)(q32;q13.1)</td>
<td>CLL</td>
</tr>
<tr>
<td>lyt-10</td>
<td>t(10;14)(q24-q32)</td>
<td>B lymphoma</td>
</tr>
</tbody>
</table>
Researchers have identified another transcription factor, the c-myb oncogene, as the transforming gene of the avian myeloblastosis virus that causes myeloblastic leukemia in chickens. The c-myb gene product is expressed in immature hematopoietic cells (14), whereas the Myb protein plays a role in the proliferation of both normal and malignant hematopoietic cells. Constitutive expression of Myb protein in cell lines inhibits differentiation. The G1/S transition in normal T-lymphocytes requires c-myb expression (15). This gene probably helps control proliferation and differentiation by regulating the expression of effector genes required for these processes.

c-myb levels are elevated in many acute leukemia cells and also in established acute leukemia cell lines. Several studies showed that patients whose leukemic blasts expressed high c-myb levels were less likely to enter remission or to have a short remission duration. Another study revealed that high c-myb transcript levels were associated with hyperleukocytosis. The measurement of both c-myb and c-myc levels could provide prognostically useful information (16-18). In the future, researchers may be able to develop treatment strategies aimed at decreasing the level of c-myb expression. For example, in vitro experiments have demonstrated that antisense oligonucleotides that inhibit Myb protein expression can also inhibit or even abolish clonogenic growth of primary acute leukemia cells (19).

MYELOID LEUKEMIAS

t(9;22) in Chronic Myelogenous Leukemia

The best-studied fusion protein is the BCR-ABL protein, which is present in virtually every case of chronic myelogenous leukemia (CML) (20, 21). In this translocation, the c-abl protooncogene translocates from its normal location on chromosome 9 to a region on chromosome 22 known as the breakpoint cluster region (BCR). The c-abl gene encodes a protein with tyrosine kinase activity. The fusion protein BCR-ABL has markedly elevated tyrosine kinase activity compared with that of the normal ABL protein. The chimeric protein most likely contributes significantly to the development of the malignant clone, but the exact role remains undefined. BCR-ABL, on the other hand, can produce a malignant proliferation in mice that resembles CML (22, 23). These studies confirm the oncogenic properties of BCR-ABL.

After several years, CML moves from the chronic phase (characterized by an expansion of differentiated neutrophils) to acute leukemia (blast crisis). Although the cause of this change remains obscure, new chromosomal abnormalities often occur during blast crisis. Recent studies suggest that in some patients, p53 (a tumor-suppressor gene) may play a role in this transformation. Approximately 25–30% of leukemic cell samples from patients in blast crisis
show abnormalities in p53, the most common of which are the loss of one allele and the development of point mutations in the other p53 allele (24, 25). This discovery is intriguing, but the molecular events leading to blast crisis remain unknown, particularly in the majority of cases where no p53 abnormality is present.

**t(15;17) in Acute Promyelocytic Leukemia**

Acute promyelocytic leukemia (APL) is a distinct subtype of acute myelogenous leukemia (AML) that is associated with disseminated intravascular coagulation. APL has a higher remission rate and a longer remission duration after chemotherapy than do other AML subtypes. In contrast to the other AML subtypes, complete remissions can be obtained in APL without marrow hypoplasia. APL is also associated with the t(15;17) translocation, and researchers have characterized the genes that participate in this process. The retinoic acid receptor-α (RARα) gene on chromosome 17 is rearranged with the pml (promyelocytic leukemia) gene on chromosome 15 (26, 27). RARs are transcription factors that bind a specific DNA sequence in the regulatory regions of genes after binding their ligand, retinoic acid. The PML protein contains a motif found in transcription factors and is thought to function as a transcription factor itself. This translocation produces two fusion proteins: PML-RARα and RARα-PML.

Retinoic acid effectively induces cell differentiation in vitro. All-trans retinoic acid produces a complete remission in the vast majority of APL patients with very low morbidity. One can obtain a complete remission without marrow hypoplasia, and clear evidence of differentiation of the leukemic cells is observed (28, 29). Unfortunately, almost all patients experience clinical relapse despite continued retinoic acid treatment. Trials are currently underway to combine retinoic acid with chemotherapy.

Even given our increased understanding of the molecular defects in APL, the mechanism by which retinoic acid induces differentiation remains unknown. The fusion proteins possibly compete with either RARα or PML function and interfere with the normal process of myeloid differentiation. The identification of both genes involved in the translocation has enabled the use of PCR to confirm the diagnosis and to monitor treatment response.

LYMPHOID MALIGNANCIES

**Translocations into an Antigen Receptor Locus**

Researchers have discovered new oncogenes by observing their juxtaposition with the genes that encode antigen receptors in lymphoid malignancies (7–9). These rearrangements take place at one of the following chromosomes: 14q32
(heavy chain gene), 2p12 (κ light chain gene), or 22q11 (λ light chain gene) in B cells. In T-cells, chromosomes 14q11 (T-cell receptor α/δ locus) and 7q35 (T-cell receptor β locus) serve as translocation sites. These translocations probably occur as errors in the rearrangement of the immunoglobulin and T-cell receptor genes. Most of the genes characterized at the breakpoints of these translocations are transcription factors. The influence of regulatory elements of the antigen receptor results in the deregulation of the expression of the translocated transcription factors.

