Knowledge of the molecular mechanisms of leukemia has important implications for treatment, prognosis, and follow-up. In the case of acute promyelocytic leukemia (APL), one of the six main subtypes of acute myeloblastic leukemia (AML), the clinical use of powerful forms of vitamin A has induced leukemia cells to relinquish their malignant phenotype and enter a program of normal cellular differentiation and death. In addition, molecular tests are available for detection of minimal residual disease after bone marrow transplantation. This method is a very sensitive way to detect small numbers of leukemic cells which may have remained even after treatment.

**Molecular genetics of acute promyelocytic leukemia**

Genes involved in the pathogenesis of cancer are thought to act by two general mechanisms: The first involves the structural alteration of a normal gene (a proto-oncogene) to generate a novel gene (an oncogene) whose protein product acts on the host cell to induce characteristics of malignancy. This protein product is usually involved in cellular proliferation, differentiation, or survival. The second mechanism involves the loss or inactivation of genes whose proteins suppress cancer. Genes of this class are known as tumor-suppressor genes or anti-oncogenes.

An example of a structural alteration of a normal gene occurs in acute promyelocytic leukemia (APL). The association of APL with a t(15;17) translocation has been known for 20 years. In 1987, it was discovered that the hallmark of the disease is the fusion of the PML gene from chromosome 15 with the retinoic acid-receptor (RAR) gene on chromosome 17. (The PML gene is an oncogene.) The t(15;17) translocation fuses two separate genes. This gene fusion causes the cell's DNA PML-RAR to produce mRNA which then constructs an abnormal protein. The abnormal fusion protein is a hybrid receptor and may contribute to leukemogenesis by dominantly antagonizing differentiation.

Retinoids, derived from vitamin A, are critical regulators of cell proliferation. A normal retinoid receptor binds to a specific DNA segment and causes transcription of mRNA. By this binding, the retinoic acid may induce maturation in promyelocytes. In normal cells, the retinoic acid-receptor (RAR) gene is switched off or regulated. The t(15;17) translocation leads to constant activation of the RAR gene. It seems counterintuitive that a defective receptor should confer spectacular clinical sensitivity to one of its ligands, retinoic acid. But that is what happens: high levels of retinoic acid induces maturation. (It is possible that at high intranuclear concentrations of retinoic acid, the normal retinoid receptors simply out-compete the aberrant receptor.)

**Clinical response, methods, disease definition**

The initial clinical response to retinoic acid is most closely correlated with the presence of the t(15;17) translocation, as assessed by conventional cytogenetic analysis or by a molecular assay using probes for RAR-alpha messenger RNA (RNA) on Northern blot analysis or by reverse transcriptase-polymerase chain reaction (RT-PCR) assays using specific primers. Each of these tests varies in sensitivity and specificity. A cytogenetic result indicating the presence of the t(15;17) is pathognomonic for APL, is associated with molecular rearrangement of the RAR receptor, and is highly correlated with clinical efficacy and retinoic acid.

A “negative” result is problematic. In some patients with equivocal morphologic characteristics and reportedly normal karyotypes, typical rearrangements of RAR have been found by molecular testing, and patients who could be evaluated clinically have subsequently responded to retinoic acid. By contrast, patients who are clearly negative by RT-PCR for the PML-RAR fusion mRNA do not have t(15;17) on cytogenetic analysis, and none of these patients have responded clinically. Other translocations involving RAR have been recently described, but they appear to be uncommon. These data argue strongly for a new molecular definition of the disease, rather than distinctions based on morphologic features, immunophenotype, or clinical manifestations.

