Molecular cytogenetic markers related to prognosis in hematological malignancies

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It has become increasingly evident that cancers are "genetic diseases" resulting from an accumulation of inherited and environmentally induced changes or mutations in the genome, i.e., the modification, activation, or inactivation of various genes, including oncogenes, tumor-suppressor genes, and genes related to cell death. Cancer genetics has, therefore, become a burgeoning area of both genetic researches and clinical application in human cancer.

This paper is intended to update researchers on the rapid advances that have been made in the understanding of prognostically significant molecular cytogenetic markers in hematological malignancies. Examples include observations of p53, RB1, and ATM gene deletions as well as 12q13 amplifications in up to 80% of patients with chronic lymphocytic leukemia (CLL) in association with significantly different prognostic groups. Greater than 80% of patients with multiple myeloma have been observed to have chromosome 13q deletions; RB1, D13S319, and p53 deletions have been reported to serve as independent adverse prognostic parameters. In chronic myelogenous leukemia (CML), large deletions (ASS and N43E1 being the two most commonly deleted loci) at the t(9;22) breakpoints have been reported to be associated with reduced time to accelerated phase; and in adult acute myelogenous leukemia (AML), molecular cytogenetic analysis as well as CEBPA, FLT3, and RAS studies predict the outcome of therapy.

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Introduction

Chromosomal changes have been established in a wide spectrum of cancers, ranging from various leukemias to lymphomas to solid tumors. In addition to the clinical application of these cytogenetic changes, the establishment of specific chromosomes or chromosomal bands affected by these changes has led molecular biologists to recognize, characterize and isolate the genes that are affected by and possibly responsible for the conditions studied.

Nonrandom chromosomal abnormalities in hematologic malignancies

Chronic myelogenous leukemia (CML)

CML is a clonal myeloproliferative disorder arising from neoplastic transformation of a pluripotent stem cell, and is characterized clinically by a marked overproduction of granulocytic cells and cytogenetically by the Philadelphia (Ph) chromosome. The Ph chromosome is the first consistent abnormality observed in the human cancer, arising from a reciprocal translocation, t(9;22)(q34;q11.2) and the molecularly by the fusion of the protooncogene ABL located on the long arm of chromosome 9 with the BCR gene of chromosome 22 known as the breakpoint cluster region (bcr).[1,2] Historically, more than 85% of patients diagnosed as having CML are found to have the Ph chromosome.[3,4] In 25% of cases, the Ph translocation is the only change noted throughout the disease.

CML should be defined by the presence of the Ph chromosome, and patients with CML-related features but without the Ph should be considered to have a different chronic myeloproliferative disorder (MPD) or some type of myelodysplastic syndrome (MDS), such as chronic myelomonocytic leukemia (CMML) or refractory anemia with excess blasts (RAEB).[3]

Variant forms of Ph translocations are seen in 5%-10% of CML cases. In general, no correlation has been found either for genomic breakpoint site or BCR/ABL RNA transcript in terms of duration of

chronic phase or survival.[5] All chromosomes except the Y chromosome have been involved in variant Ph translocations.

When CML progresses, chromosome aberrations additional to the Ph chromosome are noted in 75%-80% of cases. These may precede hematologic progression by 2-6 months or occur at blast crisis, and are therefore important prognostic markers. The most common additional chromosome changes are trisomy 8, additional Ph, i(17q), trisomy 19, trisomy 21 and loss of a sex chromosome. Molecularly, the TP53 gene appears to be affected in about 30% of cases of myeloid blast transformation.[6] However, other tumor suppressor genes, such as RB, WT1, DCC and NF2, are unlikely to be involved in the progression of CML to blast crisis in the majority of patients.[7] In addition, loss of imprinting of the insulin-like growth factor-II gene (IGF2)[8]and telomere length shortening[9] have been reported to be associated with disease progression in CML.

Recent studies also indicated that up to 29% of CML patients may have large deletions at the t(9;22) breakpoints.[10,11] Deletions on the derivative chromosome 9 may be several Mb in size with ASS and N43E1 being the two most common loci deleted. These deletions appear to occur at the time of formation of the Ph translocation and to be associated with a shorter chronic phase and overall survival time.

