GENES, CHROMOSOMES & CANCER 10:95–102 (1994)

Cell Lineage Involvement of Recurrent Chromosomal Abnormalities in Hematologic Neoplasms

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Analysis of most hematologic neoplasms indicates the involvement of one or more cell lineages in the bone marrow and/or the blood but rules out the involvement of all lineages in any one neoplasm. It is important to detect lineage involvement in order to clarify which stem cells are involved in leukemia, to predict prognosis, and to select appropriate treatment. Our aim was to study the cell lineage involvement of some of the recurrent chromosomal abnormalities seen in hematological neoplasms. The direct morphology-antibody-chromosomes (MAC) method was used. The deletion 20q in myeloproliferative diseases (MPD), the deletion of 5q and t(1;7) in myelodysplastic syndromes (MDS), and t(3;3) in acute myeloid leukemia subtype M7 (AML-M7) were seen in all or at least in two myeloid lineages. These were interpreted as stem cell abnormalities. Deletion 13q in MPD, t(8;21) in AML-M2 and t(15;17) in AML-M3 were seen in granulocytic lineages only; t(14;18) in non-Hodgkin's lymphoma and trisomy 12 as the sole abnormality in chronic lymphocytic leukemia (B-CLL) were seen only in immunoglobulin light chain clonal B cells; inversion 14 in T-CLL was seen only in T cells, whereas t(15;14) in acute lymphocytic leukemia with eosinophilia (ALL-EO) was seen in lymphoid stem cells but not in mature granulocytes or lymphocytes. Additional abnormalities (in addition to the Philadelphia chromosome) in chronic myeloid leukemia (CML) were seen in all myeloid cell lineages and also in mature granulocytes, B cells, and large granular lymphocytes. Abnormalities in Hodgkin's disease were restricted to CD30-positive Reed-Sternberg cells. Trisomy 8 and monosomy 7 are abnormalities that may be present in either stem cells or any of the single cell lineages. *Genes Chromosom Cancer 10:95–102 (1994).*

INTRODUCTION

Morphologic analysis of most hematologic neoplasms indicates the involvement of one or more cell lineages in the bone marrow and/or peripheral blood, but rules out the simultaneous involvement of all lineages. There is, however, very little conclusive evidence as to the involvement of any of the cell lineages (Fialkow, 1976; Fialkow et al., 1977, 1981a,b, 1982, 1987; Knuutila et al., 1993b). It is biologically and therapeutically important that their normality, if normal they are, be unequivocally established.

Most subtypes of hematologic neoplasms have highly specific chromosomal abnormalities (Heim and Mitelman, 1987). We selected patients with these abnormalities as well as patients with specific tumor morphology/immunophenotypes in order to find out in which lineages the chromosomal aberrations were present, so as to determine which cell lineages are involved in the different forms of leukemia. For the determination of cell lineage we used the morphology-antibody-chromosomes (MAC) method, which allows simultaneous analysis of immunophenotype, cellular morphology and karyotype of an individual interphase or mitotic cell (Knuutila and Teerenhovi, 1989).

PATIENTS AND METHODS

The methods used for cell culturing, hypotonic treatments, cytospin preparations, immunophenotyping, chromosome banding, chromosomal in situ hybridization, and interphase cytogenetics have been described elsewhere (Teerenhovi et al., 1984; Knuutila and Teerenhovi, 1989; Knuutila et al., 1993a,b).

In order to classify the cell lineages, we used May-Grünwald Giemsa, Sudan Black B (SBB), and α-naphtyl acetate esterase (ANAE) cytochemical stains, as well as immunoperoxidase and alkaline phosphatase anti-alkaline phosphatase immunostaining with monoclonal antibodies (Teerenhovi et al., 1984; Knuutila and Teerenhovi, 1989; Knuutila et al., 1993a,b).

We used G-banding or in situ hybridization techniques with chromosome-specific alphoid repetitive or whole chromosome painting probes for the cytogenetic analyses.

The alphoid probes used in the interphase cyto-

Received September 9, 1993; accepted November 25, 1993.