The induction of remission by retinoic acid is associated with the differentiation of immature neoplastic cells into mature granulocytes, followed by the emergence of normal hematopoietic cells as the patient achieves remission. After inducing an irreversible commitment to differentiation, retinoic acid may initiate programmed cell death (apoptosis) in the maturing cells. Patients with APL usually receive cytosine arabinosine and other chemotherapy besides RA.
Assessment of minimal residual disease

The possibility of detecting PML/RAR fusion transcripts by RT-PCR has yielded a specific test for a disease that is closely correlated with clinical responsiveness to retinoic acid. Since patients in first remission from acute promyelocytic leukemia historically have a high risk of relapse, several groups have explored RT-PCR as a means of detecting minimal residual disease. For the first time in any type of AML, these studies theoretically allow a real-time determination of the effects of treatment administered during remission, such as maintenance chemotherapy, bone marrow transplantation, and monoclonal antibodies.

In patients with chronic myelocytic leukemia (CML), PCR has been used to evaluate the persistence of disease after bone marrow transplantation. It appears that these tests (for the bcr/abl fusion gene) may remain positive up to one year after transplantation without risk of clinical relapse. In contrast to these patients with CML, most patients with APL who have received additional chemotherapy as consolidation have converted the RT-PCR signal of PML/RAR to negative; thus, it is unclear whether the pattern of bcr/abl persistence in CML after transplantation will prove to be the rule or the exception for molecular follow-up of minimal residual disease. The test should be useful for the identification of patients with continued positivity for PML/RAR who may benefit from further antileukemic treatment. Until these tests can be validated, many oncologists do not base treatment decisions on the results.

Methods to detect minimal residual disease

In hematological malignancies, detection of minimal residual disease is important in patients who receive chemotherapy alone or in those who undergo bone marrow transplantation. After ablative chemotherapy for a leukemia, detection of minimal residual disease may be relevant to further treatment needs, predicting early relapse, determining if the patient’s bone marrow can be harvested for future autologous transplantation or determining if bone marrow purging techniques have been successful in removing neoplastic cells.

The methods that have been used to detect minimal residual disease and the relative sensitivities are detailed in table One. These methods range from standard microscopic methods (morphology and cytochemistry) with sensitivities of one to five percent to new molecular and genetic techniques with greater sensitivities. For example, fluorescent in-situ hybridization (FISH) using specific probes can detect as few as one tumor cell in 100,000 to one in 1,000,000 normal cells. The most cost effective and objective method to monitor remission and detect clinically significant minimal residual disease will be FISH with probes specific for markers. This method is less sensitive than PCR, but more sensitive than cytogenetics. It may provide just the right amount of sensitivity for clinical practice.

With this powerful method to detect minimal residual disease comes the potential to predict success of therapy, either immediately after treatment or later. The significance of very low numbers of residual tumor cells is uncertain. It is possible that host factors prevent recurrence even though minimal residual disease is present.

Implications for risk classification

Acute promyelocytic leukemia typically presents with bleeding. The incidence of early fatal hemorrhage has ranged from eight to 47 percent. Despite the high early mortality from bleeding, long-term survival is somewhat more favorable than that for other types of acute myeloblastic leukemia, though still a disappointing 40 percent five-year survival.

Solid malignant tumors tend to show classic malignant features. At presentation, they show invasion, destruction, and necrosis. When problematic, they show local recurrence or distant metastasis. Therefore, patients with solid malignant tumors are followed clinically with radiographic studies to demonstrate, or rule out, early recurrence or metastasis. A one cm nodule of chest roentgenogram contains approximately one billion tumor cells.

Genetic information and risk classification is a complex area. For many leukemic patients, the advent of molecular and genetic techniques might result in many of them becoming insurable. Because of a generally poor prognosis for leukemic patients taken as a whole, most leukemic patients are uninsurable for roughly 10 years from the time of diagnosis. If it turns out that a leukemic patient with no minimal residual disease at five years after diagnosis has an excellent prognosis, at least that group of leukemic patients could potentially be insurable. Thus, the detection of minimal residual disease by molecular and genetic techniques might allow a more equitable underwriting variable by helping to distinguish those patients with a good prognosis from those that may need further treatment.

References


Table One

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<thead>
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<th>Non-Genetic Methods</th>
<th>Sensitivity</th>
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<td>Morphology</td>
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<td>Cytochemical stains</td>
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