Myelodysplastic syndromes (MDS)

MDS are a heterogeneous group of clonal hematopoietic stem cell disorders characterized by dysplastic and ineffective hematopoiesis as a result of progressive impairment of differentiation due to a defect at the level of multi- or pluripotent stem cells and a high risk of transformation to acute nonlymphocytic leukemia (ANLL). Etiologically, MDS occur both as primary disorders (p-MDS) and as disorders secondary to prior cytotoxic chemotherapy or radiotherapy (t-MDS). The incidence of MDS appears to be increasing in the aging population (>50 years), and already presents a perplexing and serious epidemiological problem. Clinically, to confirm the diagnosis of MDS, morphological examination of bone marrow aspirate and marrow chromosome analysis should be performed. Moreover, in general, the chromosome findings have been shown to be an independent prognostic indicator, second only to the French-American-British (FAB) subtype as a predictor of progression to leukemia and survival. An international scoring system for evaluating prognosis in MDS has also been established, primarily based on cytogenetics.[12] This system indicates that the major variables having an impact on disease outcome or evolution to acute myeloid leukemia are the presence of cytogenetic abnormalities, the percentage of bone marrow myeloblasts and extent of cytopenias, whereas for survival, in addition to the foregoing variables, also include age and gender. Thus, chromosome studies can help to indicate those cases of MDS who are likely to survive long enough to be adequately evaluated with long-term therapy or those patients who must be treated rapidly and aggressively based on chromosomal patterns associated with clinical prognosis. During therapy, the cytogenetic findings can be utilized to monitor the size of the neoplastic clone in the marrow as an indicator of response.

p-MDS

Clonal chromosomal abnormalities can be detected in bone marrow cells in 40%-70% of patients with p-MDS at presentation.[3,4] This contrasts with 70%-95% incidence of cytogenetic abnormalities found in patients with *de novo* ANLL. Although both disorders commonly have trisomy 8 and loss of chromosomes 5 or 7 or a deletion of 5q or 7q, the specific structural rearrangements that are closely associated with distinct morphological subsets of *de novo* ANLL, such as t(15;17) in ANLL-M3, are almost never seen in p-MDS. Patients with MDS may have single or multiple chromosome changes. Occasionally, several unrelated abnormal clones may be detected; the frequency of such unrelated clones may be higher than that observed in *de novo* ANLL. Additional aberrations may evolve during the course of MDS, or an abnormal clone may emerge in a patient with a previously normal karyotype; these changes appear to portend transformation to leukemia. Clinically, with several exceptions, such as the 5q- syndrome and monosomy 7 (-7) syndrome, chromosome changes in p-MDS are not correlated with specific clinical or morphological subsets using the criteria of the FAB group.

The most common molecular abnormality in MDS is activation of the RAS protooncogenes, which is found in approximately 3%-40% of patients. Although the gene mutation appears at early as well as late stages of leukemic progression, it is suggested to be a predictive factor for malignant transformation in the future. In addition, TP53 mutations are detected in 10%-15% of advanced MDS, and are preferentially associated with 17p-.[13] Other findings include observations of extensive apoptosis resulting in ineffective hematopoiesis and deficient bone marrow function,[14] as well as methylation of the p15INK4b gene (an inhibitor of cyclin-dependent kinase CDK4 and CDK6),[15] FLT3 gene (the human flt3 receptor gene) internal random duplication,[16] and increased

BCL-2 expression[17] in association with disease evolution.

MDS represents a small fraction of all hematological malignancies in children (<10%). More than 80% of childhood MDS are in the advanced stages. Childhood MDS can occur idiopathically, after chemo-/radiotherapy, or with predisposing conditions, such as constitutional chromosomal disorders (Down syndrome), neurofibromatosis, Schwachman syndrome, Kostmann neutropenia, and Fanconi anemia. Familial occurrence of MDS with -7 has also been reported.[3,4] The diagnosis and classification of childhood MDS based on cell morphology and cytochemical staining is somewhat complex and confusing. Cytogenetic analysis is essential for the evaluation of a child with proven or suspected MDS. About 64% of childhood MDS cases have an abnormal karyotype at diagnosis, a higher incidence than in adult MDS.[18,19] Monosomy 7 or deletion of the long arm of chromosome 7 [del(7q)], the most frequently observed abnormality often appears as the sole anomaly rather than part of complex karyotypes as commonly seen in adult MDS. Other commonly observed anomalies include +8, +9, +19, del(12p), and del(17p). Certain cytogenetic features have prognostic significance, such as a normal karyotype being associated with a better outcome than an abnormal karyotype and -7 alone having a survival advantage when compared to -7 with additional anomalies.

Acute leukemia

The acute leukemias, classified as either lymphoblastic (ALL) or nonlymphocytic (ANLL), result from neoplastic transformation of uncommitted or partially committed hematopoietic stem cells.