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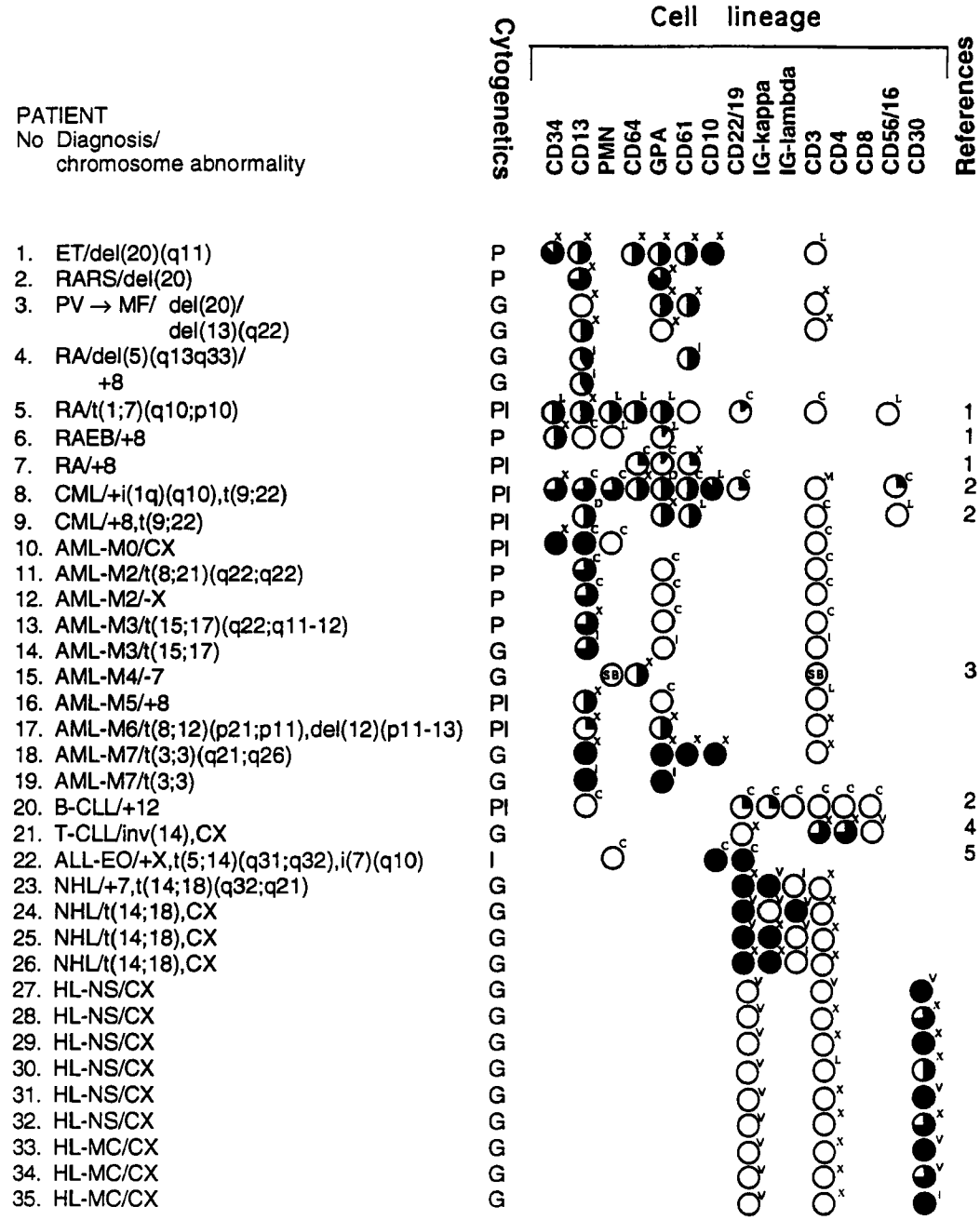