Researchers have identified several new oncogenes at translocation breakpoints at one of the T-cell receptor loci in T-cell acute leukemia (Table 1) (30–36). These genes display characteristics that are common to transcription factors. Presumably, the protein products represent transcription factors. However, the protein is not normally expressed in T-cells. How these transcription factors cause leukemogenesis is unknown, but at least three scenarios are possible: (a) transcription factors that are not normally expressed in T-cells may activate genes that in turn should not be expressed in T-cells; (b) transcription factors may reactivate a set of genes associated with a less-differentiated state; or (c) transcription factors may replace similar factors in normal T-cells and produce an opposing effect on their target genes. The characterization of these novel oncogenes contributes to our understanding of the malignant-transformation process.

**Fusion Proteins**

Chromosomal translocations with breakpoints in the introns that separate the exons or coding regions of the involved genes result in the synthesis of fusion proteins. The t(9;22) translocation in CML generates the fusion protein BCR-ABL, as discussed above. Several fusion proteins generated by chromosomal translocations are chimeric transcription factors, i.e. new transcription factors formed by the fusion of portions of two transcription factors. For example, two transcription factor fusion proteins, E2A-PBX1 (37, 38) and E2A-HLF (39, 40), occur in B-cell leukemias with translocations t(1;19) and t(17;19), respectively. The fusion proteins most likely deregulate the expression of target genes controlled by the wild-type transcription factors, and their transcriptional activity probably differs considerably from that of the normal transcription factors.

A gene encoding a protein with transcription factor motifs has been identified at the 11q23 locus (41, 42). Translocations in this region appear in acute lymphoblastic and myeloid leukemias, as well as in the secondary leukemias that develop in patients treated with topoisomerase II inhibitors. Leukemias with translocations involving 11q23 are highly prevalent in children under one year.
CONSEQUENCES FOR PATIENT MANAGEMENT

Improved Diagnostic Ability

Chromosomal translocations are associated with an increased risk of treatment failure in leukemias. Acute myelogenous leukemias with the cytogenetic abnormalities \(-7/7q^-, -5/5q^-,\) and \(11q23\) have a worse prognosis. In acute lymphoblastic leukemias, involvement of \(11q23\) and the \(t(1;19)\) and \(t(9;22)\) are associated with a worse prognosis. The specific chromosomal translocations can serve as molecular markers for the detection and classification of leukemias with similar biologic behavior and prognosis. For example, children with pre-B ALL and a \(t(1;19)\) have a higher risk of relapse than children with only pre-B ALL. It is important to identify these patients because the worse prognosis can be overcome with more intensive chemotherapy. Molecular detection techniques have enabled the identification of a \(t(1;19)\) translocation in cases where cytogenetics have not been informative (43).

Detection of Minimal Residual Disease

Histologic detection of minimal residual disease in leukemia clearly lacks sensitivity. Cytogenetic analysis and the measurement of surface or immunological markers using flow cytometry have improved sensitivity. PCR can be used to detect chromosomal rearrangements with a theoretical sensitivity of one malignant cell in a population of \(10^5–10^6\) cells (44). Molecular analysis of remission specimens apparently can predict a significant fraction of clinical relapses, but the clinical value of minimal residual disease detection at this level of sensitivity remains unclear. Researchers hope to use the results of the molecular analysis to implement a new treatment strategy for patients whose remission marrows remain positive. To clarify the role of molecular analysis in the detection of minimal disease, prospective studies are necessary.

Purging of Bone Marrow Prior to Bone Marrow Transplant

Whether the infusion of a few residual tumor cells in an autologous bone marrow transplant contributes to relapse is unclear. In a study of autologous bone marrow transplantation in non-Hodgkin’s lymphoma, researchers used PCR to detect malignant cells with the \(t(14;18)\) translocation (45). Patients with negative marrow after in vitro purging survived disease-free for a significantly longer time than did those whose marrow remained positive. The results of this study suggest that the reinfusion of malignant cells in autologous marrow contributes to disease relapse. Further studies are required to extend these observations to patients with other hematologic malignancies.
Novel Treatment Strategies

Recent advances in the molecular analysis of hematologic malignancies should lead to a better understanding of the genetic basis of these diseases. With this knowledge, researchers should be able to predict biologic behavior and develop more effective treatment regimens. In the near future, specific therapeutic modalities probably will be designed to combat the underlying abnormality in leukemic cells. In malignancies with fusion proteins, specific therapies can target these abnormal proteins as well as the fusion DNA or mRNA. Evidence indicates that fusion proteins can provoke an immune response (46). This approach may prove useful in therapeutic regimens. Anti-sense technology is being used to turn off the expression of a targeted gene. Many transcription factors are inactive as monomers. For the deregulated transcription factors that cannot function without dimer formation, researchers may be able to design analogues that interfere with dimerization of the transcription factor and thus inhibit its activity.

Literature Cited