The common chromosome changes seen in ALL and ANLL are shown in Tables 1-3.

A large array of structural and numerical chromosomal changes have been described in ANLL and ALL. However, some of these changes occur much more frequently than others. In ANLL, t(8;21), t(15;17) and inv(16) are the most common, followed by del(5q), +8 and del/t(11q23). In ALL, the most common changes are t(9;22), t(4;11) and del(6q), followed by t(8;14), t(1;19) and del(9p). A large number of other changes of much lower incidence occur in both ANLL and ALL. However, the incidence may vary in different clinics, since the changes shown may be related to age (pediatric vs adult cases), geographical location and the nature of the leukemias examined (e.g., primary vs secondary).[3,4]

Determination of the chromosome changes in acute leukemia serves a number of practical purposes, for example establishing exact diagnosis, predicting prognosis, and monitoring phases of therapy or bone marrow transplantation (BMT), as well as some basic purposes, for example supplying the molecular biologist with information on the possible location or nature of the genes affected by translocations.

ANLL

At least two-thirds of ANLL patients have demonstrable clonal chromosome abnormalities at diagnosis. In children, cytogenetic analysis reveals clonal abnormalities in 80%-85% of patients.[19] On average, about half of patients with cytogenetic anomalies have only one karyotypic rearrangement. Numerical aberrations are seen in 15%-20% of the cytogenetically abnormal cases. Trisomy 8 and monosomy 7 are particularly common. Translocations are the most common structural chromosome changes, followed by deletions and inversions. Some of the following changes are diagnostic for particular types of ANLL (Table 1).

Of interest, translocations involving 11q23 (MLL gene rearrangements) occur in 5%-10% of adult leukemia cases and in approximately 60% of infant acute leukemia cases.[3,4,19] Patients with MLL rearrangements have a significantly shorter duration

Table 1. Common chromosome changes in ANLL

der(1;7)(q10;p10)[a]	t(9;22)(q34;q11) M1 (M2) (Ph)
t(1;22)(q13;q13) M7	t(11;V)(q23;V)[b] M5 (M4)
ins(3;3)(q26;q21q26)[a,c] M1 (M7)	del(11)(q23) M5 (M4)
inv(3)(q21q26)[a,c] M1 (M7)	+11
t(3;3)(q21;q26)[a,c] M1 (M7)	del(12)(p11p13)[a]
t(3;5)(q25.1;q34)	M1, M2, M4-M6
t(3;21)(q26;q22)[a]	+13[a]
+4 M2, M4	+14[a]
-5 or del(5)(q12-13 or q31-35)[a] M1-M4	t(15;17)(q22;q12) M3
+6	del(16)(q22)[d] M4Eo
t(6;9)(p23;q34)[a] M2 (M4) (basophilia)	inv(16)(p13q22)[d] M4Eo
-7 or del(7)(q22)[a] M1-M5 t(7;11)(p15;p15)	t(10;16)(p13;q22)[d] M4Eo
+8[a]	t(16;21)(p11;q22)
t(8;16)(p11;p13) M5b (erythrophagocytosis)	i(17q)[a]
t(8;21)(q22;q22) M2 (Auer rods[a])	+19
+9	del(20)(q11-13)[a]
del(9)(q22)	+21
	idic(X)(q13)[a]

a: change also seen in MDS.
b: V = chromosomes 6, 9, 17, 19.
c: associated with platelet and/or megakaryocytic anomalies.
d: associated with marrow eosinophilia.
Where appropriate, the type of ANLL or other information associated with a particular chromosome change is also shown.

Table 2. Common chromosome changes in B-lineage ALL

t(1;9)(q23;p13)	Pre-B-cell
t(2;8)(p12;q24)	L3 (B-cell)
t(4;11)(q21;q23)	Biphenotypic, early pre-B-cell?
t(5;14)(q31;q32)	
del(6)(q13-14 or q21-27)	
t(8;14)(q24;q32)	L3 (B-cell)
t(8;22)(q24;q11)	L3 (B-cell)
del(9)(p13-22)	
t(9;22)(q34;q11)	Pre-B-cell, Ph^+
del(11)(q14-23)	
t(11;19)(q23;p13)	Mixed, early pre-B-cell?
t(12;V)(p12;V)[a]	B-lineage
t(12;21)(p13;q22)	Pre-B-cell
t(14;19)(q32;q13)	
t(14;22)(q32;q11)	

a: V = chromosomes 7, 9, 17.