Figure 1. Lineage involvement of chromosomal abnormalities in 35 patients with hematological neoplasms. A white circle indicates that the chromosomal abnormality was not seen, a black circle that all analyzed cells showed the abnormality, and a partially black circle indicates the proportion of cells with the abnormality. *Diagnosis:* AML-M0 = subtype M0 of acute myeloid leukemia; ALL-EO = acute lymphoid leukemia with eosinophilia; B-CLL = B cell-type chronic lymphocytic leukemia; CML = chronic myeloid leukemia; HL-MC = mixed cellularity Hodgkin's lymphoma; ET = essential thrombocytosis; HL-NS = nodular sclerosis type Hodgkin's lymphoma; MF = myelofibrosis; NHL = non-Hodgkin's lymphoma; PV = Polycythemia vera; RA = refractory anemia; RAEB = RA excess of blasts; RARS = RA with ringed sideroblasts; CX = complex karyotype abnormality. *Cytogenetics:* P = chromosome painting of metaphase chromosomes with library probes; I = interphase cytogenetics with repeat sequence alphoid probes; G = G-banding or Giemsa-staining of metaphase chromosomes. *Number of cells* studied (Roman numerals): I = 1–4 cells; V = 5–9 cells; X = 10–49 cells; L = 50–59 cells; C = 100–499 cells; D = 500–999 cells; M = ≥1,000 cells. *CD groups, cell lineage and monoclonal antibodies:* CD34, stem cell (anti-CD34; Becton Dickinson, Erembodegem, Belgium); CD13; granulocytic/monocytic (MY7, Coulter, Hialeah, FL); CD64, monocytic (FCR1, Serotec, Oxford, England); CD61, megakaryocytic (Y2/51 provided by Dr D. Mason, Oxford, England); CD10, lymphoid stem cells (J5, Coulter); CD22, B-lymphocytes (Leu14; Becton Dickinson); CD3, T-lymphocytes (Leu4); CD4, helper/inducer T cells (Leu3); CD8, suppressor/cytotoxic T cells (OKT8; Ortho Diagnostic Systems, Raritan, NJ, USA); CD56, large granular lymphocytes (Leu19); CD16, large granular lymphocytes (CLB, Janssen, Beerse, Belgium); CD30, Reed-Sternberg cells in Hodgkin's Disease (Dakopatts, Glostrup, Denmark); PMN = polymorphonuclear granulocytes; GPA = glycophorin A, erythrocytic lineage (anti-GPA, provided by Dr. Griffin, Boston, MA); IG-kappa, immunoglobulin kappa light chain (anti-kappa, Serotec); IG-lambda (Serotec). *Probes:* Whole chromosome painting: Library probes: 1-specific LA12NS01 (No. 57738, American Type Culture Collection, ATCC, Rockville, MD); 7-specific LA07NS01; 8-specific LL08NS02; 12-specific LA12NS01; 13-specific LL13NS02; 15-specific LL15NS01; 17-specific LL17NS02; 18-specific LL18NS01; 20-specific LL20NS01; 22-specific LL22NS01; X-specific LL0XNS01; Y-specific LL0YNS01. *Alphoid probes:* 1-specific pUC1.77 (Cooke and Hindley, 1979); 7-specific pa7t1 (Waye et al., 1987); 8-specific (generated by polymerase chain reaction (Dunham et al., 1992); 12-specific pSP12-1 (Greig et al., 1991); X-specific pXBR 325 (Willard et al., 1983); Y-specific pY431, (a kind gift from K. Smith, School of Medicine, The Johns Hopkins University, Baltimore, MD, USA). SB = Southern blotting analysis was performed on isolated cells. References: 1 = Nylund et al., 1993; 2 = Knuutila et al., 1993a; 3 = Kere et al., 1988; 4 = Larramendy et al., 1990; 5 = Knuutila et al., 1993e. These were also reported elsewhere. All other patients are novel.