Table 3. Structural chromosome changes in T-cell lymphomas and ALL

t(7;7)(p15;q11)
t(7;V)(q34;V)[a]
inv(7)(p14q35)
del(9)(p13-22)
t(9;17)(q34;q23)
t(14;V)(q11;V)[b]
inv(14)(q14q32)

a: V = 1p32-34, 9p24 or q32, 10q24, 11p13, 14q11, 19p13.
b: V = p32-34, 7q34, 8q24, 10q24, 11p13-15.

of complete remission when compared with patients without MLL rearrangements.

Acute megakaryoblastic leukemia or ANLL-M7 comprises 3%-5% of all childhood ANLL and about 20% of infant leukemias, and cytogenetically can be classified into four different subgroups, i.e., M7/Down syndrome (DS), M7/acquired trisomy 21, M7/t(1;22)(p13;q13), and M7/abnormaltiies of bands 3q21 and 3q26, and monosomy 7 or del (7q).[19] In general, patients with M7/DS have a markedly more favorable outcome compared to those without DS, and children with M7/t(1;22) or M7/other abnormalities have a poor prognosis.[19]

Normal karyotypes at diagnosis of ANLL

The infrequent limitations of conventional cytogenetic are cogently illustrated by the demonstration of genetic changes (either by fluorescence *in situ* hybridization or RT-PCR) in ANLL associated with normal karyotypes. Apparently, the group of patients with normal karyotypes at diagnosis was heterogeneous at the molecular level, and many aberrations occurred below the level of detection of conventional cytogenetic analysis, as mentioned above. Another example is the presence of an internal tandem duplication of FLT3 in patients with ANLL and a normal karyotype.[16,20] Internal tandem duplications of FLT3 were seen in 23% of 201 ANLL patients and of patients with normal karyotypes, 28% harbored internal tandem duplications of FLT3.[16,20] In patients younger than 60 years, the presence of this FLT3 mutation is the strongest poor prognostic factor for overall survival. The presence of internal tandem duplications of FLT3 in addition to the absence of the wild-type FLT3 allele were shown to have a significantly inferior disease-free survival and overall survival when compared with patients with internal tandem duplications of FLT3 and the wild-type FLT3 gene and those without internal tandem duplications of FLT3.[21] Another study of patients with ANLL and normal karyotypes identified dominant-negative mutations of the tumor suppressor gene C/EBPa in 5% of patients with normal karyotypes.[22] The clinical significance of these findings is yet to be established.[23]

Thus, the significance of a normal karyotype in patients with ANLL at diagnosis must be held in abeyance until this area is intensively explored molecularly, as already indicated above.

ALL

The karyotype is an important independent prognostic factor in ALL. At least two-thirds of ALL have clonal chromosomal anomalies. Up to 90% of children with ALL display karyotypic abnormalities.[19] Hyperdiploidy, which is present in 30%, is more common in ALL than in ANLL. Hypodiploidy is present in 10%. Several cytogenetic anomalies are associated with distinct immunological phenotypes in ALL.

Ph^+ leukemia occurs in two major forms: CML and ALL. The t(9;22) is observed in 6% of childhood and 17% of adult ALL associated with chromosome abnormalities, representing the most frequent rearrangement in adult ALL. Molecularly, there are two distinct subgroups of Ph^+ ALL. In the first group, the molecular rearrangement is identical to that seen in CML, and in the second group the breakpoint occurs in the upstream (5') of the bcr but still within the BCR gene, giving rise to an abnormal fusion mRNA (6.5-7.4 kb) and an abnormal protein (185-190 kDa).[24,25] A Ph translocation similar to that in ALL can also be seen in some cases of ANLL. Molecular BCR analysis is the approach of choice in clarifying the nature of the Ph in these leukemias. Generally speaking, Ph^+ ALL is of pre-B-cell lineage, and has an unfavorable prognosis.

Only recently has this translocation, t(12;21)(p13;q22),

been shown to be the most frequent, but cytogenetically largely undetected chromosomal anomaly in childhood ALL (25%). This translocation defines a distinct entity of childhood pre-B ALL with a good prognosis.[26] The t(12;21) results in the fusion of two genes: TEL on 12p and AML1 on 21q. Only the TEL-AML1 may play a key role in leukemogenesis.