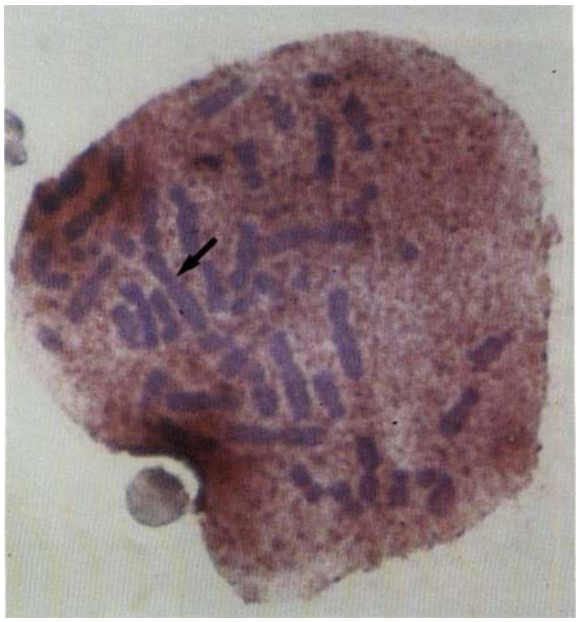


Figure 2. Megakaryocytic mitoses from patient 16 with acute myeloid leukemia, morphological subtype M7 (FAB-classification). After mild hypotonic treatment, a cytospin preparation was made and the cells were immunostained using the alkaline phosphatase anti-alkaline phosphatase (APAAP) technique with anti-CD61 monoclonal antibody (Y2, provided by Dr. D. Mason, Oxford, U.K.) (Knuutila et al., 1986). The arrow indicates one of the two derivatives of chromosome 3.

genetic analyses were detected by fluorochromes or enzymatic color reactions. Fluorescence was used to detect the chromosome painting of metaphase cells (Knuutila et al., 1993a,b).

Figure 1 shows the patients studied and the probes and monoclonal antibodies used in the analysis of cell lineage involvement.

RESULTS

Leukemia-specific Chromosomal Abnormalities and Cell Lineage Involvement

For non-lymphocytic neoplasms the aberrations, del(20), del(5),t(1;7), t(9;22) and t(3;3) were seen in all or at least in two myeloid cell lineages (i.e., the granulocytic/monocytic, erythrocytic, and megakaryocytic lineages) (Figs. 1–3). We refer to these aberrations as stem cell abnormalities. A small proportion of mature B cells and large granular lymphocytes (LGLs) in one of the patients (No. 8) also showed the abnormality [t(9;22) and +i(1q)] (Fig. 4).

The following abnormalities were seen only in single cell lineages: del(13), t(8;21), t(15;17), t(14;18), inv(14), trisomy 12, and t(5;14). We refer to them as single cell lineage abnormalities. The deletion del(13) and the translocations t(8;21) and t(15;17) were seen only in the granulocytic lineage, whereas t(14;18) and inv(14) appeared in the B-lymphocytic and T-lymphocytic lineages, respectively. t(14;18) and trisomy 12 were seen only in immunoglobulin light-chain clonal cells and t(5;14) in CD10/TdT-positive cells.

In one patient (No 7), trisomy 8 was found in all myeloid cell lineages, but in patients 16 and 6, it was found only in the granulocytic/monocytic and erythrocytic lineages, respectively. We refer to this abnormality as a variable cell lineage abnormality.

Abnormalities in a Patient With AML-M0 (Patient 10)

Chromosome painting of uncultured bone marrow cells showed that all mitotic cells were CD34- and CD13-positive. No erythroid, megakaryocytic or lymphoid mitoses were seen. All ten cells studied were abnormal.

Among CD13-positive and CD3-positive cells, a very low frequency of mitotic cells (<0.1%) was seen in the 4-day cultures, where no mitogen had been added. Phytohemagglutinin stimulation for 4 days increased the mitotic index significantly, from 0.1% or less (cultures without stimulation) to 3.5% for CD13+ cells and to 45% for CD3+ cells. The experiment was repeated twice; first on a new specimen and then on frozen and thawed cells from the first specimen. The results were essentially the same.

Abnormalities in Patients with AML-M5 and -M6 (Patients 16 and 17)

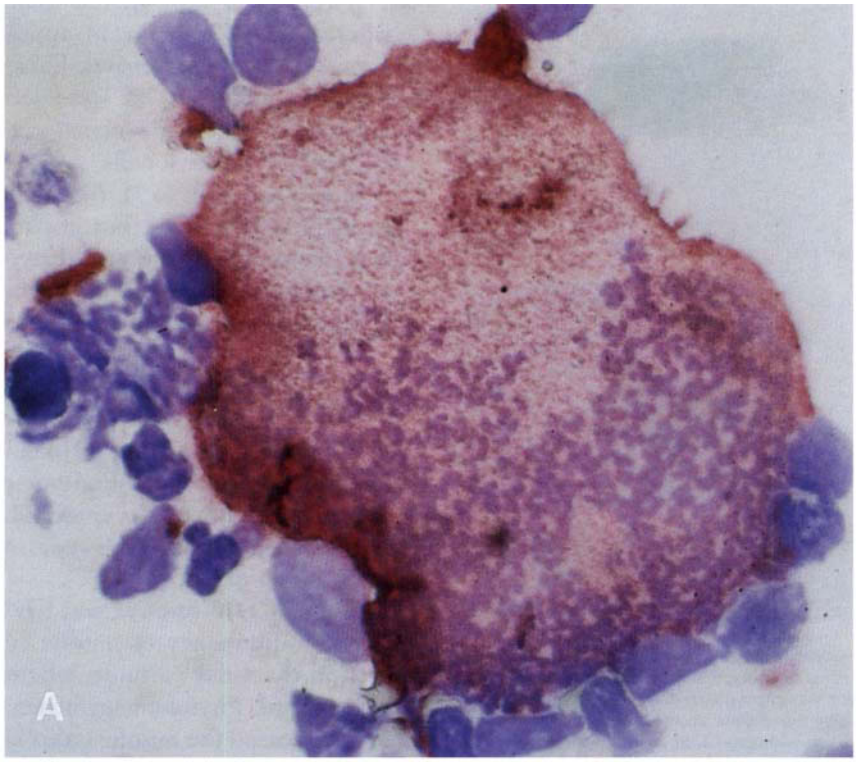
From a patient with AML-M5, only cells with CD13-positive granulocytic/monocytic mitoses showed trisomy 8 (Fig. 1), whereas from a patient with AML-M6, the erythrocytic lineage also showed the abnormality t(8;12) (Fig. 1).

Abnormalities in Patients with Hodgkin's disease

In all 9 patients with Hodgkin's disease, only CD30-positive Reed-Sternberg cells had clonal abnormalities, whereas T- and B-lymphocytes were normal (Fig. 1).

DISCUSSION

In this study, chromosomal abnormalities were used as a marker of malignancy. However, the lack of an abnormality does not necessarily mean that the cell is normal. Clonal expansion before the appearance of the Ph chromosome has been reported in CML (Fialkow et al., 1981a). Moreover, a small proportion of BCR/ABL positive CML patients and MYL/RARA positive AML-M3 patients do not