Both childhood and adult ALL patients with numerical abnormalities of 50 or more chromosomes appear to have a favorable prognosis. Such ALL is usually of L1 or L2 type, and of non-T, non-B origin. This type of anomaly is found in 25%-30% of childhood ALL.[19]

Prognostic value of molecular cytogenetics in acute leukemia

ANLL

Generally, the karyotype is an important prognostic factor in *de novo* ANLL.[3,4] ANLL cases with major karyotypic anomalies (MAKA) have a less favorable course than those with minor karyotypic changes (MIKA); patients whose marrow consists of only abnormal cells (AA) have a poorer prognosis than those patients with only normal karyotypes (NN) or a mixture of normal and abnormal karyotypes (AN). Specific chromosomal anomalies in ANLL have also been shown to correlate with response to chemotherapy. Patients with t(8;21), inv(16), t(16;16), or t(15;17) changes have high rates of complete response (CR) after initial therapy (60%-100%), whereas those with -7/7q- or -5/5q- have low rates (0-36%). In general, the former have long disease-free survivals, whereas the latter have short remission durations, even if the patients eventually achieve CR with further therapy (Table 4). The prognostic aspects of ANLL may be related to the age of the patients, and possibly to the cytogenetic changes predominant in elderly patients versus those in younger patients. Although the incidence of abnormal karyotypes appears to be about equal in both groups, the changes associated with a CR rate of more than 80% and a favorable prognosis, that is t(15;17), t(8;12) or inv(16), are seen much more frequently in the younger age group than among elderly patients, who often have -5/5q-, -7/7q- and +8 as karyotypic changes, associated with a CR rate of less than 40%. Therefore, the nature of pretreatment cytogenetics is a significant prognostic factor in determining response to induction therapy. More importantly, the relative impact of different post-remission therapies may vary according to the cytogenetic risk group; patients with favorable cytogenetics are significantly better after autologous bone marrow transplantations (ABMT) and allogenic bone marrow transplantation (allo BMT) than those with chemotherapy alone, whereas patients with unfavorable cytogenetics are better after allo BMT.[27] It has also been reported that the presence of a FLT3 internal tandem duplication (ITD) in ANLL adds important prognostic information to the cytogenetic risk group, i.e., being the most important factor predicting relapse and disease-free survival and an independent risk factor for event-free survival and overall survival.[28] Thus, detection of a FLT3/ITD should be included as a routine test at diagnosis for the evaluation of optimal therapeutic management.

ALL

Cytogenetic studies in ALL have independent prognostic value even when age, WBC count, FAB type, and immunophenotype are considered.[3,4] Hyperdiploid stem lines with greater than 50 chromosomes are seen in 30% of children with ALL, a subset that has proved to have the most favorable prognosis. Hyperdiploidy in adult ALL likewise confers the most favorable prognosis, although the rate of treatment failure is higher than that which has been observed in children. Generally speaking, a pseudodiploid karyotype confers the poorest response to therapy. Normal diploid and near-diploid cases have an intermediate prognosis, but less favorable than that for the hyperdiploid group with 50 or more chromosomes. Near-haploid ALL cases are rare in adults and children and appear to have a very poor prognosis. Cases with multiple leukemic stem lines pose a problem for prognostic classification. In general, the correct prognostic designation can be based on the leukemic line of lowest ploidy.

It is now clear that chromosomal translocations are in general, indicative of an unfavorable prognosis, whether they are found in a pseudodiploid or near-diploid karyotype. Examples include B-ALL with the t(8;14), t(2;8), or t(8;22) conferring a very unfavorable prognosis (median survival less than 6 months in both adults and children), and Philadelphia-positive ALL or ALL with the t(4;11), both conferring an unfavorable prognosis. However, the t(12;21) defines a distinct group of childhood pre-B ALL with a favorable prognosis[26] (Table 4).

Malignant lymphoproliferative disorders

Burkitt lymphoma is characterized in the preponderant number of cases (80%) by a translocation, t(8;14)

Table 4. Prognostic aspects of acute nonlymphocytic leukemia (ANLL) and childhood acute lymphocytic leukemia (ALL)

	Unfavorable	Favorable
ANLL		
Cytogenetics[a]	t(15;17), t(8;21), inv(16)/del(16q), NN, MIKA	-7, del(7q), -5, 3q or 11q23 involvement, t(9;22), +8, 20q-, AA, MAKA
Age	<45 y	Infants, >60 y
WBC count	<25 000/mm^3	>100 000/mm^3
Morphology	Auer rods present, eosinophilia, M2, M3, M4	M0, M1, M5, M6, M7
Markers	HLA-DR negative, TdT-, CD2$^+$, or CD19$^+$	CD34$^+$, HLA-DR positive, biphenotypic
ALL		
Cytogenetics[a]	Hyperdiploid (numerical changes only), t (12;21)	t(9;22), t(4;11), t(8;14), t(2;8), t(8;22)
Age	1-9 y	<1 or >10 y
WBC count	<50 000/mm^3	>50 000/mm^3
Morphology	L1, L2	L3
Immunophenotype	c-ALL, T-ALL, myeloid antigen negative	pre-pre-B-ALL, pre-B-ALL, myeloid antigen positive (biphenotypic)

a: often an independent prognostic factor. See text for details.

Table 5. Common cytogenetic and molecular genetic changes in leukemias and lymphomas

Malignancy	Chromosomal abnormality	Molecular alterations (genes involved)
CML	t(9;22)(q34;q11)	BCR-ABL
ANLL	t(8;21)(q22;q22)	ETO-AML1
	t(15;17)(q22;q21)	PML-RARA
	inv(16)(p13q22)	CBFB-MYH1
	t(11q23)	MLL-various
	t(6;9)(p23;q34)	DEK-CAN
	t(3;3)(q21;q26), inv(3)(q21q26)	EV11
	t(3;5)(q25.1;q34)	NPM-MLF1
	t(7;11)(p15;p15)	NUP98-HOXA9
	t(16;21)(p11;q22)	FUS-ERG
ALL		
B-cell lineage	t(9;22)(q34;q11)	BCR-ABL
	t(12;21)(p13;q22)	TEL-AML1
	t(1;19)(q23;p13)	PBX-E2A (TCF3)
	t(5;14)(q31;q32)	IL3-IGH
	t(8;14)(q24;q32)	IgH-MYC
	t(4;11)(q21;q23), t(11;19)(q23;p13)	MLL-various
	t(17;19)(q22;p13)	HLF-E2A
T-cell lineage	(1;14)(p32;q11), t(1;7)(p32;q35)	TAL-1-TCRD, TCRB
	t(7;9)(q35;q34), t(7;10)(q35;q24)	TCRB-TCL4, HOX-11 (TCL3)
	t(7;11)(q35;p13), t(7;19)(q35;p23)	RBTN2, LYL1
	t(10;14)(q24;q11)	TCL3-TCRD
	t(11;14)(p13;q11), t(11;14)(p15;q11)	RBTN2, RBTN1
Lymphoma	t(14;18)(q32;q21)	BCL-2-IgH
	t(8;14)(q24;q32), t(2;8)(p12;q24)	MYC-IgH, IgK, IgL
	t(8;22)(q24;q11)	
	t(11;14)(q13;q32)	BCL-1-IgH
	t(2;3)(p13;q27)	IgK-BCL-6
	t(2;5)(p23;q35)	NPM-ALK
	t(3;14)(q27;q32), t(3;22)(q27;q11)	BCL-6-IgH, IgL

(q24;q32). In a lesser number of cases (15%), the translocation is between chromosomes 8 and 22, i.e., t(8;22)(q24;q11). In about 5% of Burkitt lymphoma cases, the translocation involves chromosomes 2 and 8, i.e., t(2;8)(p12;q24). In each of these instances, the translocation results in the abnormal juxtaposition of immunoglobulin gene sequences with those of the MYC gene, leading to the creation of a chimeric gene and thus to abnormal protein products.[3,4] The latter play a key role in the genesis of the lymphoma.

More than 90% of cases of non-Hodgkin lymphoma (NHL) have been reported to have clonal chromosomal

changes. In non-Burkitt NHL, a 14q+ marker is seen in about 50% of the cases. More importantly, many of the nonrandom anomalies correlate with histology and immunological phenotypes, the most common being t(14;18)(q32;q21) in follicular (nodular) B-cell neoplasms, del(6q) in large-cell lymphomas, t(8;14)(q24;q32) in either small non-cleaved cell or diffuse large-cell lymphomas, t(2;5)(p23;q35) in Ki-1-positive lymphomas, and rearrangements of 14q32 in B-cell type neoplasms.[3,4] On the other hand, T-cell neoplasms are characterized by rearrangements of 14q11, 7q34-36 and 7p15.