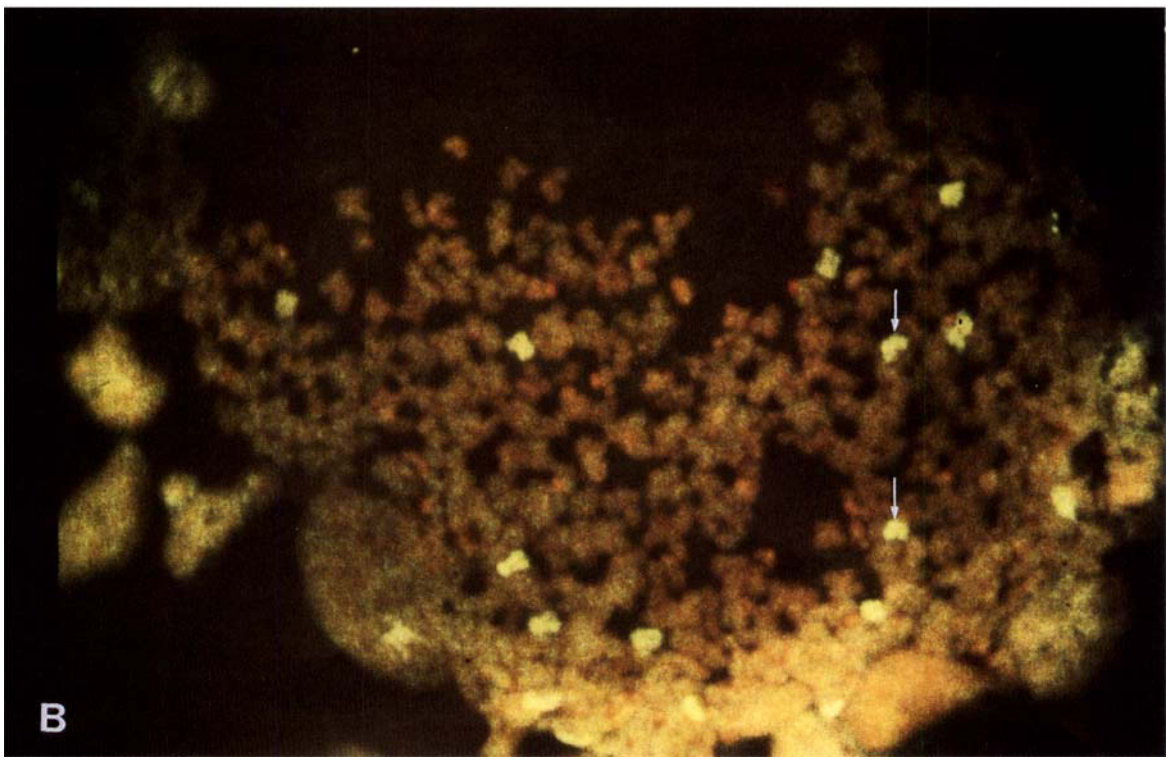


Figure 3. Megakaryocytic mitoses from patient 1 with essential thrombocytosis. **A:** As in Fig 2A, APAAP staining with anti-CD61 was used. **B:** After refixation CISS hybridization with a chromosome 20-specific library probe (ATCC LL20NS01) was used for chromosome painting. Sixteen painted chromosomes 20 are visible, of which some show del(20) (arrows).