Approximately 50% of chronic lymphocytic leukemia (CLL) cases have chromosome abnormalities, the most common of which are trisomy 12, 14q+, 13q and 11q abnormalities. With FISH, Döhner et al[29] utilized a set of DNA probes targeting at 3q26, 6q21, 8q24, 11q22-23, 12q13, 13q14, 14q32, and 17p13 and found that 268 (82%) of 325 cases of CLL had chromosomal aberrations with a frequency of 55% for a deletion in 13q, 18% in 11q, 16% for trisomy 12q, 7% for a deletion in 17p, and 6% in 6q. Importantly, five prognostic categories were also defined, i.e., a median survival of 32 months for patients with 17p- (p53-), 79 months for 11q- (ATM-), 114 months for 12q trisomy, 111 months for normal karyotypes, and 133 months for 13q- (such as Rb-1 and/or D13S25).[29] Therefore, the presence or absence of a chromosomal abnormality yields significant prognostic information. However, the exact molecular defect in CLL is largely unknown and this area remains to be further investigated.

The cytogenetic results in multiple myeloma (MM) have been heterogeneous, ranging from translocations seen in B-cell lymphoid disease, e.g., t(11;14)(q13;q32) with associated involvement of the BCL-1 and IgH genes, to complex karyotypes inconsistent in their changes.[3,4] Thus, it is possible that, at least cytogenetically, MM consists of a number of subentities. Interestingly, recent data indicate that up to 86% of patients with MM have 13q deletions as detected by FISH.[30] Of particular note is that deletions of 13q remain an independent adverse prognostic factor after conventional-dose and high-dose therapy.[30]

Summary

As described in previous sections, molecular cytogenetics not only provides key information in the care of patients with hematological malignancies, but also as a guide for the identification of genes apparently responsible for the development of these neoplastic states. Detailed analyses of the DNA sequences located at the chromosomal breakpoints have allowed investigators to identify at least two major categories of genes, namely, protooncogenes and tumor suppressor genes. With respect to this issue, some examples are listed in Table 5.

Regardless of the specificity and nature of molecular changes seen in various neoplastic conditions, the cytogenetic changes will continue to offer useful information to the clinicians in the diagnosis, prognosis and care of patients.

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References

1 Rowley JD. A new consistent chromosomal abnormality in chronic myelogenous leukemia identified by quinacrine fluorescence and Giemsa staining. Nature 1973;243:290-292.
2 Groffen J, Stephenson JR, Heisterkamp N, de Klein A, Bartram CR, Grosveld G. Philadelphia chromosomal breakpoints are clustered within a limited region, bcr, on chromosome 22. Cell 1984;36:93-99.
3 Sandberg AA. The chromosomes in human cancer and leukemia, 2nd ed. New York: Elsevier Science, 1990.
4 Heim S, Mitelman F. Cancer cytogenetics, 2nd ed. New York: Wiley-Liss, 1995: 7-18.
5 Shepherd P, Suffolk R, Halsey J, Allan N. Analysis of molecular breakpoint and m-RNA transcripts in a prospective randomized trial of interferon in chronic myeloid leukaemia: no correlation with clinical features, cytogenetic response, duration of chronic phase, or survival. Br J Haematol 1995;89: 546-554.
6 Cline MJ. The molecular basis of leukemia. N Engl J Med 1994;330:328-336.
7 Silly H, Chase A, Mills KI, Apfelbeck U, Sormann S, Goldman JM, et al. No evidence for microsatellite instability or consistent loss of heterozygosity at selected loci in chronic myeloid leukaemia blast crisis. Leukemia 1994;8:1923-1928.
8 Randhawa GS, Cui H, Barletta JA, Strichman-Almashanu LZ, Talpaz M, Kantarjian H, et al. Loss of imprinting in disease progression in chronic myelogenous leukemia. Blood 1998; 91:3144-3147.
9 Boultwood J, Peniket A, Watkins F, Shepherd P, McGale P, Richards S, et al. Telomere length shortening in chronic myelogenous leukemia is associated with reduced time to accelerated phase. Blood 2000;96:358-361.
10 Sinclair PB, Nacheva EP, Leversha M, Telford N, Chang J, Reid A, et al. Large deletions at the t(9;22) breakpoint are common and may identify a poor-prognosis subgroup of patients with chronic myeloid leukemia. Blood 2000;95: 738-744.
11 Huntley BJP, Reid AG, Bench AJ, Campbell LJ, Telford N, Shepherd P, et al. Deletions of the derivative chromosome 9 occur at the time of the Philadelphia translocation and provide a powerful and independent prognostic indicator in chronic myeloid leukemia. Blood 2001;98:1732-1738.
12 Greenberg P, Cox C, LeBeau MM, Fenaux P, Morel P, Sanz G,

et al. International scoring system for evaluating prognosis in myelodysplastic syndromes. Blood 1997;89:2079-2088.
13 Mitani K, Hangaishi A, Imamura N, Miyagawa K, Ogawa S, Kanda Y, et al. No concomitant occurrence of the N-ras and p53 gene in myelodysplastic syndromes. Leukemia 1997;11: 863-865.
14 Parker JE, Mufti GJ. Ineffective haemopoiesis and apoptosis in myelodysplastic syndromes. Br J Haematol 1998;101:220-230.
15 Quesnel B, Guillerm G, Vereecque R, Wattel E, Preudhomme C, Bauters F, et al. Methylation of the p15INK4b gene in myelodysplastic syndromes is frequent and acquired during disease progression. Blood 1998;91:2985-2990.
16 Horiike S, Yokota S, Nakao M, Iwai T, Sasai Y, Kaneko H, et al. Tandem duplications of the FLT3 receptor gene are associated with leukemic transformation of myelodysplasia. Leukemia 1997;11:1442-1446.
17 Davis RE, Greenberg PL. Bcl-2 expression by myeloid precursors in myelodysplastic syndromes: relation to disease progression. Leukemia Res 1998;22:767-777.
18 Hasle H. Myelodysplastic syndromes in childhood: classification, epidemiology, and treatment. Leuk Lymphoma 1994;13:11-26.
19 Martinez-Climent JA. Molecular cytogenetics of childhood hematologic malignancies. Leukemia 1997;11:1999-2021.
20 Nakao M, Yokota S, Iwai T, Kaneko H, Horiike S, Kashima K, et al. Internal tandem duplication of the flt3 gene found in acute myeloid leukemia. Leukemia 1996;10:1911-1918.
21 Whitman SP, Archer KJ, Feng L, Baldus C, Becknell B, Carlson BD, et al. Absence of the wild-type allele predicts poor prognosis in adult *de novo* acute myeloid leukemia with normal cytogenetics and the internal tandem duplication of FLT3: a cancer and leukemia group B study. Cancer 2001;61: 7233-7239.
22 Pabst T, Mueller BU, Zhang P, Radomska HS, Narravula S, Schnittger S, et al. Dominant-negative mutations of CEBPA, encoding CCAAT/enhancer binding protein-alpha (C/EBPalpha), in acute myeloid leukemia. Nat Genet 2001;27: 263-270.
23 Cataland SR, Caligiuri MA, Bloomfield CD. Genetic subtyping of adult acute leukemias and implications for treatment. Oncol Spectrums 2001;9:617-625.
24 Clark SS, McLaughlin J, Crist WM, Champlin R, Witte ON. Unique forms of the abl tyrosine kinase distinguish Ph-positive CML from ALL Ph1-positive ALL. Science 1987;235: 85-88.
25 Kurzrock R, Shtalrid M, Romero P, Kloetzer WS, Talpas M, Trujillo JM, et al. A novel c-abl protein product in Philadelphia-positive acute lymphoblastic leukemia. Nature 1987;325:631-635.
26 Shurtleff SA, Buijs A, Behm FG, Rubnitz JE, Raimondi SC, Hancock ML, et al. TEL/AML1 fusion resulting from a cryptic t(12;21) is the most common lesion in pediatric ALL and defines a subgroup of patients with excellent prognosis. Leukemia 1995;9:1985-1989.
27 Slovak ML, Kopecky KJ, Cassileth PA, Harrington D, Theil KS, Mohamed A, et al. Karyotypic analysis predicts outcome of preremission and postremission therapy in adult acute myeloid leukemia: a Southwest Oncology Group/ Eastern Cooperative Oncology Group study. Blood 2000;96: 4075-4083.
28 Kottaridis PD, Gale RE, Frew ME, Harrison G, Langabeer SE, Belton AA, et al. The presence of a FLT3 internal tandem duplication in patients with acute myeloid leukemia (AML) adds important prognostic information to cytogenetic risk group and response to the first cycle of chemotherapy: analysis of 854 patients from the United Kingdom Medical Research Council AML 10 and 12 trials. Blood 2001;98:1752-1759.
29 Döhner H, Stilgenbauer S, Benner A, Leupolt E, Kröber A, Bullinger L, et al. Genomic aberrations and survival in chronic lymphocytic leukemia. N Engl J Med 2000;343:1910-1916.
30 Kaufmann H, Urbauer E, Ackermann J, Huber H, Drach J. Advances in the biology and therapeutic management of multiple myeloma. Ann Hematol 2001;80:445-451.

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