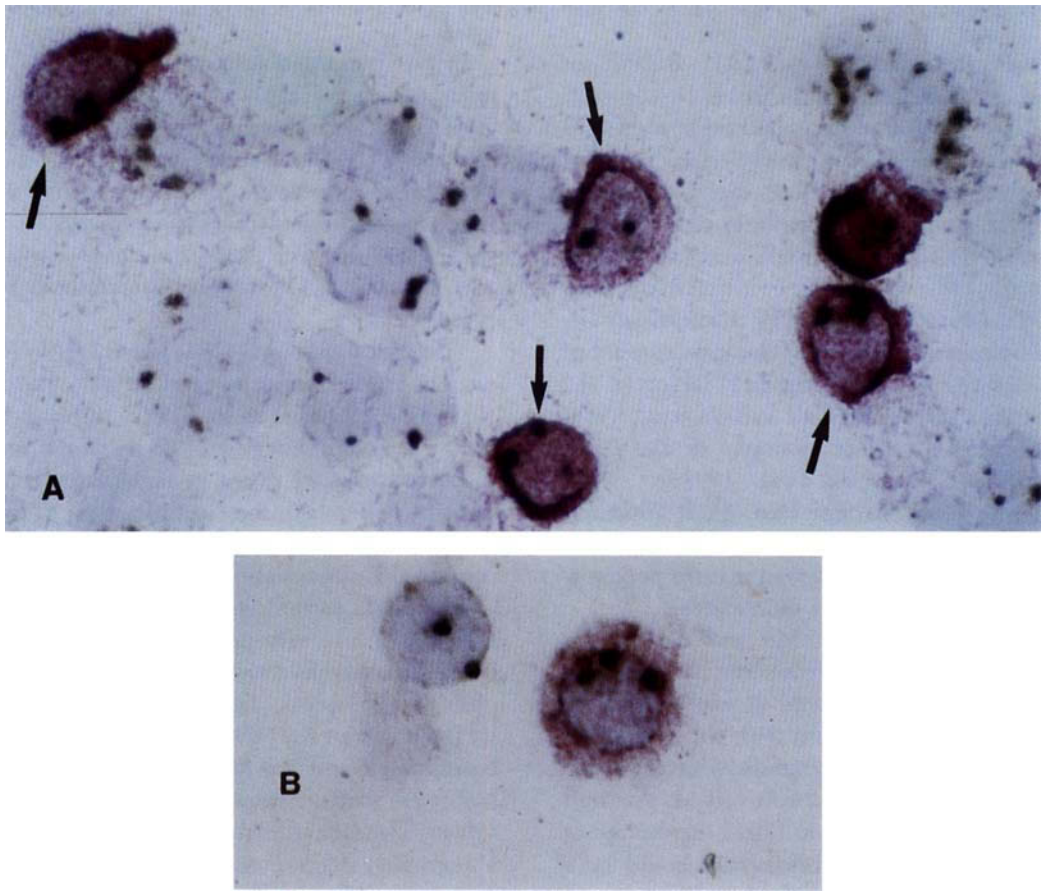


Figure 4. Interphase cells from patient 8 with CML in blast crisis. APAAP staining with anti-CD3 (A) and anti-CD16 (B) followed by in situ hybridization with the chromosome 1-specific probe pU1.77 were performed as described (Knuutila et al., 1993a). Arrows indicate that the CD3-positive T cells contain two signals (A), whereas the CD16-positive large granular lymphocytes (B) contain three signals indicating that the latter cell lineage is involved.

show the t(9;22) and t(15;17), respectively (Heim and Mitelman, 1987; Biondi et al., 1991), and some patients apparently have the AML1/ETO rearrangement characteristic of t(8;21) without having the translocation (Maseki et al., 1993).

Stem Cell Abnormalities

del(20), del(5), t(1;7), t(9;22), t(8;12), and t(3;3) were present in at least two of the myeloid cell lineages. The results with regard to del(20), t(1;7) and t(9;22) are supported by the findings of Price et al. (1992), Kanfer et al. (1992), and Kibbelaar et al. (1992). del(20, t(9;22), and t(3;3) were also seen in CD10-positive lymphoid stem cells. As myeloid leukemic blasts may show aberrant lymphoid antigen expression (Luckit et al., 1993), we cannot directly interpret the finding of CD10-positive blast-like cells containing the abnormalities to mean that a lymphoid lineage is also involved. However, the chromosome abnormalities of patient 8 with CML in blast crisis and patient 5 with myelodysplastic syndrome were seen in CD19/CD20 (both patients) and CD16/CD56 (patient 8 only) positive cells that morphologically resembled small lymphocytes and large granular lymphocytes, respectively, but not blast-like cells. Consequently these cells were considered to belong to a lymphoid lineage and not to a myeloid one with aberrant lymphoid antigen expression. Regarding the erythroid antigen glycophorin A (GPA), granulocytic/monocytic antigen CD13, and megakaryocytic antigen CD61, to our knowledge no aberrant expressions in myeloid leukemic cells have been reported. Furthermore, neither GPA, CD13, nor CD61 are stem cell markers but are seen in cells committed to the erythrocytic, granulocytic/monocytic, and megakaryocytic lineages, respectively, and no events of decommitment have been reported. Thus, the chromosomal abnormalities seen in these lineages are interpreted as having

arisen in a stem cell committed to the myeloid lineages.

The T cells from patient 8 with CML-BC did not reveal the chromosomal abnormality. In contrast, the aberration was found in a small proportion of B cells and LGL cells. To our knowledge, a similar LGL involvement has not been reported before. On the other hand, the involvement of B-lymphocytes is well documented (Fialkow et al., 1978; Martin et al., 1980; Bernheim et al., 1981; Fauser et al., 1985; Munker et al., 1989; Knuutila et al., 1993c). The previous reports of the involvement of T cells are, however, controversial (Fialkow et al., 1978; Bartram et al., 1983; Gale and Canaani, 1984; Heisterkamp et al., 1985; Konopka et al., 1985; Jonas et al., 1992; Knuutila et al., 1993d).

Besides our CML patient (No. 8), it appeared that patient 5 with myelodysplastic syndrome and t(1;7) also had the abnormality in mature polymorphonuclear granulocytes and monocytes.

In patient 17 with AML-M6 and t(8;12), involvement of the granulocytic/monocytic and erythrocytic cells was observed as well. Stamberg et al. (1988) reported a patient with childhood leukemia in whom both the granulocytic/monocytic and erythrocytic lineages were involved. As their patient probably had the same 12p13 breakpoint as our patient 17, the 12p13 breakpoint could be a recurrent aberration in erythroleukemia.

The finding of del(13) accompanied by del(20) in the granulocytic/monocytic cells of patient 3 suggests the involvement of early "pre-malignant" stem cells without any chromosomal abnormality. These cells must have undergone the first non-chromosomal step(s) towards malignant transformation. When the del(20) mutation took place in these "pre-malignant" cells, a growth advantage was presumably conferred on the erythroid and megakaryocytic cells but not on granulocytic/monocytic cells. Furthermore, when the del(13) mutation took place in the pre-malignant cells, those destined to become granulocytes gained an advantage with respect to malignant transformation.

Single Cell Lineage Abnormalities

The abnormalities t(8;21) and t(15;17) were not seen in the erythrocytic or megakaryocytic lineages of our myeloid leukemia patients. This indicates that the mutation took place in a stem cell committed only to the granulocytic lineage. Even though we could study 200 metaphase cells for patients 11 and 13, it is still possible that there is a small erythroid, non-mitotic population that does have the abnormalities. We have no direct evidence of t(8;21) and t(15;17) in mature granulocytes.

In our lymphoid leukemia cases, t(14;18) was restricted to B-lymphocytes (patients 23–26) and inv(14) to T-lymphocytes (patient 21). In the latter case, only CD4+ T cells contained the abnormality (Larramendy et al., 1990). It remains to be investigated whether inv(14) is always associated with CD4-positive helper/inducer lymphocytes and not with CD8 suppressor/cytotoxic lymphocytes.

The data from our B-CLL patients (No 20 in the present study; Knuutila et al., 1986; Autio et al., 1987; Pérez Losada et al., 1991) indicate that trisomy 12, although restricted to B cells, is not likely to be the primary event in malignant transformation. In patient 20, for example, 60% of immunoglobulin light-chain-positive clonal cells did not contain the abnormality.

The ALL patient with abnormality t(5;14) was typical of ALL with pronounced eosinophilia and basophilia. Myeloid cells, including mature eosinophils and basophils, did not contain the abnormality (Knuutila et al., 1993e). This demonstrates that eosinophilia and basophilia are mere reactions to malignant cells, rather than malignant in themselves. The lack of mature lymphocytes having the abnormality suggests that neoplastic lymphoid cells are not capable of differentiating and maturing into functioning lymphocytes.

The results in the Hodgkin's disease patients support our primary findings that only CD30-positive Reed-Sternberg cells carry chromosome abnormalities whereas the karyotypes of T- and B-lymphocytes are normal (Teerenhovi et al., 1988).

Variable Cell Lineage Abnormalities

Monosomy 7 as well as trisomy 8 seem to be the most common types of variable cell lineage abnormality. In patient 15 with AML-M4 (Kere et al., 1988), the abnormality was seen only in monocytes, not in granulocytes. Some of the patients with AML reported by Keinänen et al. (1989) showed the monosomy both in the granulocytic/monocytic and the erthrocytic lineages, whereas others showed it only in the granulocytic-monocytic lineage. Yet other patients (Baurmann et al., 1993) showed the abnormality both in the granulocytic and erythrocytic lineages. Also neutrophils, basophils, eosinophils, and monocytes, but not lymphocytes, have been reported to have trisomy B (Anastasi et al., 1993; van Lom et al., 1993).

Prognostic Implication of the Lineage Involvement in Non-lymphocytic Neoplasms

The abnormalities del(20), t(1;7), del(5), t(9;22), t(8;12), and t(3;3), which we interpret as stem cell abnormalities, are associated with subtypes of leukemia which are highly resistant to treatment; remission can seldom be achieved without bone marrow transplantation.

In contrast to stem cell abnormalities, the single lineage abnormalities [t(8;21) and t(15;17)] are associated with subtypes that respond well to chemotherapy. The knowledge that pluripotent stem cells are not involved in these diseases promises improved accuracy in the targeting of treatment.

ACKNOWLEDGMENTS

We thank Mrs. Heloisa Markkanen and Mrs. Tiina Wirtanen for their expert technical assistance. We gratefully acknowledge the support of the Sigrid Jusélius Foundation and the Finnish Cancer Society.

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